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INFORME DE ACTIVIDADES ACADÉMICAS Y CIENTÍFICAS

Dra. María Gabriela Molina

Facultad de Ciencias Agropecuarias – Universidad Nacional de Córdoba

Período de licencia: 1° de mayo de 2025 – 28 de febrero de 2026

Resolución: RHCD-2025-274-E-UNC-DEC#FCA

1. Introducción

En el marco de la licencia otorgada por la Facultad de Ciencias Agropecuarias de la Universidad Nacional de Córdoba, se desarrollaron actividades académicas y científicas en el Departamento de Bioscienze, Biotecnologie e Ambiente de la Università degli Studi di Bari (Italia).

El objetivo del período fue profundizar la formación en genética molecular aplicada a la producción animal, conservación de recursos zoogenéticos y análisis genómico de poblaciones.

Las actividades realizadas se encuentran certificadas por la institución receptora (Anexo 1).

2. Producción Científica

2.1 Publicación en revista científica

Durante el período de licencia se obtuvo la siguiente publicación científica:

Topputi R., Molina M.G., et al. (2026). Environmentally independent histological markers of wool quality: a comparative study of Gentile di Puglia and Sarda breeds. *Frontiers in Animal Science*.

Documentación respaldatoria: publicación adjunta (Anexo 2).

2.2 Presentaciones en congresos científicos

Se presentaron trabajos científicos en congresos internacionales con publicación en actas:

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- International Conference on Biodiversity, Perugia, Italia (2025)
- 27th International Congress of the Mediterranean Federation, Bologna, Italia (2025)

Documentación respaldatoria:

Certificado de participación Perugia (Anexo 3)

Certificado de participación Bologna (Anexo 4)

Libros de abstracts (Anexos 5 y 6)

3. Actividades de Investigación

3.1 Trabajo de campo

Se realizaron actividades en establecimientos productivos de la Provincia de Bari (Italia), incluyendo registro fenotípico, toma de muestras biológicas (sangre y folículos pilosos) e integración de información productiva y molecular.

Estas actividades se encuentran certificadas por la Università degli Studi di Bari (Anexo 1).

3.2 Actividad de laboratorio molecular

Se desarrollaron tareas de laboratorio vinculadas a extracción de ADN, control de calidad y preparación de muestras para análisis genómico mediante SNP.

Estas actividades se encuentran respaldadas por certificación institucional (Anexo 1).

4. Desarrollo de Competencias Técnicas

Durante la estadía se profundizó la formación en herramientas de análisis genómico y estadístico, incluyendo manejo de PLINK, imputación genómica, estimación de tamaño efectivo poblacional y análisis de estructura genética.

El desarrollo de estas competencias se encuentra respaldado por la certificación institucional (Anexo 1), la producción científica (Anexo 2) y presentaciones en congresos (Anexos 3-6).

5. Proyección Académica

Las actividades desarrolladas permitieron consolidar vínculos académicos con la Università degli Studi di Bari, generando una base para futuras instancias de cooperación científica.

Este aspecto se encuentra reflejado en la certificación institucional (Anexo 1).

6. Conclusión

El período de licencia permitió consolidar la formación en genética molecular aplicada a la producción animal, integrando trabajo de campo, laboratorio y análisis bioinformático.

La experiencia adquirida podrá ser aplicada en el ámbito académico y científico de la Facultad de Ciencias Agropecuarias de la Universidad Nacional de Córdoba.



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DNI 18175235
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ANEXOS

1. Certificación institucional – Università degli Studi di Bari
2. Publicación científica
3. Certificado de participación – Perugia 2025
4. Certificado de participación – Bologna 2025
5. Libro de abstracts – Perugia
6. Libro de abstracts – Bologna
7. Curriculum Vitae

Ciudad de Bari, Italia, 25 Febrero 2026
Departamento de Biosciencia, Biotecnología y Medio Ambiente
Università degli Studi di Bari “Aldo Moro”
Italia

Al Sr. Decano FCA UNC
Ing. Agr. Esp. Jorge Omar Dutto.

Por medio de la presente se certifica que la Dra. María Gabriela Molina desarrolló actividades de investigación en el Departamento de Biosciencia, Biotecnología y Medio Ambiente de la Università degli Studi di Bari “Aldo Moro”, durante el período de su licencia académica.

Durante su estadía participó activamente en proyectos de investigación en el área de genética molecular aplicada a la producción animal y a la conservación de recursos genéticos locales. Sus actividades incluyeron trabajo de campo en la Provincia de Bari, relevamiento fenotípico, toma de muestras biológicas y tareas de laboratorio vinculadas a la extracción de ADN a partir de sangre y folículos pilosos.

Demostró sólidas competencias técnicas en procedimientos de laboratorio molecular, incluyendo la aplicación y optimización de protocolos de extracción de ADN y control de calidad de muestras.

Asimismo, colaboró en la preparación de material para análisis genómicos basados en marcadores SNP. La Dra. Molina contribuyó también en el análisis e interpretación de datos en el marco de estudios de genética de poblaciones y diversidad genómica. Su desempeño se caracterizó por el rigor científico, la autonomía y la responsabilidad profesional.

Durante su colaboración con nuestro grupo de investigación evidenció profesionalismo, compromiso y capacidad para integrar de manera eficaz trabajo de campo, laboratorio y análisis de datos.

Se deja constancia de que su contribución a las actividades científicas del Departamento fue significativa y acorde con los estándares de la investigación académica. La colaboración desarrollada constituye asimismo una base potencial para futuras iniciativas de cooperación científica entre las instituciones involucradas.

Quedo a disposición para cualquier información adicional que se requiera.

Atentamente,

Prof. Dra. Elena Ciani
Departamento de Biosciencias, Biotecnología y Medio Ambiente
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Environmentally independent histological markers of wool quality: a comparative study of Gentile di Puglia and Sarda breeds

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Wool quality plays a role in determining the economic value of sheep breeds, amid the growing global demand for natural and sustainable material. This quality depends on both genetic and non-genetic factors, complicating breed evaluation. Among the most valued qualities is the fine wool produced by Merino and Merino-derived breeds. This study aims to identify parameters measured under controlled environmental conditions to assess the degree of merinization within flocks, meaning the extent to which a breed exhibits morphological and histological traits typical of Merino sheep. The ancient Italian sheep breed, Gentile di Puglia, derived from Merino, was compared with the non-merinized Sarda breed. Our results demonstrate that follicular structure is a key indicator for assessing merinization, under standardized environmental conditions and across different age groups. Gentile di Puglia sheep exhibited significantly higher secondary follicle density and secondary-to-primary follicle ratio ($S/P = 8,96$ vs $2,29$) compared to the Sarda breed, indicating a finer wool structure. The density of sebaceous glands was significantly higher in the adult Gentile di Puglia, and no significant differences in gland area were found between breeds. These results highlight the importance of sebaceous glands in lanolin production and provide objective criteria for evaluating wool quality. This supports the promotion of the Gentile di Puglia breed for sustainable wool production. These results provide a novel phenotypic characterization at the histological level, that could guide future genomic studies aimed at identifying the genes responsible for these traits, relevant for the conservation and

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sustainable use of these local breeds. These findings not only provide a reliable histological basis for assessing wool quality but also offer practical markers that can inform selective breeding programs aimed at enhancing merino-like traits in local sheep populations.

KEYWORDS

environmental influences, fibre quality, follicles, glands, merinization

1 Introduction

The global demand for natural and sustainable materials has reignited interest in wool production, particularly for fine wool, which is highly valued for its flexibility, breathability, and recyclability. Wool's quality is shaped by various genetic and environmental factors, such as breed, diet, and climate conditions, making breed evaluation complex and often subject to environmental bias (Champion and Robards, 2000; Khan et al., 2012; Kijas et al., 2012). Evaluating wool quality based on parameters influenced by external factors, such as diet and pasture conditions, can lead to unreliable assessments of the breed's true potential (Yeates et al., 1975). Therefore, identifying histological markers independent of environmental conditions is essential for an accurate and objective assessment of wool quality. Wool quality is predominantly determined by the density and structure of hair follicles, which are classified as primary and secondary. Primary follicles typically produce coarse wool, while secondary follicles are responsible for finer wool (Li et al., 2020). The relative density of these follicle types, referred to as the secondary-to-primary follicle ratio (S/P), is a key determinant of wool fineness (Lv et al., 2016). Primary follicles are characterized by the presence of large sebaceous glands, a retractor muscle attached to the outer root sheath just below the sebaceous gland and sweat glands.

Wool follicles develop in overlapping waves during foetal life (Moore et al., 1998). Primary follicles begin to form around the 70th day of gestation (Lv et al., 2020) and secondary follicles emerge at about day 85 of gestation, forming a distinct cluster in the skin with the primary follicles. By day 105, derived secondary follicles begin to appear as branches, so the ratio of secondary to primary follicles could be established (Lv et al., 2020). This branching can be extensive and determines the final density of the follicular population (Rogers, 2006). After birth, the histological characteristics of follicles are not influenced by exogenous factors, only by their productive activity. This makes them an interesting parameter for evaluating sheep breeds, as they provide an indication that is not influenced by environmental conditions (Lyne, 1961; Edwards et al., 1996). Recent advances in phenomics-genomics integration highlight that histological traits, such as follicle density and glandular structures, provide more precise indicators of genetic potential than fibre-level metrics alone, which are often influenced

by environmental conditions (Liang et al., 2023). By capturing intrinsic morphological features established during foetal development, these traits allow for more accurate interpretation of breed-specific genetic differences, enabling robust selection strategies and assessment of Merino-derived characteristics.

Another important characteristic, economically relevant in wool production, is lanolin, a fat secreted by the sebaceous glands that coat and softens the wool fibre, that protects it from external agents (Sengupta and Behera, 2014). Over the past decade, lanolin has become increasingly important economically, with a significant increase in its use in cosmetic, medical, and industrial applications (Sengupta and Behera, 2014). Lanolin production varies across breeds making this characteristic a distinctive and valuable factor. The Merino breed is renowned for its fine wool and has historically been used in crossbreeding programs to improve the quality of wool in local breeds across Europe and beyond (Ciani et al., 2015; Al-Atiyat et al., 2016). This process, known as 'Merinization', involves selecting and diffusing morphological and histological traits typical of Merino sheep, such as finer fibre diameter. It has had a significant influence on sheep populations around the world (Granero et al., 2022).

The Gentile di Puglia is an ancient Italian sheep breed, historically distinguished for its fine wool. Romans used these animals to improve Iberian wool production as reported from several roman authors (Ryder, 1983). The Spanish Merino breed was highly valued for its fine, soft wool and its adaptability to the Mediterranean climate, a result of centuries of selective breeding in Spain (Anaya et al., 2024). Over time, the breed shifted towards meat and milk production, but recent interest in sustainable, local materials has revived its significance for wool production. The Gentile di Puglia remains an important genetic resource, particularly for fine wool production and sustainable agricultural practices in Italy (Ciani et al., 2020). The Sarda sheep is a hardy and versatile breed from the island of Sardinia, Italy, primarily known for its high milk production, which is used in the production of the famous Pecorino Sardo cheese. Adapted to the rugged Sardinian terrain and Mediterranean climate, the Sarda sheep excels in extensive farming systems, making it an important breed for both dairy and meat production. Unlike Merino-derived breeds, the Sarda produces coarser wool, which is less commercially valuable but well-suited for traditional textile crafts. The breed's resilience and adaptability have contributed to its widespread use in

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Mediterranean regions (Serranito et al., 2021). From a demographic and conservation perspective, Gentile di Puglia (GdP) is an autochthonous Merino-derived breed with a very small current census: the most recent official data report about 3,739 registered animals in Italy (3,499 females and 240 males), and GdP is included among ovine breeds threatened by genetic erosion and extinction risk and targeted by specific conservation measures (Regione Puglia, 2022; Temerario et al., 2023). In contrast, the Sarda is the most widespread Italian dairy sheep breed: Sarda sheep account for about 80% of Italian dairy sheep and 43% of the national ovine stock and are not currently classified as at risk (Gaspa et al., 2024). The present comparison therefore contrasts a numerically limited, conservation-priority Merino-derived population with a numerically dominant, non-Merino dairy breed that plays a central role in the Italian sheep sector.

Despite the extensive literature available on Merino breeds, many studies have overlooked the evaluation of local sheep populations or failed to identify wool quality traits independent of environmental factors in Merino-derived breeds (McGregor et al., 2016; Rochus et al., 2018; Yaman et al., 2025; Megdiche et al., 2019).

This study aims to fill this gap by comparing the histological characteristics of two Italian breeds: the Merino-derived Gentile di Puglia and the non-Merino breed Sarda. The objective is to identify wool quality parameters evaluated under standardized environmental and seasonal conditions, providing a reliable method for assessing wool quality across different flocks. In particular, this study explores the secondary-to-primary follicle ratio and glandular structures, which could serve as objective indicators of the degree of Merinization and wool quality. Combining phenotypic information with genomic data is a promising way of discovering the genetic basis of complex traits related to fibre development and adaptation (Dzomba et al., 2020; Kalds et al., 2022; Liang et al., 2023).

2 Material and methods

2.1 Ethical statement

All procedures involving animals were conducted in compliance with national and institutional guidelines. Tissue samples were collected post-mortem from animals slaughtered for human consumption, and no animals were specifically euthanized for the purposes of this study. Therefore, ethical approval was not required.

2.2 Animal collection and sample preservation

To standardize environmental effects, all samples were collected from animals reared in the same commercial flock and managed under identical housing, feeding and health-care conditions. Animals were kept under uniform semi-intensive husbandry, with

group housing, outdoor access and a standardized feeding regimen based on forage with supplemental concentrate according to physiological stage. All sampled animals (adults and lambs) were females. Adult ewes (4 years of age, with no close kinship ties according to flock records) were sampled in February (late winter), whereas lambs were sampled at approximately 60 days of age in December (early winter). In this production system, adult males are few and generally retained as breeding rams rather than sent to slaughter; consequently, adult males were not available for sampling. Given the high longevity of these sheep and the limited number of adult animals slaughtered at any given time, it was not possible to further increase the adult sample size.

Ten adult Gentile di Puglia (GdP-A) ewes and ten GdP lambs (GdP-L), and ten adult Sarda ewes (SA-A) and ten Sarda lambs (SA-L) were selected. All adult ewes were in their second or third parity, and all lambs were approximately 60 days old. Skin tissue samples were collected at a commercial slaughterhouse from animals legally slaughtered for human consumption. Biopsies were taken from the dorsal region near the caudal corner of the scapula. Each tissue sample was cut into 1 cm × 1 cm pieces, immediately immersed in 10% neutral buffered formalin and kept at room temperature for 72 h. Samples were then trimmed and fixed for a further 48 h. This procedure preserved the morphological and histological integrity of the tissues and prevented cellular degradation over time. After fixation, samples were processed for histological examination as described below.

2.3 Histological analysis

The fixed samples were dehydrated and embedded in paraffin to prepare them for histological examination. To differentiate between the different follicle types, sections were cut until the sebaceous glands were reached. Histological serial sections, each 5 µm thick, were obtained using a microtome, mounted on microscope slides, and stained. Staining was performed using Harris's Hematoxylin and Eosin (H&E) (Bio-Optica), Periodic acid Schiff (P.A.S.) (Bio-Optica), and Trichrome Van Gieson (Bio-Optica), following the standard protocol. In particular, the samples were exposed to Hematoxylin for 10 min, Eosin 20 sec; to Periodic acid solution for 10 min, Schiff reagent 40 min, Mayer's Hemalum 3 min; to Weigert's iron haematoxylin for 20 min, Van Gieson's Picrofuchsin 10 sec. For quantitative analysis, the density (number per area in mm²) of primary (P) and secondary (S) follicles, the ratio of secondary to primary follicles, named as S/P ratio, and the diameter of the two types of follicles, as well as the density and area of sebaceous (SE) glands, were assessed using a Leica DMRBE microscope. Image processing and measurements were conducted with the Leica Application Suite X program in conjunction with ImageJ software (Schneider et al., 2012). Five random areas were selected from each slide for analysis. Follicle density and diameter were measured at 10X magnification, gland density and area were quantified at the same magnification. The spatial distribution of follicles was evaluated at 5X magnification. The distribution of primary and secondary follicles was evaluated

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measuring the distance between the follicle centre and the X and Y axes of the analysed images. To ensure data reliability, a subset of samples was recounted by the same observer at different times. To validate manual counts, two independent observers performed the measurements in randomly selected sections. Prior to data collection, observer calibration was carried out by jointly examining representative sections and standardizing the counting criteria, ensuring consistency and repeatability across all measurements.

2.4 Statistical analysis of follicular density

Follicle density was analysed using a repeated-measures Gaussian mixed model including the fixed effects of Breed (Gentile di Puglia, Sarda), age class (Adult, Lamb), follicle Type (Primary, Secondary), and all two-way and three-way interactions. Animal was included as a random intercept to account for repeated measurements within individuals. A compound-symmetry correlation structure was specified to model within-animal dependence, and a heterogeneous residual variance by follicle Type was incorporated following AIC-based model comparison.

The full mathematical formulation of the model, including fixed effects, random-effects structure, correlation structure, and heterogeneous residual variances, is reported in [Supplementary Figure 1 \(Supplementary Figure S1\)](#). Model diagnostics were performed using graphical inspections of residuals vs. fitted values, Q–Q plots of standardized residuals, and residual distributions by Breed, age class, and follicle Type. All diagnostic plots, ANOVA tables, and Sidak-adjusted *post-hoc* comparisons are summarized in [Supplementary Figure S1](#).

2.5 Statistical analysis of secondary and primary follicular ratio

The S/P follicle ratio was analysed using a repeated-measures Gaussian mixed model including the fixed effects of Breed, age class, and their interaction, and a random intercept for animal to account for repeated measurements within individuals. Residuals were modelled using a compound-symmetry correlation structure within animals and a heterogeneous residual variance by Breed, as supported by model comparison ($\Delta\text{AIC} = 53.35$). The full model formulation, including the mathematical expression of the random-effects structure and heterogeneous variances, is presented in [Supplementary Figure S1](#).

2.6 Statistical analysis of follicles diameter

The hierarchical mixed model for fibre diameter included the fixed effects of Breed (two levels: Gentile di Puglia, Sarda), age class (two levels: Adult, Lamb), follicle Type (two levels: Primary, Secondary), and all two- and three-way interactions. The model was fitted using the lme function (package nlme) in R (v. 4.4.3),

specifying animal as a random intercept to account for repeated measurements within individuals. A compound-symmetry correlation structure was used to model intra-animal dependence, and a heterogeneous residual variance structure across follicle Types was included to account for type-specific differences in residual variability. Model selection was based on the Akaike Information Criterion (AIC), and the model including the heterogeneous variance structure showed a substantially better fit than the homogeneous-variance model ($\Delta\text{AIC} = 305.3$). Model assumptions were evaluated graphically using residual-versus-fitted plots and Q–Q plots, which indicated adequate normality, appropriate variance modelling, and no patterns suggesting lack of fit. Overall, these diagnostics supported the suitability of the hierarchical Gaussian mixed model.

2.7 Statistical analysis of spatial distribution follicles

We evaluated the spatial arrangement of primary and secondary follicles to determine whether there was repulsion or attraction between them in GdP and SA breeds, considering both adult and lamb stages. Specifically, we analysed the cumulative inter-type of spatial distribution using the Kcross function from the spatstat package ([Baddeley et al., 2015](#)) in R. In this exploratory analysis, Ripley's K function was used to summarize inter-point dependence and clustering. For instance, the estimated K function was compared against its expected value under a completely random process (Poisson). Deviations between the empirical and theoretical K curves indicate either spatial clustering (if K is higher than expected) or spatial regularity (if K is lower than expected). The resulting plots display the K function across a range of distances, along with simulation-based envelopes. If the observed K function deviates beyond the simulated envelopes, the null hypothesis of Complete Spatial Randomness is rejected, suggesting significant spatial structure. Furthermore, the observed spatial patterns are consistent across individual animals, indicating that the relationship between primary and secondary follicles follows a stable and reproducible arrangement within each group analysed.

2.8 Statistical analysis of glands density

The hierarchical mixed model for sebaceous gland density included the fixed effects of Breed (two levels: Gentile di Puglia, Sarda), age class (two levels: Adult, Lamb), and their interaction. The model was fitted using the lme function (package nlme) in R (v. 4.4.3), specifying animal as a random intercept to account for repeated measurements within individuals. A compound-symmetry correlation structure was used to model intra-animal dependence, and a heterogeneous residual variance structure across Breed was included, as this specification substantially improved model fit over the homogeneous-variance model ($\Delta\text{AIC} = 40.8$). Model assumptions were evaluated graphically using residual-versus-fitted plots and Q–Q plots. The diagnostics showed no

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indication of departures from normality or patterns suggesting inadequate variance modelling.

2.9 Statistical analysis of glands area

Sebaceous gland area was analysed using repeated-measures Gaussian mixed model fitted to the log-transformed response, due to the strongly right-skewed distribution of the raw measurements. The fixed-effects structure included Breed (Gentile di Puglia, Sarda), age class (Adult, Lamb), and their interaction. Animal was included as a random intercept to account for repeated measurements within individuals, and a compound-symmetry correlation structure was specified to model within-animal dependence. Model selection based on the Akaike Information Criterion (AIC) supported the use of a heterogeneous residual variance structure by Breed (varIdent), which was therefore incorporated into the final model. The full mathematical specification of the model, including fixed and random effects, correlation structure, and variance heterogeneity, is provided in [Supplementary Figure S1](#). Model assumptions were evaluated using graphical diagnostics, including Q–Q plots of standardized residuals, residuals versus fitted values, and residual distributions by Breed and age class. All diagnostics, together with the ANOVA table for fixed effects and the estimated marginal means (back transformed to the original scale and presented with 95% confidence intervals), are summarised in [Supplementary Figure S1](#).

3 Results

3.1 Analysis of follicles

Analysis revealed significant differences in secondary follicle density and the ratio of secondary to primary follicles between the breeds studied, highlighting the unique characteristics of each breed. Primary follicles were identified by the presence of an apocrine sweat gland duct, the arrector muscle and sebaceous

glands, whereas secondary follicle groups were identified by the presence of sebaceous glands only ([Figure 1](#)).

Follicle density, expressed as number of follicles per mm² ([Figure 2A](#)), showed highly significant effects of Breed ($F_{1,123} = 110.46$, $p < 0.0001$), follicle Type ($F_{1,123} = 686.14$, $p < 0.0001$), and the Breed $\times$ Type interaction ($F_{1,123} = 227.31$, $p < 0.0001$). Age and all remaining interaction terms were not significant ($p > 0.05$). Estimated marginal means revealed that secondary follicles were consistently denser than primary follicles in both breeds. Secondary follicle density reached 58.7 ± 2.0 follicles/mm² in Gentile di Puglia and 19.1 ± 3.7 follicles/mm² in Sarda, whereas primary follicle density remained low and comparable between breeds (Gentile di Puglia: 7.0 ± 0.46 ; Sarda: 8.5 ± 0.81). Full ANOVA results and *post-hoc* contrasts are reported in [Supplementary Figure S1](#).

Estimated marginal means showed that Gentile di Puglia sheep had a markedly higher S/P ratio (8.96 ± 0.37) than Sarda (2.29 ± 0.31), with no age-related differences within breeds. The mixed-model analysis revealed a strong effect of Breed ($F_{1,123} = 251.72$, $p < 0.0001$), whereas neither age ($p = 0.62$) nor the Breed $\times$ Age interaction ($p = 0.65$) was significant. The complete ANOVA table and Sidak-adjusted comparisons are presented in [Supplementary Figure S1](#).

For what concerns the medium average diameter of follicles ([Figure 2B](#)), the model revealed that fibre diameter was significantly affected by Breed ($F_{1,123} = 58.59$, $p < 0.0001$), Age ($F_{1,123} = 11.52$, $p = 0.0025$), follicle Type ($F_{1,3221} = 2432.44$, $p < 0.0001$), and multiple interactions. The three-way Breed $\times$ Age $\times$ Type interaction was highly significant ($F_{1,3221} = 23.90$, $p < 0.0001$), indicating that differences between primary and secondary follicles varied across Breeds and age classes. In Gentile di Puglia, primary follicles were substantially larger in lambs (48.1 ± 1.1 μ m) than in adults (35.8 ± 1.1 μ m), whereas secondary follicles showed no significant age-related differences (lambs: 30.4 ± 1.0 μ m; adults: 26.2 ± 0.86 μ m). In Sarda, the primary follicles showed larger overall diameters (adults: 56.2 ± 1.7 μ m; lambs: 70.1 ± 3.7 μ m) compared to those in Gentile di Puglia, and there were no significant differences in size between the two age groups. Secondary follicles were smaller and relatively stable (adult: 34.8

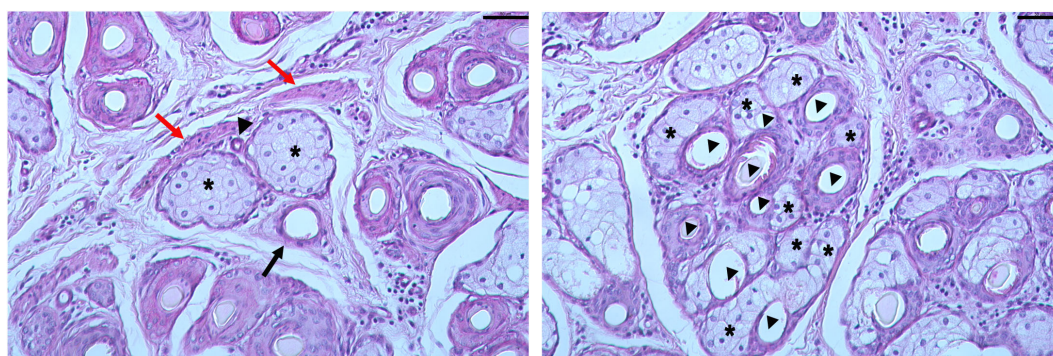


FIGURE 1

Histological features of skin from an adult Gentile di Puglia (GdP A) sheep. On the left, a primary follicle (black arrow) is shown with the sweat gland duct (arrowhead), arrector pili muscle (red arrow), and sebaceous gland (asterisk) clearly visible. On the right, a characteristic cluster of secondary follicles (arrowheads), each with associated sebaceous glands (asterisks), is marked. P.A.S. stain, 20x magnification. Scale bar: 50 μ m.

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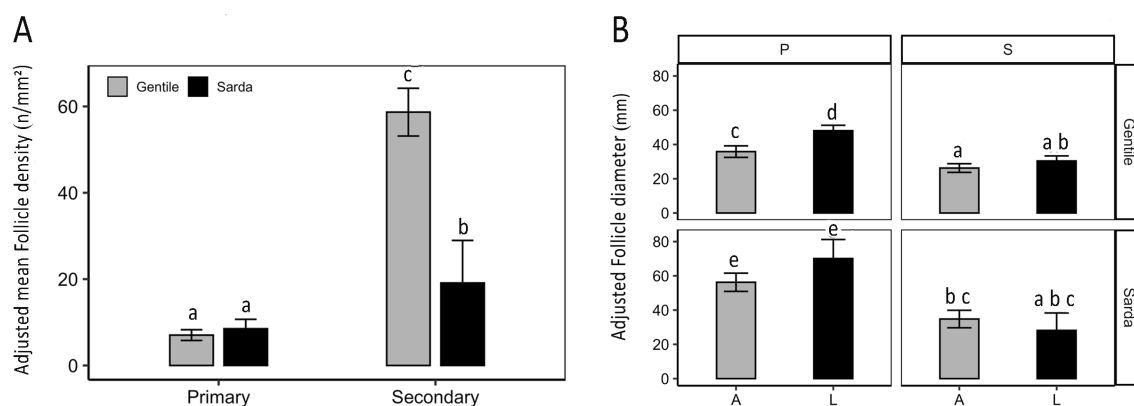


FIGURE 2

(A) Adjusted mean follicle density (follicles/mm²) for primary and secondary follicles in Gentile di Puglia (grey) and Sarda (black) sheep. Values represent estimated marginal means from a repeated-measures Gaussian mixed model including the fixed effects of Breed, Age class, and follicle Type, and a random intercept for Animal. Secondary follicles were significantly denser than primary follicles in both breeds, and Gentile di Puglia showed a markedly higher secondary follicle density than Sarda (Breed × Type, $p < 0.0001$). Different letters indicate Sidak-corrected pairwise differences. (B). Adjusted mean follicle diameter (μm) of primary (P) and secondary (S) follicles in adult (A) and lamb (L) sheep from both breeds, based on the same mixed-model framework. Primary follicles were consistently larger than secondary follicles, with diameter varying across breeds and age classes. Different letters denote significant Sidak-adjusted comparisons.

$\pm 1.7 \mu\text{m}$; lamb: $28.1 \pm 3.4 \mu\text{m}$). Across Breeds, Sarda sheep had consistently larger primary follicles than Gentile di Puglia, while Breed differences in secondary follicles emerged only in adults. Full contrasts are provided in [Supplementary Figure S1](#).

Observation of the spatial distribution of skin follicles at a scale greater than $100 \mu\text{m}$ shows that follicles tend to form clusters. In the GdP breed, these clusters consist of 1 or 2 primary follicles, each

accompanied by its own erector muscle, sweat duct and sebaceous gland, together with several secondary follicles with varying concentrations of sebaceous glands. This is observed in both GdP adults and lambs. A similar but less constant distribution was also observed in the SA lamb, whereas in the adult the primary follicles appeared to be arranged in a linear fashion, showing an orderly and uniform pattern ([Figure 3](#)).

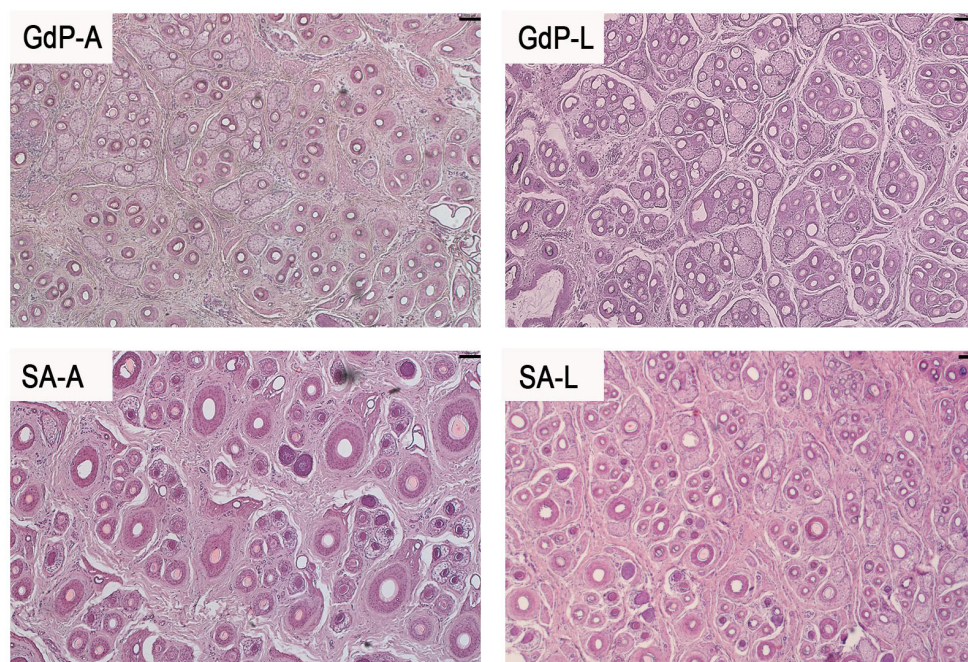


FIGURE 3

Representative skin sections from adult and lamb sheep of the Gentile di Puglia (GdP A, GdP L), and Sarda (SA A, SA L) breeds, illustrating the distribution patterns of primary and secondary follicles. Observe the differences in follicle density, spatial distribution and cluster organisation. H&E staining, 5x magnification. Scale bar: $100 \mu\text{m}$.

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3.2 Analysis of glands

Sebaceous glands associated with both primary and secondary follicles were included in the evaluation (Figure 4). Estimated marginal means showed that adult Gentile di Puglia sheep had a markedly higher sebaceous gland density (90.9 ± 7.4 glands/mm²) than all other groups ($p < 0.05$). The remaining Breed $\times$ Age combinations displayed comparably low densities: Sarda adults (5.6 ± 1.6), Sarda lambs (6.4 ± 2.5), and Gentile lambs (17.7 ± 7.6) (Figure 5). Breed had a highly significant effect ($F_{1,24} = 80.31$, $p < 0.0001$), and a very strong Breed $\times$ Age interaction was detected ($F_{1,24} = 45.05$, $p < 0.0001$). The main effect of Age alone was not significant ($p = 0.115$). These results confirm that the interaction was driven entirely by the sharp increase observed in adult Gentile individuals.

The estimated marginal means for sebaceous gland area were: GdP A = $4688 \mu\text{m}^2$ (CI95%: 3945–5570), GdP L = $5939 \mu\text{m}^2$ (5047–6990), SA A = $5594 \mu\text{m}^2$ (4594–6812), and SA L = $5497 \mu\text{m}^2$ (4078–7411). These values did not differ significantly, as none of the fixed effects were significant: Breed ($p = 0.65$), Age ($p = 0.11$), or their interaction ($p = 0.23$). These results indicate that sebaceous gland area was stable across Breeds and age classes. Full EMMs and CIs are provided in Supplementary Figure S1.

4 Discussion

4.1 Wool production

Despite the continuous development of new synthetic fibres, wool production remains an economically significant activity with high value in terms of environmental sustainability. For this reason, many countries continue to assess the quality of wool produced by different sheep breeds, often in the context of genetic improvement programs aimed at improving these characteristics. The wool production cycles in hair follicles are influenced by seasonality (Ansari-Renani et al., 2011). In addition, wool quality is also dependent on animal maintenance, which can introduce variability unrelated to the genetic potential of breeds and affect the reliability of wool-based evaluations. Strong evidence linking the presence of wool and follicle structure to environmental factors is

provided by the Pelibuey breed, a woolly and hairless sheep found mainly in tropical regions and bred primarily for its high-quality meat (Gutiérrez et al., 2005). To provide a comparative context, we included histological images of the hairless Pelibuey sheep breed in Supplementary Figure S2 (Supplementary Figure S2) of the supplemental materials. These images highlight the stark differences in follicle density and structure compared to the wool-producing Gentile di Puglia and Sarda sheep breeds, further emphasizing the environmentally influenced spectrum of follicle development.

4.2 Histological traits as indicators of merinization

The aim of this study was to evaluate histological parameters measured under controlled environmental conditions, in order to minimise the influence of short-term external factors, and to characterise a local sheep breed, Gentile di Puglia, thought to be of Merino origin, and a local non-Merino breed, Sarda, at two different stages of their lives. In general, histomorphometric analyses have consistently shown that breed is the main factor determining the follicular and glandular characteristics of sheep skin, while age and follicle type modulate specific components of this pattern. The literature on Merino breeds shows that even a small variation in the secondary to primary follicle ratio can significantly influence fleece characteristics. The S/P ratio is closely related to fibre thickness (Adams and Cronjé, 2003). When compared with published values for Merino and Merino-derived breeds, the values observed here for Gentile di Puglia (S/P: 8.96 ± 0.37 ; fibre diameter: $22.85 \mu\text{m}$) are intermediate between those of fine Merino (S/P ≈ 20.6 ; fibre diameter $\approx 17.5 \mu\text{m}$; Carter and Clarke, 1956) and those reported for other ‘merinized’ breeds such as Polwarth and Corriedale (S/P ≈ 13.2 and 10 ; fibre diameter ≈ 23.5 and $31.5 \mu\text{m}$, respectively). In contrast, the non-merinized breed analysed in this study (Sarda) exhibits a low S/P ratio (2.29 ± 0.31) and a higher fibre diameter (about $45 \mu\text{m}$). Taken together, these patterns support the classification of Gentile di Puglia as a Merino-derived, merinized local breed, and of Sarda as a coarse-wool, non-merinized type. While fibre characteristics are defined when the follicle matures, density is established earlier, during foetal life (Moore et al., 1998), and is

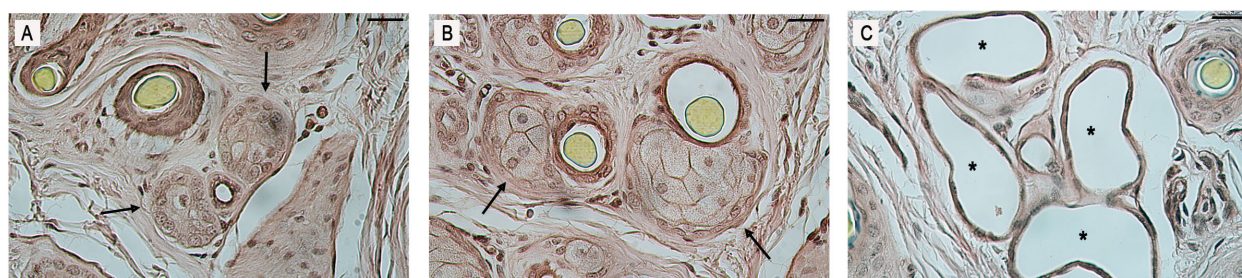


FIGURE 4

Skin section from a Gentile di Puglia adult (GdPA) sheep illustrating the distribution of glands: (A) Sebaceous glands (arrows) surrounding a primary follicle; (B) Sebaceous glands (arrows) associated with a cluster of secondary follicles; (C) Sweat glands (asterisks). Van Gieson's stain, 40x magnification. Scale bar: 25 μm .

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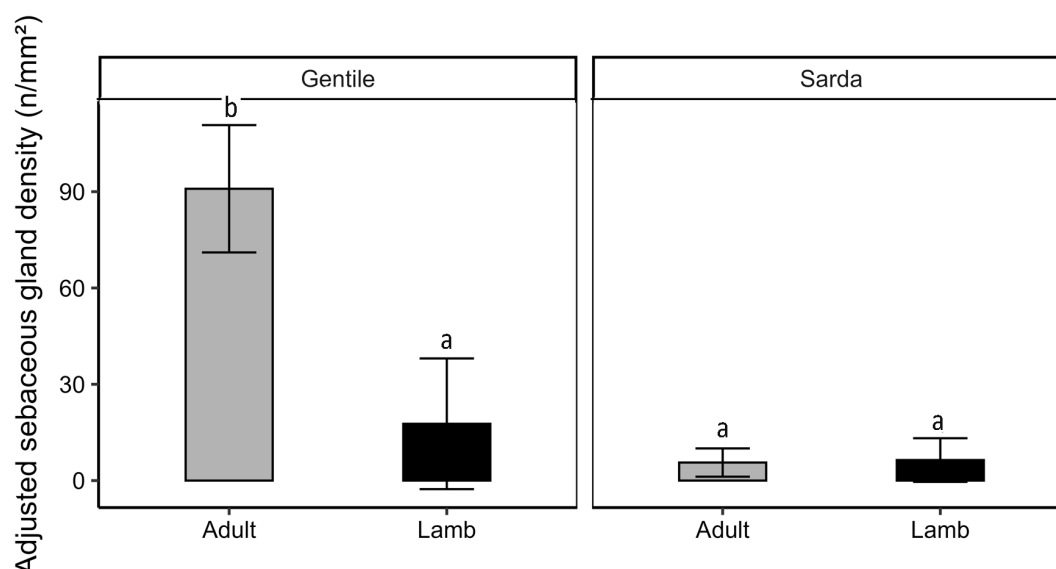


FIGURE 5

Adjusted mean sebaceous gland density (n/mm²) in adult and lamb sheep of the Gentile di Puglia and Sarda breeds. Values represent estimated marginal means obtained from the Gaussian mixed model described in the Methods. A pronounced increase in sebaceous gland density was observed only in adult Gentile di Puglia sheep, whereas all other groups showed similarly low values (Breed effect, $p < 0.0001$). Different letters indicate significant Sidak-adjusted pairwise differences.

largely determined prenatally, tending to remain relatively stable across age classes under non-extreme conditions.

It is important to note that we do not claim that follicle traits are completely insensitive to environmental or age effects. On the contrary, previous work has shown that hair follicle density can decrease with age in Merino-type sheep (Dmitrik et al., 2021) and that maternal undernutrition during late gestation can reduce secondary follicle density in Merino fetuses (Lv et al., 2020). Our results should therefore be interpreted within the specific context of this study, namely animals of similar age classes, reared in the same flock and sampled under homogeneous management and seasonal conditions. Under these standardised conditions, the large and consistent differences in S/P ratio and follicle architecture observed between Gentile di Puglia and Sarda are most likely to reflect underlying genetic and long-term selection differences rather than short-term environmental variability.

From a genetic standpoint, the contrasting fleece colours of Gentile di Puglia (predominantly white fleece with pigmented head and limbs) and Sarda (more variable pigmentation and generally coarser wool) are consistent with differences at major coat-colour loci. In sheep, coat colour is mainly controlled by genes involved in melanogenesis, particularly the melanocortin 1 receptor gene (MC1R) at the Extension locus and the agouti signalling protein gene (ASIP), which interact to regulate the balance between eumelanin and pheomelanin production (Fontanesi et al., 2010; Koseniuk et al., 2018). Sequence and association studies in Italian and Mediterranean breeds have shown that specific MC1R and ASIP haplotypes underpin the segregation of black, brown and white fleece variants, including in local breeds such as Massese and Sarda (Fontanesi et al., 2010; Fontanesi et al., 2011). More recently, work on the endangered Karamaniko sheep combined neutral

markers and MC1R genotyping, indicating that long-term selection for white fleece has canalised genetic variation at the Extension locus in an autochthonous Mediterranean population (Giantsis et al., 2022). Genome-wide and transcriptomic analyses further support a central role of MC1R, ASIP and other melanogenesis genes in shaping black-versus-white and patterned coat phenotypes in sheep (Fan et al., 2013; Zhou et al., 2023). Although comparable genomic data are not yet available for Gentile di Puglia, the histological pattern observed here, together with the breed-standard description of colour, suggests that integrating MC1R/ASIP genotyping and broader genomic scans with follicle histomorphometry would be a powerful next step to dissect the genetic basis of wool colour in these local breeds.

4.3 Follicle morphology and spatial distribution

Beyond density, follicle morphology and spatial distribution provide further information into breed-specific fleece characteristics. Previous studies on the distribution patterns of hair follicles have suggested that they tend to optimally occupy all available space and that their distribution can vary significantly during foetal development (Linsenmayer, 1972; Nagorcka and Mooney, 1985; Moore et al., 1998). These studies have provided a theoretical basis for understanding the spatial distribution of follicles in different breeds and developmental stages. The present study focuses on characterizing the follicle distribution in two sheep breeds, the merinized Gentile di Puglia and the non-merinized Sarda, revealing differences between the two. Specifically, the analysis showed that, while primary follicles density is

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comparable between the two breeds, the size of primary follicles in the Sarda breed is significantly larger than that observed in the GdP. Conversely, a higher number of secondary follicles are observed in the GdP, while the sizes remain similar in both breeds. These differences suggest a different spatial and relational organisation between follicle types in the two breeds. Merinos typically present a clustering of follicles, meaning a disposition of secondary follicles around a single primary follicle (Rogers, 2006). In the GdP breed, the follicles tend to form compact clusters, reflecting a close spatial relationship between primary and secondary follicles. In contrast, Sarda follicles are more dispersed, forming loose clusters. Specifically, in the adult, this disposition indicates a less compact cluster arrangement, which may suggest different evolutionary adaptations related to environmental conditions or selective breeding objectives of the two breeds. These observations highlight the importance of considering both the number and size of follicles in understanding fleece growth dynamics. However, while histological analysis provides objective structural insights, it does not fully account for the functional activity of follicles or glands. In this context, it is interesting to note that our data, particularly with regard to the age-related reduction in primary follicle diameter observed in Gentile di Puglia, do not entirely align with the existing literature on fibre diameter in Merino breeds (Dmitrik et al., 2021).

These histological differences also fit within the broader framework of sheep phenotypic evolution, in which domestication and subsequent breed formation have repeatedly targeted wool traits such as fibre diameter, density and crimp. Comparative genomic studies indicate that fine-wool breeds carry strong selection signatures at loci involved in hair follicle development, keratin structure and epidermal growth factor signalling, reflecting long-term selection for dense, fine fleeces (Kalds et al., 2022). Within this context, Gentile di Puglia can be interpreted as the Mediterranean outcome of historical Merino introgression and selection for improved fleece quality, as suggested by its high S/P ratio, compact follicle clusters and intermediate fibre diameter, all consistent with a merinized fine-wool phenotype. In contrast, the Sarda, with its lower S/P ratio, larger primary follicles and coarser fibres, appears closer to the ancestral dual-purpose type adapted to extensive management in the Mediterranean environment. The comparison between these two breeds therefore illustrates an evolutionary trajectory from less specialised, mixed hair-wool coats towards highly merinized fine-wool phenotypes, driven by human selection but constrained by the developmental architecture of the follicle population. Future studies combining genome-wide selection scans with histomorphometric data across a broader panel of Mediterranean breeds would help to quantify how often this “merinization pathway” has been followed and which genes have been repeatedly targeted by selection for wool traits.

However, the overall follicular architecture, density and proportions of primary to secondary follicles remain consistent, supporting the structural patterns described. These findings suggest the existence of breed-specific or sample size-related variability, which warrants further investigation in future studies.

4.4 Sebaceous glands and lanolin production potential

Another important characteristic for the enhancement of sheep breeds is the presence and activity of sebaceous glands, responsible for the production of lanolin (Zhang et al., 2017). Lanolin (wool grease or wool wax) is a complex mixture of sterol esters and other lipids secreted by the sebaceous glands attached to wool follicles; it can account for 10–25% of the weight of freshly shorn wool and represents a valuable raw material for the cosmetics and pharmaceutical industries due to its emollient, moisturising and protective properties (Jiang et al., 2014; El-Sayed et al., 2018; Bhavsar et al., 2023; Lis, 2024). Beyond its economic relevance, lanolin contributes to waterproofing and protecting the fleece and skin against climatic and environmental factors, and is involved in skin barrier homeostasis. Sebaceous glands are closely associated with primary follicles and variously distributed within secondary follicle groups. In this study we examined the distribution and size of these glands in Gentile di Puglia and Sarda sheep. While the size of sebaceous glands did not differ significantly between breeds, sebaceous gland density exhibited a pronounced Breed × Age interaction driven by an exceptional increase in adult Gentile di Puglia sheep, whereas all remaining Breed × Age groups showed similarly low values. This pattern suggests breed-specific regulation of sebaceous activity that emerges only at mature stages and is consistent with the Merino-derived, fine-wool background of Gentile di Puglia.

From a biological perspective, the pilosebaceous unit is a hormone-responsive mini-organ, integrating systemic and local endocrine signals. Studies in mammals have shown that sebaceous gland growth and function are under complex hormonal control: androgens are key stimulators of sebocyte proliferation and lipid synthesis, while other hormones and neuropeptides, such as prolactin, growth hormone, corticosteroids and components of the cutaneous neuroendocrine system, modulate sebaceous activity and interact with local paracrine factors (Deplewski and Rosenfield, 2000; Zouboulis, 2010; Szöllösi et al., 2018; Clayton et al., 2020; Datta et al., 2022; Okoro et al., 2023). Although such mechanisms have been mainly characterised in humans and laboratory animals, similar endocrine principles are expected to apply to ruminant sebaceous glands, which express a wide range of hormone receptors and enzymes for steroid metabolism. In this framework, the higher sebaceous gland density observed in adult Gentile di Puglia may reflect a breed-specific sensitivity of sebocytes to the endocrine milieu, including differences in androgen signalling or in the timing and intensity of hormonal stimuli around puberty and adult reproductive life.

Functionally, an increased density of sebaceous glands in adult Gentile di Puglia is compatible with a higher potential for lanolin production, which could enhance fibre lubrication, protection against weathering and possibly resistance to certain forms of fleece damage. Previous work in Merino and Romney sheep has linked variation in glandular structure and secretion rate to wool yellowing and sweating patterns, underscoring the role of skin

glands in determining both aesthetic and functional wool traits (Sumner and Craven, 2004). In conservation and breeding programmes, such differences should not be viewed solely as a source of variation in commercial yield, but also as part of the adaptive phenotype of local breeds to their traditional environments. For Gentile di Puglia, a Merino-derived breed of limited census and conservation concern, preserving the range of sebaceous gland phenotypes may therefore be important both for maintaining its historical wool characteristics and for sustaining its adaptive potential under changing climatic conditions.

From a genetic perspective, the marked Breed $\times$ Age pattern in sebaceous gland density suggests that this trait could be integrated into selection schemes once its molecular determinants are better understood. Recent skin transcriptome and genomic studies in fine-wool sheep have identified several candidate genes and pathways related to lipid metabolism, skin structure and hair follicle development that are associated with differences in wool traits and follicle density (Zhang et al., 2017; Bai et al., 2021; Li et al., 2020; Zhao et al., 2021). It is therefore plausible that variation in sebaceous gland density and lanolin output in Gentile di Puglia and Sarda is at least partly under genetic control and may be linked to genomic regions affecting sebocyte differentiation and lipid synthesis. In a conservation-oriented context, the histological markers proposed here could serve as phenotypic anchors for future genome-wide association or selection-scan studies aimed at identifying loci influencing sebaceous activity. Once such loci are characterised, they could be incorporated into marker-assisted or genomic selection strategies that enhance wool and lanolin-related traits in Gentile di Puglia while explicitly constraining inbreeding and preserving genome-wide diversity. In this way, sebaceous gland density would become not only a useful descriptor of wool quality, but also a biologically meaningful target trait within integrated conservation and breeding programmes for Merino-derived local breeds.

4.5 Concluding remark and future perspectives

In conclusion, the histological analysis of hair follicles has proven to be an effective tool for assessing the degree of merinization in sheep breeds, when performed under standardized environmental and management conditions. This approach allows for an accurate and objective characterization of breeds, enabling clear distinctions between merinized and non-merinized breeds based on intrinsic biological parameters, such as the ratio of secondary to primary follicles (S/P), and the density and distribution of the follicles themselves. The Gentile di Puglia clearly exhibits the histological characteristics typical of merinized breeds, with an S/P ratio and follicle structure like those observed in Merino breeds. This supports its classification as a local genetic resource of high value for sustainable wool and lanolin production. Further studies should combine histological data with genomic and transcriptomic analyses. This integrated approach could help to identify the genetic determinants of key morphological traits. These integrative

analyses would be crucial for conservation strategies and for developing breeding programmes that enhance fibre quality while preserving the genetic diversity of local sheep breeds.

From an applied perspective, our findings support three main recommendations for research, conservation and breeding. First, we suggest that the secondary-to-primary follicle ratio (S/P), together with primary and secondary follicle densities and sebaceous gland density, should be integrated into phenotypic recording schemes and breed databases for fine-wool and local sheep breeds. When measured under standardised conditions, these histological indicators provide intrinsic information on the degree of merinization and on the potential for wool and lanolin production, complementing conventional fleece traits. Second, we recommend that future histological studies on wool breeds adopt simple, standardised sampling protocols, including the use of animals of the same sex and clearly defined age classes (e.g. adult ewes and lambs), reared under homogeneous management and seasonal conditions, and skin biopsies collected from a consistent body site (such as the dorsal region near the caudal corner of the scapula), with a sufficient number of animals per Breed $\times$ Age class to ensure robust inference. Third, we highlight the potential to link these histological markers with emerging genomic and transcriptomic information, including functional variants at genes such as KRT74 and EDAR, which have recently been shown to synergistically drive finer and denser wool production in Chinese fine-wool sheep (Liang et al., 2024). In this context, Gentile di Puglia and related Merino-derived populations represent ideal candidates for future integrative studies that combine histomorphometric data with candidate-gene and genome-wide markers to inform conservation-oriented, marker-assisted or genomic selection programmes for wool and lanolin traits.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because The samples came from animals intended for human consumption and collected during slaughter operations.

Author contributions

RT: Conceptualization, Investigation, Validation, Writing – original draft. MM: Conceptualization, Formal analysis, Software, Writing – original draft, Writing – review & editing. GV: Conceptualization, Resources, Writing – original draft. JQ: Resources, Software, Writing – review & editing. MR: Resources, Validation, Writing – review & editing. SB: Formal analysis, Methodology, Writing – original draft. EC:

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Conceptualization, Formal analysis, Funding acquisition, Resources, Writing – original draft, Writing – review & editing. LG: Formal analysis, Methodology, Writing – original draft. AC: Software, Writing – review & editing. VL: Conceptualization, Formal analysis, Funding acquisition, Resources, Writing – original draft, Writing – review & editing. TM: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fanim.2025.1670137/full#supplementary-material>

SUPPLEMENTARY FIGURE 1

Mixed-model structures, assumption checks, ANOVA tables, and *post-hoc* estimated marginal means for all histomorphometric variables. ¹ Models. All traits were analyzed using hierarchical Gaussian mixed models fitted with the lme function (nlme package, R v. 4.4.3). For each variable, fixed effects included Breed (two levels: Gentile di Puglia, Sarda), age class (Adult, Lamb), and follicle Type (Primary, Secondary) when applicable. Animal (VETRINO) was included as a random intercept to account for repeated measurements within individuals. A compound-symmetry correlation structure (corCompSymm) was used to model within-animal dependence. When supported by Akaike Information Criterion (AIC) a Residual variance structure (varIdent): Density and Diameter: heterogeneous residual variance by follicle Type (Primary vs Secondary); S/P ratio, Sebaceous gland density, Sebaceous gland area: heterogeneous residual variance by Breed (Gentile vs Sarda). ² Assumptions. Normality and homoscedasticity were evaluated graphically through residual-versus-fitted and Q–Q plots. “Normality” and “Homoscedasticity” panels indicate that diagnostic plots did not show departures from model assumptions. ³ ANOVA columns. numDF = numerator degrees of freedom; denDF = denominator degrees of freedom; F-value = test statistic; p-value = significance level. Asterisks (*) indicate statistically significant effects at $\alpha = 0.05$. ⁴ *Post-hoc* contrasts *Post-hoc* estimated marginal means (EMMs). emmean = estimated marginal mean; SE = standard error; df = degrees of freedom; LCL/UCL = lower/upper 95% confidence limits. Grouping letters are generated by compact letter display (CLD): Groups sharing at least one symbol are not significantly different (Sidak-adjusted $\alpha = 0.05$).

SUPPLEMENTARY FIGURE 2

Skin histology of Pelibuey sheep. The right panel shows a low magnification view of general skin architecture with visible primary follicles. The left panel highlights a region containing sweat glands. Notice the absence of secondary follicles, common in wool-producing breeds. H&E staining, 5x magnification. Scale bar: 100 μ m.

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Certificate of Attendance

This is to certify that

MARIA GABRIELA, MOLINA

has attended the

15th National Conference on Biodiversity

2nd International Conference on Mediterranean Biodiversity

June 3-6, 2025, Perugia, Italy

The Chair of the Organizing Committee
Prof. Pietro Buzzini

A handwritten signature in black ink, appearing to be 'P. Buzzini', written over a horizontal line.

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**Mediterranean Federation
Health and Production of Ruminants**



We certify that

MARIA GABRIELA MOLINA

attended the 27th Congress of the
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President of the Organizing Committee

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**BIODIVERSITY
2025**

**15TH NATIONAL CONFERENCE
ON BIODIVERSITY**

**2ND INTERNATIONAL CONFERENCE
ON MEDITERRANEAN BIODIVERSITY**



BIODIVERSITY IN THE XXI CENTURY: NEW PARADIGMS FOR NEW CHALLENGES

PROGRAMME

JUNE 3-6, 2025

**HOTEL GIÒ - PERUGIA CONGRESS CENTRE
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**15th National Conference on
Biodiversity
2nd International Conference on
Mediterranean Biodiversity**

***Biodiversity in the XXI century: new paradigms
for new challenges***

Perugia, Italy

June 3-6, 2025

BOOK OF ABSTRACTS

Edited by:

Pietro Buzzini
Benedetta Turchetti
Ciro Sannino
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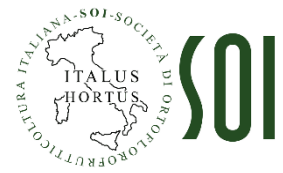
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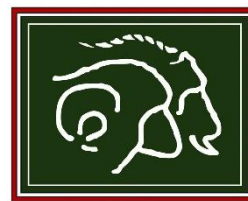


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WELCOME TO PERUGIA

Dear attendees of the 15th National Conference on Biodiversity - 2nd International Conference on Mediterranean Biodiversity,

Welcome to Perugia!

Perugia, the capital of the beautiful Umbria, is one of the most evocative destinations of the region, a city of artists such as Perugino, Pinturicchio and Raffaello, as well as the contemporary art of Burri and Beuys). Built on a hill in the valley of the Tevere river, the city, with its history and artistic and cultural heritage, is one of the most popular destinations.

Starting from its Etruscan walls and walking through the streets of Old Town, Perugia present a succession of monuments. It is also an important cultural centre, with its historic University and the oldest and most prestigious University for Foreigners in Italy.

In addition to its artistic and cultural riches, Perugia offers tasty itineraries to try food and wine, with all the flavour of simple regional cuisine. Perugia also offers many opportunities for fun, with pubs and discos, or by visiting it on the occasion of its major events, such as the renowned international festival Eurochocolate, which transforms Perugia in October in the most coveted destination for lovers of the food of the gods. Equally noteworthy is the Umbria Jazz Festival in July, which offers ten days of shows and jazz, ranking among the most important jazz events in Europe.

Besides, the beauty of the surrounding towns (Assisi, Todi) and nature (Trasimeno lake, Marmore falls) and the rich cultural heritage of the region will provide an excellent opportunity for scientific presentations and discussions as well as for the informal encounters.

FROM THE ORGANIZING COMMITTEE

Dear Friends and Colleagues,

welcome to the 15th National Conference on Biodiversity - 2nd International Conference on Mediterranean Biodiversity, Perugia, Italy, organized by the Department of Agricultural, Food and Environmental Sciences of the University of Perugia, in cooperation with the Accademia delle Scienze della Biodiversità Mediterranea (ASBM).

Current human activities are leading to a significant loss of biodiversity of both natural and agricultural ecosystems. The new millennium has led to new opportunities for the conservation and the sustainable use of biodiversity, but at the same time has meant about the emergence of new threats. The rapid progress of scientific knowledge (including new and more advanced study methodologies), the impact of climate change, the increasing consumption of land, the spread of invasive alien species and the emergence of new pathogens have profoundly changed the overall picture (including the human perception) of the strategic value of biodiversity, stimulating the research and development of new and more effective solutions for its sustainable use.

The assessment of biological diversity is a key indicator for monitoring the health of terrestrial ecosystems, often subjected to a growing human impact, including the effects of global warming. The correct functioning of the biosphere is based on the presence of multiple biological networks that are resilient to changes, ensuring the continuity of animal, plant and microbial life. The extinction of one or more species can lead to unexpected impacts, sometimes threatening the loss of entire ecosystems. Climate change, loss of biodiversity and degradation of ecosystems are interconnected phenomena, with devastating consequences for the economic, environmental and social stability of the planet.

Globally, plants constitute over 80% of the human diet: 30,000 plant species are considered edible, 7,000 are cultivated for food, but only 30 are used for food purposes on a large scale. Among them, rice, wheat, corn, millet and sorghum ensure 60% of global food resources. Among animals, of the 30 domesticated species, only 14 ensure 90% of food of animal origin. About microorganisms, it has been estimated that less than 1% of total microbial biodiversity has been described and studied so far.

The 15th National Conference on Biodiversity will disseminate the latest basic and applied scientific knowledge on the study, conservation and sustainable use of animal, plant and microbial biodiversity. The topic of the Conference, "Biodiversity in the 21st century: new paradigms for new challenges", was chosen to provide answers to the new challenges imposed by the growing impact of human activities and climate change on animal, plant and microbial biodiversity in natural and agricultural ecosystems, suggesting possible solutions and defining innovative intervention models, both aimed at in-situ and ex-situ conservation, as well as sustainable enhancement, also in light of the principles of circular economy, in line with the EU strategies envisaged within the European Green Deal.

The 15th National Conference on Biodiversity is also associated with the 2nd International Conference on Mediterranean Biodiversity. The Mediterranean basin represents not only an area important for its history, cultures and traditions, but also a complex of natural and agricultural ecosystems that are hotspots of plant, animal and microbial biodiversity still understudied.

INVITED LECTURES

Biodiversity monitoring and knowledge in the implementation of the nature restoration regulation – NRR

June 3, 2025, 16:00

CARLO BLASI

Sapienza University of Rome, Italy

Professor Emeritus of Sapienza University of Rome He is currently Director of the scientific council of the Interuniversity Center for Biodiversity, Ecosystem Services and Sustainability" (CIRBISES); president of the Foundation for Italian Flora (from 2008); member of the National Observatory for Biodiversity; He was also president of the Italian Botanical Society (SBI); president of the Italian Society of Vegetation Science (SISV); president of the Fédération internationale de phytosociologie (FIP); president of the Italian Federation of Natural and Environmental Sciences (FISNA) Furthermore on behalf of the Ministry of the Environment (MASE) he is member of the National Commission for Natural Capital (since 2016); member of the Control Room of Measure M2C4 I 3.1 (Protection and enhancement of urban and extra-urban greenery) of the NRRP dedicated to urban, peri-urban and extra-urban forestry (2021); His most recent interests include the ecological classification of the territory, the dynamics of vegetation and the evaluation of the state of conservation, with research aimed at producing cartographic and descriptive syntheses at national and regional scale, such as the Vegetation of Italy (with the Map of the Vegetation Series of Italy), the Vegetation Prodrome of Italy, the Map of the Eco-Regions of Italy and the Flora of Italy. He is currently interested in restoration ecology applied in particular to the urban system and the definition of the national ecological network. He is the author of over 440 publications, most of which are in international journals and 40 book chapters. He is also the author of several volumes on flora, vegetation and plant landscape, is a member of the editorial board of numerous international journals and was Editor-in-chief of the international journal Plant Biosystems.

INVITED LECTURES

Nature based solutions for ecosystem restoration: the experience of the National Biodiversity Future Centre

June 3, 2025, 16:45

CARLO CALFAPIETRA

Institute of Research on Terrestrial Ecosystems (IRET), CNR, Italy

Director of the Institute of Research on Terrestrial Ecosystems (IRET) of CNR. PhD in Forest Ecology at University of Padova and Marie Curie fellow at University of Madison- Wisconsin (USA). His main interests are the biosphere-atmosphere interactions and the effects of global change and air pollutants on these with a particular focus on the biogenic VOC emission both in urban and rural environments. He is expert for the EU Commission for Green infrastructure and Nature Based Solutions (NbS). He has published more than 100 research papers on international journals/books on these topics (H-index: 58) and has coordinated past or ongoing international and national projects including a PRIN project and a COST Action on Urban forests, two Infrastructural PON projects, vice coordinator of a H2020 project on NBS. He is the Italian Focal point of the European Research Infrastructure ICOS and Coordinator of the Italian Hub of the NbS. He is in the Coordination team and CNR Responsible of the National Centre of Biodiversity of the PNRR. He is regularly invited as key-note speaker in a number of international events. (www.carlocalfapietra.com).

INVITED LECTURES

10 years after the Nagoya Protocol: the road ahead

June 3, 2025, 17:30 pm

VALENTINA VENEROSO

Udine Bar Association, Italy

Valentina Veneroso is an Italian lawyer, with a Post Graduate Certificate in Environmental Law. She gives advice and assistance in the field of environmental law and bio law. Over the last 10 years, she has gained in-depth knowledge and experience in the field of biodiversity law and "access and benefit sharing" (ABS") and has been active in capacitating users of genetic resources (e.g. academic research, collections, private companies and Associations) on ABS legal framework. From 2013 to 2015 she collaborated with the Ministry of the Environment by providing assistance and advice to the relevant Directorate in the activities aimed at the ratification and implementation of the Nagoya Protocol at national level and at European level in the writing of the EU ABS Regulation (n. 511/2014) and derived acts, participating as an expert in the related work in the EU Commission. During the European semester of the Italian presidency of the EU Council (1 July – 31 December 2014), she was appointed as a member of the Biodiversity Italian team of the EU Presidency and consultant to the WPIEI-Biodiversity Italian core team for the ABS issues. In 2014, as a member of the Italian Delegation, she attended the Nagoya Protocol COP-MOP1, with the role appointed ABS expert. She is the author of articles and publications on biodiversity law and ABS, as well as a speaker in courses and conferences on the subject at Italian research institutions and universities.

PROGRAMME

Tuesday, June 3

Auditorium of S. Francesco al Prato Church

Welcome Introduction

10:00 - 15:00 Registration

15:00 - 16:00 Welcome Introduction - Major of Perugia, Rector of the University of Perugia, Director of the Department of Agricultural, Food and Environmental Science, University of Perugia, Chair of the ASBM, Chair of the Organizing Committee

Auditorium of S. Francesco al Prato Church

Opening Session – Chairs: P. Buzzini (University of Perugia), L. De Bellis (University of Salento)

16:00 - 16:45 Carlo Blasi

Biodiversity monitoring and knowledge in the implementation of the nature restoration regulation - NRR

16:45 - 17:30 Carlo Calfapietra

Nature based solutions for ecosystem restoration: the experience of the National Biodiversity Future Centre

17:30 - 18:15 Valentina Veneroso

10 years after the Nagoya Protocol: the road ahead

Auditorium of S. Francesco al Prato Church

Welcome Party

18:15 - 22:00

Wednesday, June 4

Hotel Giò - Perugia Congress Centre - Auditorium

Plenary session: Impact of climate change on biodiversity – Chairs: A.C. Elia (University of Perugia), L. De Bellis (University of Salento)

8:30 - 8:50 Claudia Coleine

Global-scale human pathogenic fungi under climate change

8:50 - 9:10 Marta Azzolin

Bottlenose dolphins (*Tursiops truncatus*, Montagu 1821) in the Adriatic Sea: an analysis of their habitat shifts over the last two periods of the habitats directive

9:10 - 9:30 Greta Giovagnoli

Microbiomes in cold soils: novel strategies for conserving biodiversity and promoting sustainable development

9:30 - 9:50 Barbara Caldaroni

Fifty years of changes in phytoplankton diversity in a closed lake. Cyanobacteria like it salty?

9:50 - 10:10 Maria Landolfi

Plant-microbial interactions: tracing a 2 million-year-old alliance with ancient DNA

Hotel Giò - Perugia Congress Centre

Coffee/Tea Break

10:10 - 10:40 Coffee/Tea Break

Hotel Giò - Perugia Congress Centre - Auditorium

Parallel session: The challenges of crop biodiversity in 21st Century – Chairs: E. Albertini (University of Perugia), S. Salvi (University of Bologna)

10:40 - 10:52 Aaron Anderson

Exploring the process of polyploidization through structural genomic changes in neotetraploid Alfalfa

10:52 - 11:04 Chiara Delvento

Haplotype-resolved assembly and resequencing of wild and cultivated genomes shed light on the almond evolutionary history and the genetic basis of self-compatibility

11:04 - 11:16 Eleonora Grassi

Taxonomical and functional biodiversity of nematodes to assess the eco-friendly potential of alternative approaches in crop protection

11:16 - 11:28 Giacomo Bongiorno

A Pan-Genome of the diplosporous grass *Eragrostis curvula* to investigate genetic diversification among genotypes with different ploidy levels and reproductive modes

11:28 - 11:40 Marzia Guerriero

Establishment of a comprehensive germplasm collection of chate melon informed with whole-genome sequence and phenotypic data

11:40 - 11:52 Paola Semenzato

LIFE Olivares Vivos+: A scalable model for reconnecting olive farming with biodiversity conservation and supporting the Common Agricultural Policy (CAP)

11:52 - 12:04 Pasquale Venerito

Varietal characterization and valorization of traditional fruit germplasm in Puglia

12:04 - 12:16 Francesco Arcieri

Resistance mechanisms to *Orobanche crenata* in pea (*Pisum sativum* L.): beyond strigolactones

12:16 - 12:30 Marco Maccaferri

The challenge of developing the tetraploid wheat pangenome and leveraging it to improve wheat resilience to climate change

Hotel Giò - Perugia Congress Centre - Room A

Parallel Session: Innovation in vine and wine microbiology – Chairs: G. Cardinali (University of Perugia), P. Romano (Universitas Mercatorum, Rome)

10:40 - 10:50 Vittorio Capozzi

Italian Geographical Indications, wine science, microbial diversity and autochthonous resources

10:50 - 11:00 Laura Moretti

Yeasts in the vineyard: a promising tool for biocontrol action

11:00 - 11:10 Angela Capece

Role of apiaries located in the vineyard on biodiversity of culturable yeast population associated with grape must

11:10 - 11:20 Tiziana Nardi

The grape microbiome throughout post-harvest withering and its correlations with berry metabolites

11:20 - 11:30 Vasileios Englezos

Investigating the role of temperature in shaping the microbiota and its functionality during a wine spontaneous alcoholic fermentation

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11:30 - 11:40 Nicola Francesca

Use of novel non-conventional yeasts *Candida oleophila* and *Starmerella lactis-condensi* for profiling the fruity and floral aroma of Catarratto wines: focus on microbial interaction and metabolome

11:40 - 11:50 Alessio Pio Rossetti

Effect of *Starmerella bacillaris* strains on Montepulciano d'Abruzzo wine color

11:50 - 12:00 Gianluigi Cardinali

Microbial wine protection and exploitation in view of the new Italian plan for the biodiversity of agricultural interest

12:00-12:30 Discussion

Hotel Giò - Perugia Congress Centre – Room B

Parallel session: Fungal diversity and bioprospecting (part 1) – Chairs: S. Tosi (University of Pavia), G. Venturella (University of Palermo)

10:40 - 11:02 Lucia Muggia

The diversity of the lichen mycobiota

11:02 - 11:24 Pamela Anelli

Characterization of secondary starters occurring on traditional Apulian cheese: a chance to improve and control the aging process

11:24 - 11:46 Alessia Romano

Graphite for graphene: digging into the diversity of degrading fungi from graphite-containing soils and rocks of NW-Italy

11:46 - 12:08 Lobna Hajji-Hedfi

Mycoconservation in arid and Mediterranean North Africa: biodiversity, bioprospecting, threats, and SDG synergies in Tunisia and Egypt

12:08 - 12:30 Simone Buratti

Two different realities come together: how woody decay fungi can lead to safe hydrogen use

Hotel Giò - Perugia Congress Centre - Room C

Parallel session: New frontiers in the studies of animal biodiversity – Chairs: E. Lasagna (University of Perugia), M. Albenzio (University of Foggia)

10:40 - 10:58 Francesca Bertolini

Genomic diversity and signatures of selection in Italian heavy pig breeds: insights into the genetic foundation of dry-cured ham production

10:58 - 11:16 Daniele Colombi

Genetic diversity and selection signatures in three Italian local beef cattle breeds

11:16 - 11:34 Lucia Sepe

Chemical characterization of goat meat obtained from native breeds in inland areas of the Basilicata Region – Italy

11:34 - 11:52 Fabio Formenti

IoT Technologies for the sustainability of Suino Nero Cinghiato farming: innovation and biodiversity

11:52 - 12:10 Vittorio Sarchese

Complete genomic sequencing of Canine Distemper Virus (CDV) with nanopore technology in wild animals in northern Italy

12:10 - 12:30 Laura Garzoli

Bats as bioindicators of environmental levels of trace elements and legacy organic contaminants through non-invasive approaches

Hotel Giò - Perugia Congress Centre

Lunch Break

12:30 - 14:00 Lunch Break

Hotel Giò - Perugia Congress Centre - Auditorium

Plenary session: Biodiversity, invasive species, and multitrophic interactions – Chairs: E. Conti (University of Perugia), P. Pollegioni (CNR-IRET, Porano)

14:00 - 14:22 Paola Pollegioni

Understanding the invasion mechanisms of the alien tree species *Ailanthus altissima* (Mill.) Swingle across Italy: the role of Arbuscular Mycorrhizal Fungi

14:22 - 14:44 Claudio Cucini

Exploring *Popillia japonica* through molecular lenses

14:44 - 15:06 Elena Chierici

Manipulating the foraging behaviour of the egg parasitoid *Trissolcus japonicus*: possible non-target effects on local biodiversity

15:06 - 15:28 Francesca Boero

Monitoring of insect invasive alien species *Scyphophorus acupunctatus* and *Xylosandrus* spp: (Coleoptera: Curculionoidea) in Western Liguria (Northern Italy)

15:28 - 15:50 Irene Tatini

Invasive species monitoring and community engagement: a multilevel approach within the RICONOSCERE Project

Hotel Giò - Perugia Congress Centre

Coffee/Tea Break

15:50 - 16:20 Coffee/Tea Break

Hotel Giò - Perugia Congress Centre - Auditorium

Parallel session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy – Chairs: B. Turchetti (University of Perugia), G. Perrone (CNR-ISPA, Bari)

16:20 - 16:42 Maria Grazia Caruso

Assessment of relationships with indolamines in a collection of tomato culturable endophytic bacteria

16:42 - 17:04 Luciana De Vero

New insights into the long-term preservation of microbial communities associated with Apulian table olives

17:04 - 17:26 Annamaria Bevivino

Enhancing the ENEA Microbial Culture Collection: a valuable resource for advancing biotechnology and bioeconomy development

17:26 - 17:48 Sandro Gepiro Contaldo

Empowering Microbial Research: SUS-MIRRI.IT Collaborative working environment platform

17:48 - 18:10 Marino Moretti

The Future Sustainability of the Research Infrastructure MIRRI-IT: an overview on objectives to pursue, KPIs to reach, and strategies to adopt

Hotel Giò - Perugia Congress Centre - Room A

Parallel session: Insect genetic and functional biodiversity – Chairs: M.A. Bologna (University of Rome3), F. Frati (University of Siena)

16:20 - 16:35 Ilaria Latella

Biodiversity of diurnal and nocturnal Lepidoptera in different environments of South Italy

16:35 – 16:50 Martina Falagiarda

How diversity of stink bugs and their parasitoids is affected by habitats and host plants

16:50 – 17:05 Antonio Gugliuzzo

Increasing plant diversity into Mediterranean tomato greenhouses: benefits for natural enemies and effects on key pest management

17:05 – 17:20 Roberto Catania

Towards integrated pest and pollinator management: the role of functional biodiversity

17:20 – 17:35 Alessandra Ricciari

Patterns of endemism of Italian insects: different biogeographic histories uncovered by phylogenetic and phylogeographic approaches

17:35 – 17:50 Emiliano Mancini

Conservation of saproxylic beetles in the genomics era

17:50 – 18:10 Davide Badano

Pit-traps and pitfalls: reconstructing the evolutionary history of neuropteroid insects

Hotel Giò - Perugia Congress Centre - Room B

Parallel session: Fungal diversity and bioprospecting (part 2) – Chairs: S. Tosi (University of Pavia), G. Venturella (University of Palermo)

16:20 - 16:38 Giuseppe Venturella

Bioprospecting of medicinal mushrooms: a bet for the future

16:38 - 16:56 Francesco Bergese

Exploring the plastisphere: a culturomic approach to uncover the floating microplastic-related fungal biodiversity in the Mediterranean Sea

16:56 - 17:14 Mara Rondolini

Unraveling the soil fungal diversity of a white truffle-producing floodplain forest

17:14 - 17:32 Segula Masaphy

Diversity of *Morchella* species: effect of ecosystem changes on proliferation of two ecotypes of *Morchella* spp. in Israel

17:32 - 17:50 Francesco Di Serio

Viroid-like RNAs infecting fungi: an unforeseen source of biodiversity and of potential innovative biotechnological tools

17:50 - 18:10 Carolina Elena Girometta

Diversity of corticoid fungi in the relict *Abies alba* population of Monte Nero (North Apennines)

Hotel Giò - Perugia Congress Centre - Room C

Parallel session: Animal biodiversity hotspots and conservation strategies for endangered species – Chairs: F.M. Sarti (University of Perugia), A. Sevi (University of Foggia)

16:20 - 16:35 Caterina Spiezio

Breeding success of the critically endangered red-headed vulture (*Sarcogyps calvus*): an international partnership to save the species from the extinction

16:35 – 16:50 Lorenzo Attili

Genetic variability and differentiation of an endangered Mediterranean falcon (*Falco biarmicus feldeggii*) through a multi-marker approach: a support for conservation efforts

16:50 – 17:05 Francesca Maria Sarti

MuVal – The future of tradition: an immersive journey through Valnerina

17:05 – 17:20 Morena Carlentini

Characterization of heterozygosity-rich regions in Sicilian horse breeds

17:20 – 17:35 Marco Bertoli

Ecology of the lasca *Protochondrostoma genei* (Bonaparte 1939) in the Chiarò di Cialla Creek (Northeast Italy): new insights from a biodiversity hotspot affected by human impacts and alien species

17:35 – 17:50 Francesco Perini

Genetic characterization and conservation strategies for Mediterranean trout (*Salmo ghigii*) in Italian freshwater ecosystems

17:50 – 18:10 Serena Tumino

Genomic insights into runs of homozygosity and heterozygosity of Sicilian cattle breeds

Hotel Giò - Perugia Congress Centre

Poster Session

18:10 - 19:30 Poster session

Thursday, June 5

Hotel Giò - Perugia Congress Centre – Auditorium

Plenary session: Biodiversity in anthropized environments: issues and prospects – Chairs: M. Rebora (University of Perugia), P. Mattarelli (University of Bologna)

8:30 - 8:43 Gianni Gilioli

Discovering unexpected pollinators suitability in urban environment

8:43 - 8:56 Anna Poli

Enrichment procedures for the selection and isolation of promising polycyclic aromatic hydrocarbons (PAHs) - degrading fungal strains: towards a Nature Based Solution (NBS) soil restoration

8:56 - 9:09 Beatrice Falcinelli

The effect of biodiversity on phenolic content and antioxidant activity of sprouts from fruit-tree species

9:09 - 9:22 Giulio Barone

An updated Plant Landscape Map of the Oreto river basin (Palermo, NW Sicily)

9:22 - 9:35 Ilaria Fracasso

Impact of ski slope management on soil properties and biodiversity in mountain grasslands

9:35 - 9:48 Laura Scirè Calabrisotto

Novel aerobic 1,2-DCA degrading consortia from a contaminated aquifer for enhanced bioremediation purposes

9:48 - 10:01 Antonella Trisorio

A strategic tool for agrobiodiversity: the national portal of genetic resources for food and agriculture

10:01 - 10:15 Raffaella Pergamo

The role of organic districts in nature and biodiversity protection and climate change mitigation. The example of the biodistricts in the Lazio Region

Hotel Giò - Perugia Congress Centre

Coffee/Tea Break

10:15 - 10:45 Coffee/Tea Break

Hotel Giò - Perugia Congress Centre – Auditorium

Parallel session: Biodiversity in cropping systems – Chairs: C. Moretti (University of Perugia), A. Ferrante (Sant'Anna School of Advanced Studies, Pisa)

10:45 - 11:06 Ilaria Bruno

From diversity to multifunctionality: Integrating marginal area and cover crops for enhanced agroecosystem performance

11:06 - 11:27 Luigi De Bellis

Physiology and endophytic communities of *Xylella*-resistant and susceptible olive tree cultivars

11:27 - 11:48 Alessandro Frontini

Structural and metabolic changes in *Xylella fastidiosa* resistant and susceptible olives

11:48 - 12:09 Beppe Benedetto Consentino

Solanum aethiopicum gr. gilo accessions and their potential use as eggplant rootstock

12:09 - 12:30 Francesco Vitali

Multilevel assessment of soil biodiversity after application of biostimulant based on arbuscular mycorrhizal fungi in three cropping systems

Hotel Giò - Perugia Congress Centre - Room A

Parallel session: Insect biodiversity in the modern era - Chairs: R. Romani (University of Perugia), G. Burgio (University of Bologna)

10:45 - 11:02 Matteo Montagna

Insect taxonomic and functional diversity: opportunities from genetic approaches

11:02 - 11:19 Daniele Sommaggio

Hoverfly (Diptera, Syrphidae) biodiversity in Italian National Parks

11:19 - 11:36 Adriana Poccia

Using biosolutions in fruit orchards against the invasive *Halyomorpha halys* to reduce pest control impact on biodiversity

11:36 - 11:53 Stefano Vanin

Funerary archaeoentomology: a discipline to investigate biodiversity, local extinction and new arrivals

11:53 - 12:10 Giuseppina Carta

The use of archaeoentomology and cartography to investigate the landscape transformation in the modern era

12:10 - 12:30 Barbara Manachini

Small creatures, big impact: unveiling the power of nematode diversity in the Mediterranean ecoregion

Hotel Giò - Perugia Congress Centre - Room B

Parallel session: Microbial biodiversity in foods: evolving insights and uncharted horizons – Chairs: M. Gullo (University of Modena and Reggio Emilia), V. Capozzi (CNR-ISPA, Foggia)

10:45 - 11:06 Maria De Angelis

Phenotypic and genomic assessment of sugar assimilation in the pomegranate yeast *Hanseniaspora valbyensis*

11:06 - 11:27 Davide Gottardi

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From by-product to innovation: exploiting biodiversity of *Yarrowia lipolytica* strains to valorize cheese whey and develop adjunct cultures for improved cheese ripening and quality

11:27 - 11:48 Chiara Nasuti

Development of a new brewing strain with improved aroma through hybridization and UV mutagenesis

11:48 - 12:09 Michela Pellegrini

Microbial resources for sustainable meat productions in the green deal era

12:09 - 12:30 Giovanni De Francesco

Yeast biodiversity in brewing: a comprehensive screening of 30 strains of *Saccharomyces cerevisiae* isolated from different ecological niches

Hotel Giò - Perugia Congress Centre

Lunch Break

12:30 - 14:00 Lunch Break

Hotel Giò - Perugia Congress Centre – Auditorium

Parallel session: Plant biodiversity: environmental preservation to face global change – Chairs: L. Reale (University of Perugia), A. Canini (University of Rome Tor Vergata)

14:00 - 14:22 Giuseppe Bonanno

Climate changes, habitat transformation and germination behavior. A complex story from three Mediterranean plants of conservation interest: *Muscari gussonei*, *Petagnaea gussonei* and *Poterium spinosum*

14:22 - 14:44 Laura Bassolino

Shading lights on *Silybum* genus biodiversity: chemodiversity, genetic and genomic characterization of a wide germplasm collection

14:44 - 15:06 Waed Tarraf

The potential of the terpene profile of Mediterranean fir species in the chemotaxonomic study and the conservation prospects

15:06 - 15:28 Daniela Gigante

Conservation status of Annex I habitats in Italy: first overview from the Fifth National Report under Article 17 of the Habitats Directive

15:28 - 15:50 Camilla Wellstein

Plant biodiversity and conservation of endemic species of the Dolomites and Southeastern Alps

Hotel Giò - Perugia Congress Centre - Room A

Parallel session: Round table - Biodiversity and PNRR infrastructures: what future beyond projects? – Chairs: P. Buzzini (University of Perugia), A. Bevivino (ENEA, Rome)

14:00 - 15:50

Discussion with F. Frati (NATIONAL BIODIVERSITY FUTURE CENTER), F. Pennacchio (AGRITECH CENTER) and A. Moretti (SUS-MIRRI.IT)

Hotel Giò - Perugia Congress Centre - Room B

Parallel session: Agro-environmental microbiology: emerging perspectives and new frontiers – Chairs: C. Sannino (University of Perugia), L. Borruso (Free University of Bozen)

14:00 - 14:16 Silvia Gattucci

Occurrence and distribution of yeast communities related to honeybees system: an ecological overview

14:16 - 14:32 Sara Del Duca

Enhancing soil bacterial community profiling: PMA treatment and long-read sequencing across biogeographic regions and land uses

14:32 - 14:48 Debora Casagrande Pierantoni

Plant Growth-Promoting Yeasts (PGPYs) as a sustainable solution to mitigate salt-induced stress on zucchini plant growth

14:48 - 15:04 Oussama Bouaicha

Cascade effect of microplastic aerial deposition affects tomato growth, root metabolome and exudome, and rhizosphere microbiome

15:04 - 15:20 Michela Paglialunga

Characterisation of *Pseudomonas syringae* pv. *syringae* Italian strains causing severe wilting in eggplants

15:20 - 15:35 Simone Cutajar

The environmental context of honey bee gut microbiota: insights from the endemic Maltese subspecies, *Apis mellifera ruttneri*

15:35 - 15:50 Daniele Alberoni

Microbial drivers of nectar flows: the case study of sunflower

Hotel Giò - Perugia Congress Centre

Coffee/Tea Break

15:50 - 16:20 Coffee/Tea Break

Hotel Giò - Perugia Congress Centre - Room A

ASBM General Assembly

16:20 - 18:00

Hotel Giò - Perugia Congress Centre

Poster Session 2

16:20 - 19:00 Poster session

Hotel Giotto - Assisi

Gala Dinner (optional)

19:30 - 00:00

Friday, June 6

Hotel Giò - Perugia Congress Centre – Auditorium

Plenary session: Challenges and opportunities for the implementation of the EU Nature Restoration Law – Chairs: L. Rocchi (University of Perugia), C. Catalano (CNR-IRET), L. Servadei (CREA-PB)

9:00 - 9:30 Chiara Catalano, Luigi Servadei

The challenges and opportunities of the EU Nature Restoration Law and the role of research. The national project for the development of a catalogue and planning support tool for Nature-Based Solutions

9:30 - 10:00 Luisa Paolotti

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The role of integrated LIFE programmes in restoring ecosystems: a case study from the “Integrated management and actions for a green and Innovative nature” Project

10:00 - 10:30 Matteo Pallottini

The LIFE IMAGINE UMBRIA project for the conservation of insect species of community importance in Umbria

10:30 - 11:00 Franziska Zimmermann

Tipping points of biodiversity and sustainability of South Tyrol traditional pasture systems (BIOPAS)

Hotel Giò - Perugia Congress Centre

Coffee/Tea Break

11:00 - 11:30 Coffee/Tea Break

Hotel Giò - Perugia Congress Centre – Auditorium

Farewell considerations and introduction of the next meetings

11:30 – 12:00 Awards

Chair of the ASBM, Chair of the Organizing Committee

Hotel Giò - Perugia Congress Centre – Auditorium

12:00

End of the Conference and Departure

ORAL LECTURES ABSTRACTS

Opening Session

Biodiversity monitoring and knowledge in the implementation of the nature restoration regulation – NRR

Carlo Blasi

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The Farm to Fork Strategy is at the heart of the Green Deal, which aims to make food systems fair, healthy and respectful of the natural environment. For the first time at European level, the strategy for a sustainable national agricultural system and the biodiversity strategy is directed to protecting and enhancing nature have several objectives in common. In some points of the Farm to Fork strategy the need to protect the Natura 2000 Network and the Habitats Directive in general is highlighted in addition to biodiversity.

In fact, the agricultural system intends to support the vision of restoration ecology by joining the Nature Restoration Regulation, which considers, among other things, bringing nature back to 10% of the agricultural system by 2030. The territorial models obtained from the knowledge of the flora, vegetation and ongoing dynamics analyzed with the phytosociological method contribute to making the agricultural system increasingly resilient. They provide indeed the necessary elements to define both the state of conservation of habitats and ecosystems and the most suitable serial stages to recover the resilience of natural ecosystems. With the right and expected integration of skills, rural systems typical of internal areas can provide ecosystem services and enhance sustainable activities also in terms of tourism and food production, suitable for mitigating the effects of the ongoing climate crisis.

Opening Session

Nature based solutions for ecosystem restoration: the experience of the National Biodiversity Future Centre

Carlo Calfapietra

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The role of Nature-based Solutions (NBS) in restoring urban ecosystems has now been widely recognized since the EU has made NBS one of the critical pillars in its political and executive agenda in 2015. Nature Restoration Law and Green Deal targets of Climate Neutrality and reduction of biodiversity loss provide now an unprecedented chance.

At the same time in Italy a unique initiative has started in 2022, thanks to the PNRR funds, The National Biodiversity Future Centre (NBFC) which is involving more than 2000 researchers and is focusing on a variety of topics of the Terrestrial, Marine and Urban Biodiversity.

While Monitoring, Conservation and Valorization represent three important keywords of this initiative, Restoration assumes a priority topic thanks to the recent international mandate related to the new policies. For this reason a new Report on Restoration is being released by the NBFC providing an important contribution to the effort that each member state is carrying out. It is particularly relevant to identify gaps and protocols to validate scientifically innovative and practical solutions for successful and effective NBS applications also quantifying the benefits of the NBS implementations in European cities.

Opening Session

10 years after the Nagoya Protocol: the road ahead

Valentina Veneroso

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On October 12, 2024, the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization ("ABS") celebrated its 10th anniversary since entering into force. The Nagoya Protocol, an additional treaty to the Convention on Biological Diversity (CBD), was adopted in 2010 to combat biopiracy and reduce inequalities between countries providing genetic resources (typically developing nations) and those utilizing them (generally industrialized nations). Over the past decade, more than 130 national ABS laws have been adopted worldwide, turning the acquisition and exchange of genetic resources into a complex and challenging process. These regulations directly impact anyone conducting research on animal, plant, or microbial biodiversity.

In addition, in 2022, the Kunming-Montreal Global Biodiversity Framework (GBF) recognized Access and Benefit Sharing as one of its four overarching goals for 2050. The GBF calls for a substantial increase, by this deadline, in the amounts of the benefits shared. For the first time, the framework also addresses the need to share benefits derived from the use of digital sequence information (DSI) on genetic resources. To this end, a multilateral benefit-sharing mechanism for open-access DSI was approved and the 'Cali Fund for the Fair and Equitable Sharing of Benefits from the Use of Digital Sequence Information on Genetic Resources' was officially launched on February 25, 2025, during the resumed 16th Conference of Parties (COP16) to the CBD in Rome. This fund will collect contributions from industries relying on DSI from plants, animals, and microorganisms, including sectors such as pharmaceuticals, cosmetics, agriculture, and biotechnology.

The presentation will explore the lessons learned in the 10 years since the Nagoya Protocol came into force, as well as the current and future challenges for ABS in the context of biodiversity and scientific research.

Session: Impact of climate change on biodiversity

Global-scale human pathogenic fungi under climate change

Claudia Coleine¹, Federico Biagioli¹, Tadeo Saez-Sandino², Eleonora Egidi², Antonis Rokas³, Leho Tedersoo⁴, Manuel Delgado-Baquerizo⁵

¹University of Tuscia, Italy, ²Western Sydney University, Australia, ³Vanderbilt University, USA, ⁴University of Tartu, Estonia, ⁵CSIC-IRNAS, Spain

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Soils are the largest reservoir of human fungal pathogens, yet their global biogeographical distribution and environmental drivers remain poorly understood. Using high-resolution PacBio sequencing on 3,200 soil samples from 108 countries, we performed the first global-scale assessment of soil-borne human pathogenic fungi. Our results reveal that warmer and more mesic ecosystems harbor a higher relative abundance of key pathogens, including *Fusarium solani*, *Candida tropicalis*, and *Cunninghamella echinulata*. We observed species-specific climatic preferences, with thermotolerant taxa more prevalent in arid zones, while others favored cooler and wetter climates. Predictive models under future climate scenarios forecast a substantial redistribution of pathogenic fungi by 2050, with expansions expected in tropical and coastal regions. This global atlas of fungal pathogen diversity and projected shifts under climate change provides a critical foundation for risk assessment and the development of long-term surveillance and mitigation strategies.

Keywords: Fungal pathogens, Climate Change, Global Future Projections, Soils

Session: Impact of climate change on biodiversity

Bottlenose dolphins (*Tursiops truncatus*, Montagu 1821) in the Adriatic Sea: an analysis of their habitat shifts over the last two periods of the habitats directive

Marta Azzolin^{1,2,3}, Matteo Costantino^{1,3}, Livio Favaro^{1,3}, Marco Gamba^{1,3}, Cristina Giacomini^{1,3}, Andrea Giovannini², Sonia Silvestri⁴, Maria Aurora Iorfino⁴, Antonella Arcangeli⁵

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Studying the distribution of suitable habitats for bottlenose dolphins (*Tursiops truncatus*, Montagu, 1821) in the Adriatic Sea is essential for management reasons. Any change might signal shifts in prey availability or water quality, represent an early sign of climate-related modifications, or increased human pressure. Bottlenose dolphins' presence data collected from ferries by the FLT-Med network, before and within the framework of the LIFE Conceptu Maris project, were analysed employing the software Maxent to develop Species Distribution Models (SDM) for the last two reporting periods of the Habitat Directive, 2013-2018 and 2019-2024. Ten environmental variables (bathymetry, seabed slope, distances from the coast, the 200-meter platform and canyons, currents, sea surface temperature, salinity, chlorophyll concentration, and primary productivity) were used as models' explanatory factors. Respectively, 38 and 103 sightings were employed to develop the SDMs for the two periods. The Sightings Per Unit of Effort is 0.0023 sight/km for 2013-2018 and 0.0041 sight/km for 2019-2024, indicating an increased presence of bottlenose dolphins in the study area in the second period, which is statistically significant (Mann-Whitney Test, N=241; U=5909; p=0.0177). The 2013-2018 SDM (AUC, Area Under the Curve, = 0.85) shows suitable habitats extending throughout the central and southern Adriatic. In contrast, 2019-2024 (AUC = 0.92) SDM shows a more restricted distribution, primarily along coastal areas. The analysis of the dynamic variables of the two periods shows that the shift in suitable habitats likely follows variable's changes over time. In fact, in the 2019-2024 period, there is a general increase of temperature and salinity in the northern and central Adriatic, while the chlorophyll concentration is reduced and concentrated along the Italian coasts. It would be worth investigating if anthropogenic threats other than maritime traffic may also have affected the distribution of suitable habitats.

Keywords: Bottlenose dolphin, Species Distribution Model, Climate change

Session: Impact of climate change on biodiversity

Microbiomes in cold soils: novel strategies for conserving biodiversity and promoting sustainable development

Greta Giovagnoli¹, Pietro Buzzini¹, Ciro Sannino¹, Gianmarco Mugnai², Benedetta Turchetti¹

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Cold soils found in low-temperature regions host distinctive microbiomes that are vital for ecosystem functions and hold notable biotechnological potential. With the effects of global warming, these habitats are experiencing ongoing changes and have emerged as climate change hotspots. They support psychrophilic biological diversity that has adapted to cold environments but is particularly sensitive to rising temperatures. It is crucial to preserve these microbial communities for a deeper understanding of their ecological significance, while also facilitating environmental knowledge and biotechnological innovations. This study sought to assess the effectiveness of various “standard” preservation techniques over a period of 180 days, tracking shifts in microbial composition and functionality to evaluate the microbiome's stability and resilience. In order to determine the best conservation strategies, multiple analyses were performed, which included: (i) examining community structure and quantifying microbial populations, (ii) characterizing taxonomic diversity by considering both total and transcriptionally active populations, and (iii) analysing the metabolic activity and functional profiles of the microbial communities. By linking preservation techniques to microbial stability and functional performance, we identified the most effective conservation method. These results help establish best practices for ex-situ soil microbiomes preservation, ensuring the reliability and reproducibility of microbiome studies across various temporal and spatial conditions, while also promoting long-term environmental biodiversity conservation.

Part of this work was granted by the European Commission - NextGenerationEU, Project SUS-MIRRI.IT "Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy", code n. IR0000005.

Keywords: Microbiome, Preservation, Biodiversity, Bacteria, Fungi

Session: Impact of climate change on biodiversity

Fifty years of changes in phytoplankton diversity in a closed lake. Cyanobacteria like it salty?

Alessandro Ludovisi, Barbara Caldaroni, Mariantonietta Brancale, Chiara Todini, Antonia Concetta Elia

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Long-term ecological research (LTER) plays a crucial role in tracing and predicting ecosystem trends in response to environmental changes and anthropogenic disturbances. Lake Trasimeno is an LTER site which has been the subject of naturalistic and ecological investigations since the beginning of the last century. The availability of historical data and the strict dependence of the water balance of the lake on meteo-climate conditions make Lake Trasimeno a special site for evaluating climate-driven effects.

Herein, we present the results of an analysis of monthly data series of phytoplankton collected over the last 50 years in Lake Trasimeno.

Overall, the results revealed significant long-term changes in phytoplankton, including species composition, community structure, seasonal succession, and diversity. A general shift of dominance towards Cyanobacteria was recorded, which resulted in a marked reduction in the number of represented algal classes, orders, and equitability.

A statistical analysis identified salinity and water column stability as the main potential driving forces behind the observed changes along the course of the seasonal succession, while temperature and water depth seem to play an uncertain role.

Keywords: Climate change, Algae, Salinity, Lake Trasimeno

Session: Impact of climate change on biodiversity

Plant-microbial interactions: tracing a 2 million-year-old alliance with ancient DNA

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¹Free University of Bozen, Italy, ²Lund University, Sweden, ³University of Milan, Italy

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Microbial communities, mainly bacteria and fungi in the rhizosphere, play a crucial role in plant health. Nitrogen-fixing microorganisms are of particular importance, as their ancient associations with plants likely contributed to the colonization of terrestrial environments approximately 450 million years ago. While fossil records support these long-standing interactions, direct molecular evidence from ancient DNA (aDNA) remains limited. Here, we reanalyzed the Kjær et al. (2022) dataset, where a variety of tree and shrub species were found associated with the early Pleistocene in the Kap København Formation permafrost (North Greenland). Metagenomic sequencing data from forty-one soil samples across four distinct sites, with depths ranging from 10 to 60 meters, were re-analyzed to detect microbial aDNA. Our analysis focused on uncovering potential plant-microbe associations in 2-million-year-old DNA derived from the original dataset. We identified 318 statistically significant co-occurrences between bacteria and various plant genera. Most of these co-occurrences involved bacteria from the Rhizobiales and Burkholderiales orders, known for their plant growth-promoting and nitrogen-fixing abilities. Additionally, we observed that certain plant-microbe co-occurrences were conserved across sites, while others were site-specific, suggesting that rhizosphere microbial communities dynamically adapt to environmental changes. These results reinforce the ancient origins and dynamic nature of plant-microbe interactions in the rhizosphere, highlighting their resilience and adaptability to climatic and environmental shifts.

Keywords: Ancient DNA, Rhizosphere, Plant-microbe associations, Permafrost

Exploring the process of polyploidization through structural genomic changes in neotetraploid Alfalfa

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Many agronomically important crop species are polyploids. The process of polyploidization can cause a genetic bottleneck of sorts where genes and alleles present in diploid progenitors are not present in an established polyploid population. Therefore, some of the biodiversity for these species can only be found in diploid populations. The *Medicago sativa* complex encompasses several diploid and tetraploid species and subspecies which are able to interbreed resulting in cultivated alfalfa, an autotetraploid ($2n=4x=32$). Many studies on the mechanisms of polyploidization have focused on inbred allopolyploid species, so we believe focusing on an outcrossing autopolyploids species will provide unique insights. This work seeks to understand the process of polyploidization to bridge the gap between diploid and tetraploid germplasm to ease the introduction of more biodiversity into breeding programs. By comparing a unique population of neotetraploids that arose from bilateral sexual polyploidization by crossing two diploid meiotic mutants to their diploid full siblings, we sought to better understand the structural genomic changes between diploids and their polyploid siblings. Using long read consensus sequencing from the Pac Bio Revio system, we have constructed telomere to telomere genomes of 3 neotetraploids, 3 diploid full siblings, and the 2 diploid parents to determine regions of interest for genes and structural changes that allow for the formation of viable polyploids. This natural polyploidization pathway can circumvent the formation of adverse gene mutations through chemical polyploidization and may allow for prebreeding gains in diploid wild relatives to be more effectively introduced into polyploid cultivated varieties while addressing a lack of research on obligate outcrossing autopolyploid evolution.

Keywords: Neotetraploid, Polyploidization, Structural Genomics, Alfalfa, Breeding

Haplotype-resolved assembly and resequencing of wild and cultivated genomes shed light on the almond evolutionary history and the genetic basis of self-compatibility

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Almond (*Prunus amygdalus* Batsch) is a major nut crop worldwide and one of the oldest domesticated trees; however, its evolutionary history and phenotypic diversification still require thorough investigation. Moreover, there is limited genomic, genetic and phenotypic information on wild almond relatives, which hinders studies on almond domestication, and restricts the use of the extensive wild almond gene pool in breeding. Herein, we present haplotype-resolved assemblies of the almond cv. Tuono, dominating global breeding programs as a source of self-compatibility, and the self-compatible wild almond species *Prunus webbii*, together with a genetic variation map obtained by *P. amygdalus* and *P. webbii* whole-genome resequencing. Population genomic analyses indicated that *P. webbii* did not contribute to the evolution of self-compatible *P. amygdalus* cultivars, in contrast with a previous assumption. Rather, haplotype profiling revealed that the self-compatibility alleles of Tuono and *P. webbii* arose from parallel evolution, as they originated from independent losses of the stelar RNase gene involved in the *Prunus* self-incompatibility system. The analysis of nutritional components in genetically homogeneous samples of *P. amygdalus* and *P. webbii* revealed significant differences in volatile compounds and amygdalin content. Genome-wide scan for selective sweeps resulted in the detection of several loci and genes putatively associated with *P. amygdalus* domestication and breeding. Overall, our study highlights the potential of haplotype-resolved genomic information for the genetic analysis of highly heterozygous species and provides information of broad interest for plant biologists and breeders.

Keywords: Almond, Wild relative, Genome assembly, Selective sweeps, Nutritional components

Session: The challenges of crop biodiversity in 21st Century

Taxonomical and functional biodiversity of nematodes to assess the eco-friendly potential of alternative approaches in crop protection

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The preservation of biodiversity is essential to ensure the resilience of agroecosystems, which are increasingly threatened by anthropogenic pressures and climate change. Nematodes, among the most abundant and functionally diverse soil invertebrates, are ideal indicators for assessing soil ecological quality. In line with the Farm to Fork strategy, essential oils (EOs) are emerging as promising alternatives to synthetic pesticides, as their high volatility and biodegradability suggest a low environmental risk. However, their actual effects on non-target soil fauna remain largely unknown. To address this gap, a field study was conducted to evaluate the effects of EOs on the taxonomic and functional structure of nematode communities. Specifically, the experiment was carried out on chickpea crops and aimed to assess the potential disturbance caused by basil EO on free-living nematodes. The seed coating was obtained using chitosan (CHI), a natural, non-toxic, and edible polymer derived from the deacetylation of chitin. CHI is used in combination with EOs to reduce their volatility and to form a protective coating on seeds. A Before-After-Control-Impact design was adopted to distinguish biodiversity changes due to temporal dynamics from those caused by treatments. Overall, 44 genera were identified, and Tylenchidae, Dorylaimidae, Cephalobidae, and Hoplolaimidae were the most abundant families. The results revealed that basil EO + CHI seed coatings had no significant negative impact on nematode taxonomic diversity or functional indices. The taxonomic community structure, considered one of the most sensitive detection metrics, showed that the observed effects were mainly driven by temporal variations, likely reflecting the natural progression of the crop system rather than the EO treatment itself. These findings are particularly promising, as they suggest that basil EO-based coatings can preserve soil biodiversity, ecosystem functioning, and natural capital.

Keywords: Biodiversity, Free-living nematodes, Functional diversity, Ecological quality assessment, Sustainable alternatives

A Pan-Genome of the diplosporous grass *Eragrostis curvula* to investigate genetic diversification among genotypes with different ploidy levels and reproductive modes

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As large-scale genomic studies have progressed, it has become clear that a single reference genome cannot capture the genetic diversity present at the species level. The pan-genome approach offers an efficient system to detect missing genetic information from a single reference genome, identify large-scale structural variants, and better understand how different evolutionary trajectories drive changes in reproductive strategies. *Eragrostis curvula* (Weeping lovegrass) exemplifies these processes, as it includes genotypes with diverse ploidy levels (2x to 8x) and reproductive modes ranging from sexuality to apomixis. In this study, we constructed a pan-genome of *E. curvula* to detect genomic variation, establish phylogenetic relationships, and analyze the effects of ploidy on genome evolution and reproductive mode. To achieve this, we used the genome assembly of cv. Victoria and genomic data obtained through Illumina sequencing from 10 genetically diverse accessions. The pan-genome was constructed using an iterative mapping-and-assembly approach. First, reads from each accession were mapped to the reference genome. Unmapped reads were then assembled de novo to identify novel genomic fragments and annotate new genes. These newly assembled sequences were integrated into the reference genome. The process was repeated iteratively with each accession, resulting in a final pan-genome comprising both the reference genome and newly assembled sequences. This approach proved to be efficient for constructing a pan-genome using an already published reference genome and short-read sequences from genetically distant *E. curvula* genotypes. Ultimately, the genomic resources and insights generated in this work provide a comprehensive basis for understanding the phylogenetic relationships among genotypes and offer new perspectives on the correlation between their path of genetic diversification culminating in a shift in ploidy and transitions between sexual and apomictic reproduction.

Keywords: Pangenomes, Genetic diversity, Apomixis

Establishment of a comprehensive germplasm collection of chate melon informed with whole-genome sequence and phenotypic data

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The species *Cucumis melo* L. includes several minor crops, such as the chate melon (*C. melo* subsp. *melo* var. *chate*), found in Apulia (Southern Italy) as a relic of its former wider cultivation, spanning from the time of Ancient Egypt to the Middle Ages. Recently, the consumption of chate melon has been gaining popularity both inside and outside the regional boundaries as a substitute for cucumber. Here, we describe the collection and characterization of fifteen open-pollinated, farmer-maintained chate melon populations, known by the folk names "meloncella", "spuredda" and "cucummaru", which are representative of the germplasm cultivated in Salento (Southern Apulia). Whole-genome resequencing of population DNA pools was performed to investigate genetic diversity within and between populations. A high level of variation was found in terms of heterozygosity. Notably, the lowest heterozygosity was associated with the population known as meloncella striata, characterized by deep grooves on the pepo and more widely distributed in commerce, indicating a greater selective pressure exerted by farmers. A total of 1,352 alleles were found to be fixed privately in the populations under study, including 170 private alleles fixed in the meloncella striata population, which are valuable for its traceability. Replicated field trials enabled the selection of superior populations based on key agronomic and commercial traits, including fruit number per plant, fruit shape, yield per plant, and earliness. Overall, this study secures the chate melon gene pool from further genetic erosion. Additionally, genomic and phenotypic data reported here offer a basis for utilizing the chate melon gene in breeding and for introducing this crop into mainstream agrifood systems.

Keywords: *Cucumis melo* L., Chate melon, Whole-genome resequencing, Genetic diversity, Phenotypic diversity

LIFE Olivares Vivos+: A scalable model for reconnecting olive farming with biodiversity conservation and supporting the Common Agricultural Policy (CAP)

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Introduction-The LIFE20NAT/ES/001487 Olivares Vivos+ (OV) aims to reconnect olive cultivation with biodiversity conservation, addressing the decline in biodiversity and soil health. It seeks to identify evidence-based good practices for agronomically, and socio-economically sustainable farming that contributes to halting biodiversity loss, improving environmental governance and to support the transition towards a CAP increasingly oriented to providing public goods.

Methods-The agri-environmental scheme was tested in olive farms across Spain, Italy, Portugal, and Greece and focused on sustainable herbaceous cover management, restoration of non-productive areas, and installation of fauna-supporting structures. Biodiversity monitoring assessed species richness, abundance, and ecological functions (pollination, pest control).

Results-Olive groves are key refuges for Mediterranean biodiversity. First tested in Spain, OV model increased species richness by 7% and abundance by 18% over three years. The greatest gains occurred on farms with poorer initial conditions. Key drivers were landscape complexity and well-managed ground covers. Ants showed high pest control efficiency, while insectivorous birds were limited by lack of nearby vegetation. Solitary bees proved reliable sustainability indicators, with even small floral patches enhancing ecosystem services. These results support CAP2023/27 goals and contribute the design of agro-climatic-environmental measures in both the CAP and the Vision for Agriculture and Food 2040 EC COM(2025)75.

Discussion-OV model proves that biodiversity restoration and profitability are compatible. It works across farm sizes and encourages adoption through policy-linked ecosystem services. Although training gaps remain, many farmers are willing to transition when properly supported. OV offers a robust foundation for eco-schemes aligned with CAP biodiversity goals, positioning olive farming as a driver of nature-positive agriculture in the EU.

Keywords: LIFE Olivares Vivos+, Olive cultivation, Biodiversity conservation, CAP, Sustainable farming

Session: The challenges of crop biodiversity in 21st Century

Varietal characterization and valorization of traditional fruit germplasm in Puglia

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A significant boost to the recovery, conservation and characterization of old fruit germplasm has been achieved in Puglia since 2012 with the implementation of integrated projects for biodiversity financed by the Puglia Region with the Rural Development Program. With the aforementioned projects it was possible to strengthen the laboratories functional to the conservation, characterization, evaluation of the health status and sanitation of the recovered germplasm. Through the new integrated projects for Biodiversity ReGEFRUP 2.1, Stonefruits and Pome Fruits, and REGEFRUP 2.2 Minor and Citrus Fruits, financed by the PSR Regione Puglia 2014/2022 on a selection of fruit varieties worthy of valorization, preliminarily chosen on the basis of the tradition of different products in the area and the preliminary results of the previous pomological and genetic characterizations, agronomic, productive, chemical and technological characterization actions were carried out on different accessions at risk of genetic erosion for their valorization. For this purpose, agro-food transformation tests were also conducted on different fruit varieties to verify their technological aptitude, in particular for the production of dried fruits, concentrates, juices, jams, fermented drinks, extracts. Both fresh and processed products were also subjected to organoleptic analyses in order to draw up the sensory profile of the fruit or product. The main objective of the work was to significantly support the protection and valorization of the biodiversity of the regional native fruit species, with a view to supporting agricultural companies in the production, transformation and distribution of specific local varieties, with a view to developing new supply chains.

Keywords: Technological characterization, Traditional fruit germplasm, Recovery of fruit germplasm, Agronomic characterization, Valorization of germplasm

Resistance mechanisms to *Orobanche crenata* in pea (*Pisum sativum* L.): beyond strigolactones

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Strigolactones (SLs) are a group of carotenoid-derived compounds occurring in plant root exudates, known to stimulate the germination of parasitic weeds belonging to the large family of Orobanchaceae, such as broomrapes and witchweeds. In several economically important crops, resistance to Orobanchaceae is associated with reduced SL biosynthesis and/or altered blend of individual SL molecules. In pea (*Pisum sativum* L.), we previously selected the line ROR12, associated with low SL levels and nearly complete field resistance to the parasitic weed *Orobanche crenata* Forsk. (Oc). Here, we performed field trials to select pea genotypes with contrasting response to Oc, starting from: 1) a population of recombinant inbred lines (RILs) originating from the cross between ROR12 and the susceptible cultivar Sprinter; 2) a germplasm population. Notably, liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of root exudates indicated that, in both populations, genotypes showing lower parasitization rates also display significantly lower (although detectable) levels of the pea SLs orobanchol, orobanchyl acetate and fabacyl acetate, suggesting that SLs play an effective role in field resistance against Oc. Unexpectedly, a third field trial carried out with the pea wild-type cultivar T  r  se and its mutants rms5 and rms1, impaired for key SL biosynthetic genes, indicated that complete lack of SLs only confers partial resistance to Oc. Overall, our results indicate that ROR12 resistance might be partially due to mechanisms that are independent from SL biosynthesis. In addition, considering negative pleiotropic effects associated with severe SL deficiency, such as extensive branching and dwarfism exhibited by the rms mutants, we envisage that the exploitation of post-germination and post-attachment defense mechanisms to Oc might be valuable for pea breeding.

Keywords: Strigolactones (SLs), *Orobanche crenata* (Oc), Pea, Field resistance

The challenge of developing the tetraploid wheat pangenome and leveraging it to improve wheat resilience to climate change

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Within the Genus *Triticum*, the tetraploid wheat (carrying the BBAA and GGAA genomes) group includes up to 11 highly diverse species and subspecies that underwent two independent and consecutive events of domestication and subsequent evolution. Tetraploid genetic diversity was shaped by exposure to diverse environments, pathogens and human selection, thus providing valuable alleles for resilience to abiotic and biotic stresses. To retrieve this valuable adaptive diversity, the durum wheat community developed a long-term collaborative project. A comprehensive, highly diverse Global Durum Genomic Resource (https://wheat.pw.usda.gov/GG3/global_durum_genomic_resources) was retrieved from genebanks and breeders, purified, and genotyped with a common genotyping platform. At the same time, the Svevo Platinum reference sequence was used as a framework. Haplotype-based analysis led to unfolding the genomic and demographic structure. A Tetraploid wheat Core Collection (TCC; 432 accessions), effectively capturing most of the diversity in tetraploids, was assembled and the tetraploid wheat pangenome was developed, including 40 accessions PACBIO25X sequenced. The GDGR is currently under distribution and phenotypic evaluation for: (i) phenology, (ii) resistance to diseases, (iii) root architecture, (iii) grain Yield components and grain quality. GWAS allowed to discover loci relevant for wheat breeding and haplotype-tagging KASP markers were developed. Main results will be presented and discussed.

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Session: Innovation in vine and wine microbiology

Italian Geographical Indications, wine science, microbial diversity and autochthonous resources

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Geographical Indications (GIs) in oenology represent a strategic sector for the European agri-food sector. In this context, Italian GIs are a relevant sector and a good model of innovation trends in the sector, aimed at protecting tradition and improving the quality and safety of production. An overview of the relevant scientific literature is proposed to highlight the research and development directions in this field. A particular focus is dedicated to microbial diversity as a reservoir of bio-resources useful for positively modulating the global quality of wines with GIs status. Specifically, an examination of the isolations of autochthonous yeasts and bacteria that have been isolated from Italian wines is reported. The importance of microbial collections in the management, enhancement and conservation of the microbiodiversity associated with oenological production is highlighted.

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Keywords: Wine, Innovation, *Saccharomyces*, non-*Saccharomyces*, Malolactic bacteria

Session: Innovation in vine and wine microbiology

Yeasts in the vineyard: a promising tool for biocontrol action

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In vineyard many fungal infections can affect the grapes, roots, trunk, canes, leaves and berries. Generally, chemical antifungals have been used, but in recent decades the European Union has been taking measures to reduce the use of chemical compounds in vineyards. For this reason, the research focused to identify and characterize new microorganisms as natural biocontrol agents. The aims of the present study were characterizing a wide range of yeasts, isolated from different environmental and food sources, for their antimicrobial activity against *Botrytis cinerea*, *Aspergillus carbonarius*, *Penicillium expansum*, *Penicillium digitatum* and *Cladosporium* spp. A preliminary screening evaluated the metabolites and volatile organic compounds produced by yeasts for their antimicrobial action. The results showed that *Brettanomyces bruxellensis* and *Metschnikowia pulcherrima*, had antifungal activity. A selected strain of *M. pulcherrima* was then evaluated in vineyard. Fifteen days before the harvest, the strain was sprayed on both red and white grape. At harvest time grape colonization and biocontrol action the grapes were evaluated. Results showed significant colonization of the *M. pulcherrima* strain and significant mold control. These findings highlight the potential of yeast-based biocontrol strategies as sustainable and effective tools for vineyard management.

Keywords: Biocontrol, Molds, Vineyards, Yeasts, *Metschnikowia*

Role of apiaries located in the vineyard on biodiversity of culturable yeast population associated with grape must

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The studies evaluating the agents carrying the yeasts on the grapes demonstrated that animal vectors, such as insects and birds, play a key role in yeast spreading in the environment. Furthermore, social insects (such as wasps and bees), can host the yeasts in their gut, contributing to their survival and biodiversity. This study, performed for two consecutive years, investigated the influence of the presence of apiaries in the vineyard on culturable yeast community of both grape/wine and honey bees collected in these apiaries. The yeast population isolated from spontaneously fermented grapes randomly collected in two vineyards with apiaries on the borders were compared to those isolated from spontaneously fermented grapes collected from a vineyard without apiary. At the same time, yeast community was analyzed on bees collected in each apiary placed in the vineyards, in comparison to yeasts isolated from an apiary located far from the vineyards. The isolated yeasts were identified by restriction analysis of amplified ITS region, followed by sequencing. Results showed that the presence of apiaries increased the occurrence of oenologically relevant yeasts on grapes; furthermore, during the second vintage, *S. cerevisiae* strains were found also among the yeasts isolated from bees. These findings corroborate the role of bees as both yeast vectors and reservoirs for *S. cerevisiae* during unfavorable seasons. The approach proposed in this study was aimed to contrast the loss of biodiversity in the agroecosystems, such as vineyards. The new yeasts isolated in this study represent an important bioresource which will be further characterized to select new strains for production of distinctive wines or for other biotechnological applications.

Keywords: Culturable yeast community, Honey bees, Yeast dispersion, Biotechnological application

The grape microbiome throughout post-harvest withering and its correlations with berry metabolites

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Grape withering is a post-harvest process for producing reinforced and sweet wines. The microbiome associated with withering grapes is closely linked to the process and its resulting wines. This study investigated the mycobiome and bacteriome of grape carposphere throughout withering, aiming to: (a) detail bacterial and fungal community evolution during grape withering using NGS metabarcoding, (b) compare two dehydration methods (non-controlled and controlled environments), and (c) correlate microbial communities with grape metabolites, including volatile organic compounds (VOCs).

Samples were collected from Valpolicella, Italy, where Amarone wine is produced. Drying was performed in traditional well-aired rooms (non-controlled, NC) or warehouses with set airflow and humidity (controlled, C). NGS metabarcoding (ITS and 16S) depicted fungal and bacterial species at four time points, and VOCs were measured using GC-MS and UHPLC-QTOF.

Microorganism evolution during withering was variable, with slight differences between the two systems. Overall, 144 bacterial genera (32 classes) and 176 fungal genera (19 classes) were found. Betaproteobacteria was the main bacterial class throughout the process, followed by Sphingobacteria. Dothideomycetes was the dominant fungal class, with *Aureobasidium* and *Alternaria* as major genera. *Botrytis*, which can damage grapes, grew during withering, showing significant diversity between NC and C.

Microbial taxa correlated with grape metabolites: for instance, aliphatic alcohols linked with four fungi genera, monoterpenes with 3, D-viniferin correlated with 16 bacterial genera, and trans-resveratrol with 11.

Understanding the microbial ecology of the grape carposphere can significantly inform winemaking practices. NGS metabarcoding has proven to be an effective tool for studying the microbiome of withering grapes, revealing changes in microbial communities due to different drying systems and drawing their correlation with grape metabolites.

Keywords: Grape carposphere microbiome, Post-harvest withering, Volatile Organic Compounds

Session: Innovation in vine and wine microbiology

Investigating the role of temperature in shaping the microbiota and its functionality during a wine spontaneous alcoholic fermentation

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Winemaking is a complex process in which fermentation temperature plays a crucial role, influencing the rate of the process, the microbial population dynamics as well as wine quality. In this study, shotgun metagenomic sequencing was combined with chemical analyses to investigate the impact of temperature on the microbiome, its functional profile, and on wine quality. Spontaneous fermentations of *Vitis vinifera* L. cv. Nebbiolo grapes were conducted in laboratory scale at 28 °C and 18 °C, joined with a maceration process from the grape solid parts. At different time points, the chemical properties of the wine and its metagenome were analysed. No significant differences were observed in microbial composition based on alpha diversity, while temperature clearly shaped bacterial community beta diversity. The relative abundances of certain species differed significantly between the two temperatures. *Tatumella ptyseos* and *Komagataeibacter saccharivorans* dominated high-temperature fermentations, while fermentations at 18°C exhibited high variability over time, with *Gluconobacter* spp. prevailing at days 2 and 4, and *Bradyrhizobium* spp. becoming dominant at the end of fermentation. Regarding fungal communities, *Saccharomyces cerevisiae* established dominance earlier in high-temperature trials, leading to lower biodiversity. The evolution of chemicals over time differed between the two temperatures and reflected significant differences in the metabolism of both bacterial and fungal communities, as evidenced by the analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology functional categories and Carbohydrate-Active Enzymes (CAZymes). The higher temperature led to a significant modification of the sugar consumption rate. An accelerated grape skin softening in the 28°C trials promoted tannin and anthocyanin extraction, but the latter content underwent a pronounced degradation, achieving way less pigments concentration (-40%) at the end of the monitored period.

Keywords: Wine fermentation, Temperature, Microbiome evolution, Wine color

Session: Innovation in vine and wine microbiology

Use of novel non-conventional yeasts *Candida oleophila* and *Starmerella lactis-condensi* for profiling the fruity and floral aroma of Catarratto wines: focus on microbial interaction and metabolome

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Volatile organic compounds (VOCs) are fundamental to wine quality. Although it is known that significant influences are carried out by the microbial component in the volatile fraction of wine, little is known about the effects of microbial interactions in the production of aromas. Therefore, the aim of this work was to promote microbial interactions using *Candida oleophila* (*C. oleophila*) and *Starmerella lactis-condensi* (*St. lactis-condensi*) to bio-stimulate the production of VOCs in Catarratto wines. The combination of *St. lactis-condensi* and *C. oleophila* with *Saccharomyces cerevisiae* (*S. cerevisiae*) induced significant increases in ethyl esters and acetates in the theses compared to the control tests fermented in pure culture. The co-presence of *Oenococcus oeni* (*O. oeni*) in the microbial consortia together with the non-*Saccharomyces* stimulated the microbial interactions and thus the effects. The use of *St. lactis-condensi* in the *O. oeni* - *S. cerevisiae* consortia significantly lowered the ester hydrolysis reactions while preserving their quantity. In contrast, the introduction of *C. oleophila* in *O. oeni* - *S. cerevisiae* consortia stimulated the biosynthesis of ethyl esters by increasing them by about 110% compared to the control. It is conceivable that this behavior can be traced back to the ability of *C. oleophila* to improve the biotechnological performance of the entire microbial consortia. Indeed, untargeted metabolomic investigations detected in *C. oleophila* the ability to bio-synthesise vitamins and their precursors such as vitamin B6 and B5 respectively. For the first time studying the impact of two novel non-conventional yeast on wine quality, both volatilome and exo-metabolome is reported. The use of *C. oleophila* and *St. lactis-condensi* constitutes a potential biotechnological resource to cope with the wine quality decay stimulated by the current climate change scenarios in terms of fresh fruity and floral aroma.

Keywords: non-conventional yeast, *Candida oleophila*, *Starmerella lactis-condensi*, Metabolome, Carattatto wine

Session: Innovation in vine and wine microbiology

Effect of *Starmerella bacillaris* strains on Montepulciano d'Abruzzo wine color

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Yeasts can influence wine color through several mechanisms e.g. direct adsorption of pigments on yeasts' cell wall, and producing metabolites such as pyruvic acid and acetaldehyde that have been found to react with different phenolic compounds. In this study, the influence of 10 *Starmerella bacillaris* strains inoculated as either planktonic or biofilm-detached cells on the chromatic properties of Montepulciano d'Abruzzo wine was evaluated. Wines obtained with biofilm-detached cells exhibited higher glycerol content and cell counts, and lower ethanol levels compared to those inoculated with planktonic cells. These wines were also characterized by higher concentrations of pyruvic acid, polyphenols, and anthocyanins than the others. These differences in anthocyanins and polyphenols notably influenced wine color, with wines obtained with biofilm-detached fermentations displaying lower b* (yellowness) and h* (hue) values, and higher a* (redness) values, indicative of a stronger red color, along with reduced clarity (lower L* values). Moreover, a regression model predicting wine color was developed. Future studies will expand on these findings by examining the effects of other non-*Saccharomyces* yeasts grown in various aggregation states on wine color.

This work has been funded by the European Union - NextGenerationEU, Mission 4, Component 1, under the Italian Ministry of University and Research (MUR) National Innovation Ecosystem grant ECS00000041 - VITALITY - CUP: C43C22000380007

Keywords: Wine color, *Starmerella bacillaris*, Montepulciano d'Abruzzo

Session: Innovation in vine and wine microbiology

Microbial wine protection and exploitation in view of the new Italian plan for the biodiversity of agricultural interest

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The upcoming National Plan for the Biodiversity of Agricultural Interest poses several challenges at the ruling, scientific, technological and economic level for the preservation and the exploitation of the microbial diversity bound to the agricultural and food chain. Several aspects must be considered to guarantee an efficient conservation of the microbial diversity, respectful of the rural conditions and of the farmers to guarantee an effective long-term sustainability of the measures undertaken.

This specific work calls for a real “convergence” approach considering microbial biology, ecology, conservation technology and strategies, as much as rural conditions and respect of the current rulings.

Conservation strategies are basically those *ex situ*, i.e. in collections and *in situ*, i.e. in specific areas with strong restrictions for the agricultural activities to avoid perturbation of the environment and therefore of the biodiversity. Already in 2012, the “Guidelines for the conservation and the exploitation of the biodiversity of agricultural interest” (<https://www.reterurale.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/9580>), in the part relative to microorganisms suggested the inclusion of the in farm and in factory conservation, where the former attains to the genetic resources used in agriculture and mostly bound to the soil, whereas the latter is specific for food transformation activities. Both strategies aim at maintaining the microbial diversity of interest without affecting the economic activity, as it could happen in some *ex situ* stringent conditions. All strategies require a deep understanding of several scientific and technical issues that are necessary to individuate, conserve, register and exploit Microbial Genetic Resources of agricultural interest, that will be presented and discussed in the presentation.

Keywords: Biodiversity, Agricultural interest, in factory, in farm, in situ and *ex situ*

Session: Fungal diversity and bioprospecting

The diversity of the lichen mycobiota

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Lichen thalli are multi-kingdom symbioses in which fungi, algae and bacteria interact to form communities of variable composition, often shaped by the environmental conditions under which the lichen thalli develop. Particularly under extreme environmental conditions, lichens are one of the most successful groups of organisms, where they serve as unique niches for microbial diversification. The lichen-associated microfungi, i.e. the lichen mycobiota, have been investigated at different geographic scales, including cosmopolitan lichen species as well as endemic lichens from Antarctica, using both a culture-dependent approach and environmental DNA metabarcoding. We show that the lichen mycobiota are predominantly composed of Ascomycota belonging to the classes Chaetothyriomycetes and Dothideomycetes, while some key representative taxa were recognized as basidiomycetous yeasts. The variation of the mycobiota is extremely high, and a stable species-specific core mycobiota is not detected with the methods we applied. Most taxa were present in a low fraction of the samples, indicating that the mycobiota are thus composed of heterogeneous fungi, of which some taxa are detectable only by culture-dependent approaches. Among these, eight new species have been recognized at a worldwide scale, which seem to select lichens as their preferred niche. Indeed, the major representatives of the lichen mycobiota are fungi that do not show any specificity toward a particular lichen host species, rather toward the lichen thallus in general, as a structure in which spores, yeast cells and mycelia fragments thrive or rest.

Keywords: Extremophiles, Fungi, Lichens, Metabarcoding, Symbiosis

Session: Fungal diversity and bioprospecting

Characterization of secondary starters occurring on traditional Apulian cheese: a chance to improve and control the aging process

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Filamentous fungi contribute significantly to the organoleptic characteristics of mature cheese when used as secondary starters during the aging process, because their metabolic activities can positively influence aroma, texture, colour, and appearance. However, uncontrolled fungal colonisations may include species capable of producing mycotoxins, secondary metabolites that pose risks to human and animal health. To mitigate these hazards and enhance cheese quality, the deliberate addition of selected secondary starters can be an effective strategy in leading the aging process.

In this study aiming to select appropriate strains, we evaluated the pro-technological activities of nineteen *Penicillium* spp. strains, isolated from the surface of Apulian cheese aged in cave and refrigerated cell. The fungal strains were DNA-based identified and further characterized in vitro, through polyphasic approach. Metabolic activities, such as proteolysis and lipolysis, responsible for flavour development, and the capacity to grow on a cheese-like synthetic substrate were evaluated. A subset of strains was further assessed for the volatile organic compounds (VOCs) emission, providing insights into related potential role in the sensory profile of aged cheese and in the identification of markers for traceability.

Additionally, the interaction between *Penicillium* spp. strains and *Aspergillus westerdijkiae*, an unwanted ochratoxin A-producing species often associated with natural-aged cheese, was evaluated in vitro. Among the selected strains, *Penicillium biforme* ITEM 19069 emerged as good candidate as potential secondary starter, due to its compatibility with the cheese-like environment, its high proteolytic and lipolytic activities, as well as a characteristic VOCs emission, and ability to affect the growth of *A. westerdijkiae*.

Keywords: Food safety, Cheese ripening, Selected cultures, Fungal biodiversity

Session: Fungal diversity and bioprospecting

Graphite for graphene: digging into the diversity of degrading fungi from graphite-containing soils and rocks of NW-Italy

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Graphene is a carbon-based nanomaterial with extraordinary physical, mechanical, and electronic properties and, for this reason, it is used in numerous applications, from optoelectronics and energy storage to medicine. Although graphene-based materials bring several advantages, they will eventually accumulate into the environment, but their fate in terrestrial ecosystems is unclear. Graphene is strictly derived from graphite and usually commercialised in different forms such as few-layer graphene (FLG). Therefore, graphite mining sites may be potential hotspots of graphene degraders. In this work, graphite-containing samples of soil and rocks were collected from three sites in Piedmont (Roure, San Germano Chisone, Villasecca; TO, Italy), along with control (not graphite-containing) samples. From the over one thousand fungi isolated, six species (*Penicillium citreosulfuratum*, *P. virgatum*, *Penicillago nodositata*, *Exophiala bergeri*, *Aaosphaeria arxii* and *Didymella exigua*) were selected which were not retrieved from the control samples and were found in at least two sites. One strain per species was incubated in 0.1% Malt Extract Broth or water with FLG powder at a concentration of 50 mg/mL. The mycelia of all strains were observed to aggregate FLG, to a greater extent than an isolate (*Trichoderma* sp.) from control soil. Raman spectroscopy showed FLG alteration (lattice disordering under irradiation associated with vacancy containing regions) after one month of incubation with five out of the six strains, *P. citreosulfuratum* being the most active. These preliminary findings suggest that FLG is prone to natural soil biodegradative processes.

Keywords: Biodegradation, Soil fungi, Environmental fate

Session: Fungal diversity and bioprospecting

Mycoconservation in arid and mediterranean North Africa: biodiversity, bioprospecting, threats, and SDG synergies in Tunisia and Egypt

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North Africa, encompassing six nations (Algeria, Egypt, Libya, Morocco, Sudan, and Tunisia), spans ~6 million km² and supports over 275 million inhabitants. Fungi are invisible ecosystem engineers, living as hyphae and mycelium. Globally, fungal mycelium in soil's top 10 cm exceeds 450 quadrillion km² *id est* about half our galaxy width. Yet fungal conservation remains overlooked in North Africa; only Algeria and Egypt acknowledge fungi in CBD reports. Tunisia's fungal diversity includes 400-500 macrofungi, thousands of microfungi, vital mycorrhizal/endophytic species, and 200+ lichenized fungi. Egypt has recorded 2,502 fungal species. Climate change threatens fungal biodiversity, disrupting ecosystem services tied to SDGs. Rising temperatures harm mycorrhizal networks (SDG 2), while pathogenic fungi spread (SDG 3). Saprobic fungi aid carbon sequestration (SDG 13) and bioremediation (SDG 15), but desertification risks extinction (SDG 15.5). This study highlights fungal diversity in Tunisia and Egypt, exposing gaps in research, conservation, awareness, and policy. It advocates sustainable use, legal protections, bioprospecting oversight, and protected-area management. Prioritizing fungal resilience in climate strategies can advance SDGs while safeguarding this overlooked kingdom.

Keywords: Climate resilience, Endemism, Ethnomycology, Policy frameworks, Soil restoration

Session: Fungal diversity and bioprospecting

Two different realities come together: how woody decay fungi can lead to safe hydrogen use

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Wood decay fungi (WDF) have great morphological, trophic, and adaptive diversity that results in broad application prospects. WDF are studied in many fields, but their use to prepare H₂ storage materials is a novelty never tried before. H₂ transport normally occurs through procedures requiring high safety measures, thus representing an issue. H₂ transport on solid and stable compounds, such as by adsorption on carbon-based materials, has been proposed as a safe and affordable solution. Biochar (BC) is a solid carbon powder produced by pyrolysis of biomasses and is attracting attention as gas storage medium. To improve the preparation yields and the storage efficiency, plant materials can be pre-treated with chemical and physical methods, but also with biological ones.

The aim of this study was to investigate how a biological pre-treatment of vegetable wastes by chosen WDF strains could optimize the pyrolysis process of vegetable biomasses and enhances their H₂ storage performance.

Different vegetable wastes were collected in Lombardy region (Italy) and used as a growth substrate for WDF strains belonging to the fungal culture collection of Pavia University (MicUNIPV). The pre-treated materials were subjected to pyrolysis to obtain BC and then chemically activated by KOH treatment to increase porosity.

The fungal action was monitored via FT-IR technique, evidencing changes in the IR spectra with respect to the starting biomass and with particular concern to the main constituting polymers, i.e. lignin, cellulose and hemicellulose. Fungal pre-treatment obtained several effects: 1) increasing the BC production yield compared to control (up to 40% in rice bran); 2) increasing H₂ storage capacity (up to 4.2 wt%); 3) higher H₂ sorption rates (<1 min for adsorption and around 1.5 min for desorption).

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Session: Fungal diversity and bioprospecting

Bioprospecting of medicinal mushrooms: a bet for the future

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Many species of Basidiomycetes, and to a lesser extent Ascomycetes, fall into the category of so-called “medicinal mushrooms”, or those fungi that are used for prevention, alleviation, or healing of diseases and/or for nutritional purposes. Due to the presence of high content of biologically active compounds they are used as (a) dietary food, (b) dietary supplement products, (c) a new class of drugs called “Mushroom Pharmaceuticals”; (d) natural biocontrol agents in plant protection; and (e) cosmeceuticals. Eco-sustainable reuse of agricultural wastes allows to obtain growth substrate for producing edible and medicinal mushrooms. Besides, the food industry has been incorporating natural components into traditional products to improve and prevent diet-related diseases. There has been a growing interest in adding mushroom powders and extracts because their content of bioactive molecules. Fortifying cereal-based foods with fungal mycelia or sporophores enhances the availability of vitamins, minerals, fibers, beta-glucans, and antioxidants in baked goods. In addition to illustrating the above uses, an assessment of the medicinal mushroom market is provided. This market has been growing in recent years due to consumer demand for natural health remedies and the potential health benefits of mushrooms.

Keywords: Medicinal mushrooms, Bioprospecting, Human Health

Session: Fungal diversity and bioprospecting

Exploring the plastisphere: a culturomic approach to uncover the floating microplastic-related fungal biodiversity in the Mediterranean Sea

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Microplastics (MPs) are widespread in natural environments and represent a sign of the human impact. In marine environment they introduce allochthonous microorganisms and chemicals creating a new ecological niche (plastisphere), where fungi and other microorganisms form a biofilm. Investigating floating MPs is essential for understanding the composition of the fungal community and its ecological role. Since marine fungal biodiversity is still largely unexplored, a culturomics approach is pivotal to discover and preserve taxa with potential biotechnological applications.

To collect MPs, Manta nets were towed along 6 transects located between the Livorno harbour and the protected area "Secche della Meloria" (Tuscany, Italy). Surface water samples were also collected to compare the culturable mycobiota between the two matrices. MPs were sonicated or directly plated, using three culture media (CMA, MEA and SNA) supplemented with sea salts and incubated at 15°C. Fungi were identified through a polyphasic approach combining morphological observation and molecular analyses.

Overall, the nearly 400 strains isolated were ascribable to about 100 taxa, 55 were exclusively found on MPs, while 22 were retrieved in the surface water; only 20% of the taxa was shared. The two fungal communities were clearly distinct. Most of the taxa were ascribable to the phylum Ascomycota (87%), whereas Basidiomycota (11%) were more represented in the MPs mycobiota than in the surface water, where only two Basidiomycota spp. were retrieved.

Many taxa were rare/or unexpected in the marine environment, and their preservation will increase the ex-situ biodiversity of the Mycotheca Universitatis Taurinensis. Preliminary observations suggest the presence of a putative new *Penicillium* species. In the near future, strains isolated from MPs will be characterized for their potential degradation capabilities toward different xenobiotics.

Keywords: Plastisphere, Marine fungi, Culturomics, Microplastics, Mediterranean Sea

Session: Fungal diversity and bioprospecting

Unraveling the soil fungal diversity of a white truffle-producing floodplain forest

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Floodplain forests are unique ecosystems that harbor exceptional biodiversity. In the Mediterranean region, the Motovun forest in Istria (Croatia) represents one of the last remaining examples of this habitat. Among its ecological rarities is the prized white truffle (*Tuber magnatum* Pico), a hypogeous mushroom famous for its unique flavor and significant economic value. Due to its limited natural distribution and the challenges of cultivation, the white truffle remains one of the most expensive fungi in the world. In Istria, it has become a symbol of the region's natural heritage, playing a crucial role in the local economy through direct harvesting and associated industries such as gastronomy and tourism. This study explores the biotic and abiotic factors that characterize the Motovun forest, aiming to define the environmental characteristics of a truffle alluvial forest. Understanding these interactions is essential for evaluating potential conservation practices that safeguard truffle resources and overall forest biodiversity. In addition to assessing vegetation and soil physico-chemical properties, we employed amplicon sequencing to analyze soil fungal biodiversity. Our findings emphasize the ecological conditions that promote white truffle growth, while also identifying significant threats related to land management practices, such as alterations in the river's course, which can greatly impact its survival. The characterization of soil fungal communities allowed the identification of dominant taxa (Mortierellomycota, Basidiomycota and Ascomycota), and further analysis of the trophic classes revealed that ectomycorrhizal fungi are highly present with an important presence of *Sebacina*, *Membranomyces*, and *Naucoria*. These insights enhance our understanding of the complex ecological network surrounding the white truffle environment, offering valuable knowledge that could aid in the sustainable management and conservation of this unique alluvial forest ecosystem.

Keywords: White truffles, Soil, Amplicon sequencing, Fungi, Floodplain forest

Session: Fungal diversity and bioprospecting

Diversity of *Morchella* species: effect of ecosystem changes on proliferation of two ecotypes of *Morchella* spp. in Israel

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An increasing understanding of the impact of environmental factors on mushroom temporal and spatial proliferation in nature is important for conservation and control of mushroom populations, especially but not exclusively, with regard to edible mushrooms. Both local climate changes and site-related environmental changes such as disruptive activities, may affect mushroom proliferation. Morels (*Morchella* spp., Pezizales) are important edible mushrooms known for their delicate taste and aroma, as well as their medicinal properties. Range of *Morchella* species exhibit are found in the Mediterranean zone, including in Israel. Morels usually appear for only a short duration in a season and in wide range of habitats. The proliferation pattern of two distinguished *Morchella* species, exhibiting mycorrhizal and free-living saprotrophic modes, was monitored in Israel together with climatic and soil condition data. Results: air climatic changes were found to affect the temporal fruiting pattern of morels as well as the mushroom phenotypic and chemical composition properties such as color and phenolic content. Moreover, both population types were affected by disruptive activities such as fires and site forest management activities.

Keywords: *Morchella* species, Morel, Trophic mode, Environmental conditions, Phenotypes

Viroid-like RNAs infecting fungi: an unforeseen source of biodiversity and of potential innovative biotechnological tools

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Viroids are circular non-coding RNAs infecting plants. Some of them contain ribozymes, which are catalytic elements self-cleaving the multimeric RNA replicative intermediates. Only about 50 viroids were known till a few years ago, when, by screening publicly available metatranscriptomes for circular and ribozyme-bearing RNAs, tens of thousands of new viroid-like RNA elements, here referred as ribozycirculome, were discovered in several ecological niches. The largest elements coincided with ambiviruses, which were found in isolates of orchid endomycorrhizal fungal collections and in *Cryphonectria parasitica*. The genome of ambiviruses is composed of 5 kb encoding a putative viral RNA-dependent RNA polymerase and a highly conserved protein of unknown function, thus being a first example of a hybrid between RNA viruses and viroid-like elements. We showed that the ambivirus infecting *Cryphonectria parasitica* interfere with the fungus pathogenicity. Other circular RNA elements carrying ribozymes in each polarity strand were some mitoviruses (around 3kb in size), circular elements encoding delta-like antigens (circa 1.5-2 kb), and thousands of smaller (< 1kb) viroid-like RNAs. The circularity of genomic and anti-genomic RNAs and their self-cleaving capability mediated by the embedded ribozymes was shown for ambiviruses as well as for several non-protein coding viroid-like RNAs from fungi, including the plant pathogen *Rhizoctonia solani* and *Trichoderma spirale*. Infectivity and replication of viroid-like RNAs into *Trichoderma* species were confirmed by a reverse genetics method and by horizontal transfer via anastomosis. Interestingly, viroid-like RNAs reduce the antagonistic potential of *Trichoderma* species against *Rhizoctonia solani*, providing further evidence of interference with the host behaviour. We, therefore, propose that the ribozycirculome is a new layer of biodiversity in the fungal mycobiota and a source for identifying potential new biocontrol agents.

Keywords: Infectious circular RNAs, Ribozymes, Mycovirus, Mycobiota

Session: Fungal diversity and bioprospecting

Diversity of corticioid fungi in the relict *Abies alba* population of Monte Nero (North Apennines)

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The relict *Abies alba* Mill. population in Mt. Nero (Ferriere-Bedonia municipalities, SIC IT4010003) is placed on ophiolites in an Alpic temperate-oceanic, hyper-humid orotemperate phytoclimate. It is a never managed, native relict in the Apennine Subprovince disjuncted from the main Alpic corpus and includes few hundred individuals up to 350 years old.

Aim of the work was to survey the diversity of corticioid fungi growing on *Abies alba* in this population.

Field surveys were conducted in October-November especially in 2022, 2023 and 2024. Living fir individuals were surveyed as well as fallen decaying trunks. Samples were identified based on both macro and micromorphology.

Less than 10 cumulative lignicolous species were recorded from the *Abies alba* population in Mt. Nero including the corticioids: *Cyphella digitalis* (Alb. & Schwein.) Fr., *Hymenochaete cruenta* (Pers.) Donk, *Aleurodiscus amorphus* (Pers.) Rabenh., *Radulomyces confluens* (Fr.) M.P. Christ., *Gloiothele citrina* (Pers.) Ginns & G.W. Freeman. The latter was found in decaying trunks in the South side only. *C. digitalis* recorded 1.82 id/ha Northward, 1.59 id/ha Southward and co-occurred with other species on the same branch as follows: with *H. cruenta* in 4/19 samples; *A. amorphus* 2/19; *A. amorphus* + *H. cruenta* 1/19. The outgroup localities in Trentino (Noana and Lavarone) yielded *C. digitalis* by 0.1 id/ha and 0.052 id/ha respectively, each one including *A. amorphus* too on the same branch.

The poor lignicolous fungal spectrum lacks significant decayers and includes strictly saproxylic species only; 3/5 corticioids are considered monophagous (*C. digitalis*, *H. cruenta*) or almost monophagous (*A. amorphus*) on *A. alba*. Before these surveys, *C. digitalis* had been recorded only once in Italy in 1901, whereas *H. cruenta* is really scattered and perhaps overlooked. This study confirms the value of the relict *A. alba* population in Mt. Nero for fungal conservation.

Keywords: Corticioid fungi, *Abies alba*, *Cyphella digitalis*, Relict, Mt. Nero

Session: New frontiers in the studies of animal biodiversity

Genomic diversity and signatures of selection in Italian heavy pig breeds: insights into the genetic foundation of dry-cured ham production

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The Italian pig farming industry focuses on producing high-quality dry-cured hams through crossbreeding Italian Duroc, Italian Landrace, and Italian Large White pigs, which have been subjected to distinct selection pressures over the past four decades. This study explores the genomic landscape of these breeds using high-density SNP data from over 9,000 pigs genotyped for over 60,000 markers. We applied four different selection scan methods, two within breeds (runs of homozygosity and integrated haplotype score) and two across breeds (Fixation index and cross-population extended haplotype homozygosity) to identify genomic regions under selection, relevant genes within these regions and biological pathways. Our results reveal that Italian Duroc exhibits greater genetic differentiation (pairwise $F_{ST} = 0.20$) from Italian Landrace and Italian Large White, which are more closely related (pairwise $F_{ST} = 0.13$). This likely reflects distinct origins and breeding goals. Despite clear differentiation, few selection signatures reached fixation, likely due to the polygenic nature of selected traits. Enrichment analyses revealed genes and pathways linked to growth and fat deposition, suggesting a common selection direction across breeds but involving distinct biological mechanisms. These genes may play a crucial role in defining breed-specific characteristics and influencing traits in crossbred pigs. Overall, this study enhances our understanding of how selective breeding has shaped the genome of Italian heavy pig breeds and provides valuable insights for optimizing selection strategies to support the dry-cured ham industry.

This project has received funding from the European Union - NextGenerationEU under the National Recovery and Resilience Plan (PNRR) - Mission 4 Education and research - Component 2 From research to business - Investment 1.1 Notice PRIN 2022 PNRR (DD N. 1409 del 14/09/2022), from FEEDTHEPIG, proposal code P2022FZMJ9 - CUP J53D23018310001.

Keywords: Pig breeds, Single Nucleotide Polymorphisms, F_{ST} , Runs of Homozygosity

Genetic diversity and selection signatures in three Italian local beef cattle breeds

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Marchigiana (MAR), Chianina (CHI), and Romagnola (ROM) are three autochthonous Italian beef cattle breeds which are selected for meat production. These breeds have a very ancient history, CHI breed is reported to exist in Italy for 22 centuries, while ROM originated after barbaric invasions in the IV century AD. MAR breed originated by crossbreeding of CHI, ROM, and local Marche cattle at the beginning of the last century. Nonetheless, their genetic background is still mostly unknown. To fill this gap, we analysed the genetic diversity of these breeds using 980 MAR, 1000 CHI, and 967 ROM young bulls from performance test at ANABIC genetic station and genotyped with a 33K SNP-chip array. Principal components, structure, neighbour-joining tree, and minor allele frequency correlations were computed with PLINK, ADMIXTURE, ape and pcadapt packages on R, and suggest that CHI and ROM are the most differentiated breeds, while MAR falls in between of the two. We finally observed selection signatures by iHS and XP-EHH methods in these breeds using rehh package on R, within non-overlapping windows of 100kbp. Among all, several regions resulted for both statistics: more specifically on BTA1 and BTA13 in MAR, harbouring respectively DPP2 and DPP4 and CDH6 genes. Regarding CHI, multiple regions resulted under selective pressure on BTA2, and on BTA5 harbouring EMP1, GSG1, FAM234B, HEBP1 genes. Two windows in BTA6 and BTA23 resulted finally for ROM, harbouring respectively ABCG2, PKD2, SPP1, and ILRUN, SNRPC, SNORA70 genes. The same window on BTA6 also resulted under selective pressure for MAR and CHI, according to XP-EHH. Indeed, a bigger region of 3.2Mbp on BTA6, harbouring several genes associated with muscularity and growth ability, was observed selected, also for XP-EHH, for the derived alleles in ROM, while selection for the ancestral ones is observed for MAR and CHI. These results deepen our knowledge on these breeds history and could be considered for selection strategies.

Keywords: Autochthonous breeds, Genomics, Adaptation, iHS, XP-EHH

Session: New frontiers in the studies of animal biodiversity

Chemical characterization of goat meat obtained from native breeds in inland areas of the Basilicata Region – Italy

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Lesser-known goat breeds are recognized to exhibit high nutritional quality in their meat. Although these breeds are economically less sustainable for farmers, their meat quality is relevant but not well-documented. The COLAUTOC 2.0 project (PSR 2014-2020, Basilicata Region, CUP C63D24000140002) investigates the quality of kid meat raised in Basilicata region. Ten kids from each breed (Garganica, GR; Derivata di Siria, DS; Capra di Potenza, CP) aged 55±2 days, were selected. The left half-carcasses were chosen, and the Longissimus dorsi (LD) and Quadriceps femoris (QF) muscles were sampled. The pH of the carcasses was measured at 30 minutes and 24 hours after slaughter, showing an acidification of the meat at the second measurement. Samples underwent wet aging for 7 days at 4°C and were analyzed at 0, 3, and 7 days of aging for chemical and sensory properties. The average dressing percentages for GR, DS, and CP were 59%, 49%, and 51%, respectively. Results showed that cut selection influenced weight loss (higher in the LD) regardless of breeds. Among the breeds, meat of DS showed a lower brightness, while the redness was higher in QF at the end of the aging period, regardless of the breed. The trend of the aw, for all breeds, appears constant over time for both LD and QF. No differences in nutritional quality (protein and fat) were observed. The QF of CP showed a higher amount of secondary oxidation products over time compared to the other breeds. Shear strength tests revealed an increasing trend in the shear strength of QF over time in GR and CP, while DS showed the opposite trend. Regarding sensory properties, analysis using the electronic nose showed that both cuts were more sensitive to sulfur compounds for all breeds. Preliminary results underscore that studying local breeds is essential for preserving biodiversity and supporting sustainable farming practice.

Keywords: Kid's meat, Wet aging, Local breed, Nutritional quality, Electronic nose

Session: New frontiers in the studies of animal biodiversity

IoT Technologies for the sustainability of Suino Nero Cinghiato farming: innovation and biodiversity

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The enhancement of native breeds is crucial for biodiversity conservation and the sustainability of agri-food production systems. This project focuses on the free-range breeding of the Suino Nero Cinghiato, a reconstituted breed closely tied to its territory, requiring innovative and sustainable conservation methods.

Our initiatives involve farms specializing in extensive breeding of the Suino Nero Cinghiato and an IT company developing Internet of Things (IoT) technologies. The goal is to optimize free-range management through smart devices that enable real-time monitoring of environmental and physiological parameters. Technologies such as the Smart Feeding Breed scale and the FollowPig System enhance animal welfare and address challenges in extensive farming.

The FollowPig system aids breeders in genetic selection and provides traceability for end consumers throughout the supply chain. The integrated Smart Feeding Breed system monitors weight variations by tracking feed consumption per animal at the feeder. This data is invaluable for farm management, enabling yield monitoring and analysis of environmental impacts on nutrition and growth, as well as health monitoring and early disease detection. The collected data can be processed with R, Python, SAS, and Tableau, facilitating strategic scientific research. R&D in IoT technologies for free-range farming is essential for improving operational efficiency and reducing environmental impact, while ensuring high-quality products. The synergy between innovation and biodiversity fosters a sustainable farming model, preserving native breeds and contributing to ecosystem conservation, thereby minimizing challenges and enhancing genetic and cultural heritage with significant economic and environmental benefits.

Keywords: Biodiversity, Suino Nero Cinghiato, IoT (Internet of Things), Sustainability, Production Management

Session: New frontiers in the studies of animal biodiversity

Complete genomic sequencing of Canine Distemper Virus (CDV) with nanopore technology in wild animals in northern Italy

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Canine distemper virus (CDV) is an RNA virus that belongs to the Paramyxoviridae family in the *Morbillivirus* genus. CDV is a significant viral pathogen capable of triggering epizootic events, resulting in a negative impact on wild animal populations. Over the last two decades, CDV caused local epizootics among wild carnivores in Europe. Herein, we report the results of a surveillance study aimed at detecting CDV RNA among various wild animal species and performing complete genomic sequencing. Between 2023 and 2024, as part of a PRIN project 2022 “Investigating Hepatotropic Viruses in carnivores and humans in a One Health perspective (HVOH)”, duodenal samples were collected from a total of 108 wild animals found dead, including 10 wolves (*Canis lupus*), 80 foxes (*Vulpes vulpes*), 6 stone martens (*Martes foina*), 10 Eurasian badgers (*Meles meles*), 1 squirrel (*Sciurus vulgaris*), and 1 otter (*Lutra lutra*). Sampling was conducted under a National passive surveillance programme by the National Reference Centre for Wild Animal Diseases (CeRMAS-IZS PLV) in North-Western Italy. Molecular screening was carried out using primers targeting a highly conserved region of the nucleocapsid (N) gene. Two positive samples underwent a sequence-independent enrichment protocol and were sequenced using the MinION Mk1C platform (ONT, Oxford, UK). Viral RNA was detected in 11 animals (10.2%, 11/108). In detail, CDV RNA was identified in samples collected from ten foxes (12.5%, 10/80) and one marten (16.7%, 1/6). The complete genome sequences of two fox strains were generated. Upon sequence analysis, strains exhibited the highest identity to Europe lineage CDV strains recently identified in martens and otters in Hungary. We provided novel CDV genomic sequence data and demonstrated the reliability of ONT sequencing in genomic epidemiological studies, which may facilitate rapid response actions during future epizootic situations that can threaten animal biodiversity in a One Health perspective.

Keywords: CDV, Animal biodiversity, Surveillance, Nanopore, Sequencing

Bats as bioindicators of environmental levels of trace elements and legacy organic contaminants through non-invasive approaches

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Bioindicators play a crucial role in revealing the environmental effects of human activities. Occupying high trophic levels, insectivorous bats are sensitive to accumulation of pollutants such as trace elements and persistent organic contaminants. Maternity colonies are at particular risk, as reproductive females consume large amounts of prey to meet their increased energy needs. At the same time, they are especially valuable for studying the impact of contaminants and can serve as proxies for evaluating environmental pollution. Finally, due to their roost fidelity, maternity colonies are optimal bioindicators for short- and long-term monitoring.

In the framework of the PRIN “BAT-SIGNALS”, we developed a non-invasive biomonitoring protocol to evaluate the exposure of bat colonies to environmental contaminants. We sampled maternity colonies of different species (*Myotis blythii*, *M. capaccinii*, *M. myotis*, *M. emarginatus*, *Rhinolophus ferrumequinum*, and *R. hipposideros*) in NW Italy in 2024.

We collected guano under all colonies prior and after parturition, and faeces and hair samples from a limited number of captured individuals. We characterized bat diet by guano metagenomics and quantified different kind of pollutants: legacy organic contaminants (DDT and PCBs) and trace elements (As, Ni, Pb, Cd, Hg, Se, and Hg), comparing concentration ratios in guano and hair. Finally, we investigated the presence of possible oxidative stress biomarkers in bat hair, using proteomics. We explored relationships among several environmental factors (urbanisation level, crop type, pesticide use, environmental conditions, bat diet) and biological responses (contaminant levels in bat guano and hair, stress biomarkers), with the ultimate aim of proposing bats as bioindicators of environmental contamination, while also promoting their conservation by highlighting emerging threats posed by trace elements and pesticides.

Keywords: Bats, Legacy contaminants, Trace elements, Mercury, Proteomics

Session: Biodiversity, invasive species, and multitrophic interactions

Understanding the invasion mechanisms of the alien tree species *Ailanthus altissima* (Mill.) Swingle across Italy: the role of Arbuscular Mycorrhizal Fungi

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Ailanthus altissima (Mill.) is a fast-growing species native to Asia, introduced to Europe for ornamental purposes in the late 18th century. It is classified as one of the most invasive woody plants in Europe and is listed as a species of EU concern. With strong pioneering traits, *A. altissima* grows successfully across a range of ecosystems, from urban landscapes to forests, and is widely distributed throughout temperate regions. Its rapid spread is facilitated by wind-dispersed seeds, extensive root suckers, and adaptability to various soil and water conditions, particularly in disturbed areas such as roadsides. This species threatens biodiversity by altering native tree diversity, community composition, and ecosystem functions. Effective management is crucial to mitigate its impact and limit further expansion. Recent studies highlighted the facultative ability of *A. altissima* to establish and modulate various forms of endomycorrhizal associations, which confer significant ecological plasticity. This, consequently, would promote the evolution of an increased competitive ability (EICA) in *A. altissima*. While the beneficial effects of arbuscular mycorrhizae on the early development of *A. altissima* seedlings have already been demonstrated, these mutualistic relationships warrant further exploration. This study integrates high-throughput amplicon sequencing of the fungal internal transcribed spacer (ITS) region with stable isotope analysis of soil samples to examine soil fungal communities across an *A. altissima* density gradient (Absence, Front, Infested) in three pilot sites (Urban Park, Rural, Natural) in central Italy. Our results revealed the lowest AMF diversity in the Urban Park compared to the Natural and Rural sites. Although significant differences in Glomeromycota composition were found across sites, two of the most abundant genera, *Glomus* and *Septoglomus*, progressively increased with *A. altissima* density in both the Natural and Urban Park sites.

Keywords: Invasive species, *Ailanthus altissima*, Microbiome, Increased competitive ability, AMF

Session: Biodiversity, invasive species, and multitrophic interactions

Exploring *Popillia japonica* through molecular lenses

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Invasive species pose an increasing global threat due to their ability to rapidly spread and adapt to novel environments. The ecological and economic impacts of these species emphasize the urgent need to understand the mechanisms driving their invasion processes and success. *Popillia japonica* is a notable invasive pest, feeding on hundreds of economically valuable plant species. Since the last century, it has expanded beyond its native range in Japan, colonizing large areas of North America and Europe. Due to the significant threat it poses to agriculture and biodiversity, *P. japonica* has been designated as a quarantine pest while the EU has funded different research lines aimed at developing innovative strategies to combat this invasive species.

To improve the reference genome, we de novo sequenced and assembled *P. japonica* using ONT and Illumina libraries, annotating it with RNA-seq data from various life stages. We analyzed duplication levels of key gene families (CYPs, IRs, GRs, ORs). Population genomics involved resequencing individuals across the beetle's range, variant calling, and SNP dataset analysis to trace invasion routes. Lastly, RNA-seq was used to study gene expression responses to three treatments - *Bacillus thuringiensis*, LLINs, and *Metarhizium robertsii* - at two time points. Genome analysis revealed significant expansion of CYP genes, linked to detoxification, and ORs, associated with olfactory perception. Population genomics confirmed *P. japonica*'s invasion route from Japan to the US, followed by two independent introductions into the Azores and Italy, respectively. RNA-seq showed that the response to deltamethrin focusses on detoxification and Ca²⁺ modulation, whereas response to *M. robertsii* and *B. thuringiensis* is systemic and entails a complex immune response.

These molecular insights enhance our understanding of *P. japonica* biology, providing a foundation for developing targeted control strategies.

Keywords: Invasive species, Japanese beetle, Population genomics, Gene expression, Genomics

Manipulating the foraging behaviour of the egg parasitoid *Trissolcus japonicus*: possible non-target effects on local biodiversity

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Classical biological control is a promising strategy for sustainable pest management, offering pest suppression while reducing the ecological impact of chemical pesticides. However, the introduction of exotic natural enemies may pose risks to biodiversity, particularly through unintended effects on native species closely related to the target. The egg parasitoid *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), an effective biocontrol agent against the invasive brown marmorated stink bug *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), has been approved for release in Italy as part of a classical biocontrol programme. The foraging behaviour of parasitoid females is mediated by host-associated cues, such as herbivore-induced plant volatiles and adult host odours. Behavioural attraction may be influenced by the host used for rearing the parasitoid and by parasitoid conditioning during its adult life. Fundamental host range tests demonstrated that *T. japonicus* can develop on native non-target stink bug species. Using the tri-trophic system *Vicia faba* - stink bugs - *T. japonicus*, we assessed how the rearing host and exposure to host-related volatiles influence parasitoid foraging. *Trissolcus japonicus* females responded positively to *H. halys* oviposition-induced volatiles only when reared on this target host. Parasitoids reared on alternative hosts did not respond to either plant or host cues. Notably, female conditioning through exposure to *H. halys*-associated cues enabled recognition of volatiles associated to an intense stink bug feeding activity. These findings highlight the importance of parasitoid conditioning in modulating host specificity, with key implications for biocontrol efficacy and biodiversity conservation.

Keywords: Hemiptera, Invasive species, Learning, Scelionidae

Monitoring of insect invasive alien species *Scyphophorus acupunctatus* and *Xylosandrus* spp. (Coleoptera: Curculionoidea) in Western Liguria (Northern Italy)

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Invasive alien species are one of the main causes of biodiversity loss and species extinction. Arthropods invasions are one of the most pervasive, impacting human health, food, clean water, and sustainable economies. This work focuses on the study of insect invasive alien species, *Scyphophorus acupunctatus* and *Xylosandrus* spp. (Coleoptera: Curculionoidea). The first one, known as the black agave weevil, is native to America and is a polyphagous species that feeds on species of Asparagaceae, causing important damage to ornamental plants. The bark beetles of the genus *Xylosandrus*, of tropical origin, are highly polyphagous species infesting woody, arboreal and shrubby plants, impacting both ornamental and native plants. Hanbury Botanical Gardens (Ventimiglia, Italy) has suffered extensive damage to historic plant collections by the arrival of these new phytophagous insects. Therefore, a monitoring of the species is being carried out since 2024.

Scyphophorus acupunctatus monitoring is carried out during all the years using pitfall traps, baited with the species aggregation pheromones, and a visual control of the weevils on the plants. Monitoring of *Xylosandrus* spp. is carried out using a multifunnel trap, triggered with ethanol, α -pinene, α -copaene, and quercivorous.

A total of 1177 adults of *S. acupunctatus* were captured throughout the whole year but peaking in spring and autumn. The bark beetles monitoring has detected the presence of *X. compactus*, *X. crassiusculus* and *X. germanus* peaking in May. In addition, the exotic species *Dactylotrypes longicollis* (n=124), *Coccotripes dactyliperda* (n=7) and *Hypotenemus eruditus* (n=329) were trapped.

The monitoring data suggest a good adaptability of the exotic species to the mediterranean environment and their increase in the Hanbury Botanic Gardens, currently hosting already a large number of exotic arthropods, making the management and the conservation of botanical collections difficult.

Keywords: Invasive alien species, Coleoptera, Pest, Monitoring

Session: Biodiversity, invasive species, and multitrophic interactions

Invasive species monitoring and community engagement: a multilevel approach within the RICONOSCERE Project

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The increasing spread of invasive alien species (IAS) across Italy poses significant threats to native biodiversity and ecosystem functioning, and calls for updated conservation policies. Addressing these challenges requires both rigorous ecological monitoring and the integration of local knowledge. Since November 2024, researchers from the University of Pisa, Scuola Superiore Sant'Anna, and the University of Florence have been collaborating with the Ecoistituto delle Cerbaie on the RICONOSCERE Project, a citizen science initiative active in the Special Area of Conservation "Cerbaie" (IT5170003). The collaborators have implemented a multitaxa monitoring framework combining traditional field protocols and environmental DNA (eDNA) analysis, with a focus on aquatic habitats and cryptic or neglected taxa. This integrative approach enables non-invasive detection of native and allochthonous species, contributing to the development of an updated species checklist for the study area. In parallel with data collection, the project offers a series of educational activities. Some are specifically designed to address IAS-related issues, aiming to foster ecological awareness and informed dialogue, with particular attention to communicating the concept of alien species without emphasizing stigmatization. Although preliminary, results from evaluation and self-assessment forms collected during the educational activities and field sessions suggest an improvement in participants' understanding of concepts related to IAS and their interactions with native species. In this preliminary phase, the RICONOSCERE Project reaffirms the potential of participatory approaches, combined with molecular tools, to enhance IAS surveillance while simultaneously empowering local communities in long-term biodiversity monitoring.

Keywords: Citizen science, Biodiversity monitoring, Neglected taxa, Naturalist, Metabarcoding

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

Assessment of relationships with indolamines in a collection of tomato culturable endophytic bacteria

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Endophytes can benefit plants by promoting growth and nutrition and by inducing tolerance to biotic and abiotic stresses, thus they are often described as the "second functional genome" of host plants. The composition of endophytic communities is shaped by both plant genotype and environmental factors, and it may differentially impact plant phytochemical profiles. Recent evidence suggests that bacterial endophytes produce indolamines in vitro and that they may affect the expression of melatonin-related biosynthetic genes in plants exposed to abiotic stress.

Lycopersicum esculentum MicroTom plants known to differentially accumulate serotonin and tryptamine depending on genotypes, tissues and plant growth stages, were used to isolate endophytic bacteria. Culturable endophytic bacterial strains isolated from roots, stem, leaves and fruits of tomato plant were maintained in the CNR-MLIP culture collection, identified by 16S rDNA sequencing and characterized for their sensitivity in vitro to indolamines.

The analysis of strains growth responses in the presence of different doses of serotonin and tryptamine revealed that tryptamine did not significantly affect bacterial growth, whereas high concentrations of serotonin inhibited growth. Interestingly, a potential growth-promoting effect of low serotonin levels was detected in several strains. Investigation on indolamine production by the isolated strains may provide further data on the complex and multifunctional roles of endophytes in plant indolamines physiology.

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Keywords: Culture collections, Plant endophytic bacterial communities, Melatonin biosynthesis, Indolamine production

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

New insights into the long-term preservation of microbial communities associated with Apulian table olives

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Microbial biodiversity associated with fermented foods is a crucial resource for promoting human health and boosting innovation in the food industry. Scientific research and biotechnological valorization of specific microbial consortia require the preservation of their viability and functionality, which are the traits guaranteed by Microbial Culture Collections. However, assessing the best practices to promote the maintenance and revitalization of complex microbial communities is still challenging. Here, we present the strategies designed for the long-term preservation of microbiomes associated with table olives, a relevant example of plant-based fermented food worldwide, a key product in the Mediterranean diet, and among the main heritage foods in the Apulian region. The fermentation of these matrices is driven by a complex community involving mainly lactic acid bacteria and yeasts. To assess the best condition for their preservation, naturally fermented olives cv. Leccino, a typical Apulian cultivar, were sampled. Two cryopreservation protocols were developed by using 15% glycerol or DMSO as cryoprotectants and a storage temperature of -135 °C. The microbial consortium was studied before and during a 1-year storage period using a culture-dependent approach, RNA-based metabarcoding analysis, and metabolic profiling evaluation by Biolog Ecoplate®. The results showed that after 1 year of cryopreservation, the viability of the microbial consortium slightly decreased, regardless of the cryoprotectant used, and no significant changes in the metabolic profile were observed. Moreover, the metabarcoding analysis showed no significant differences in relative abundances after the storage period. Thus, we confirmed the effectiveness of the developed cryopreservation protocols, proper preservation of the microbial consortium, and its functionality during a long-term storage period.

This research is related to a specific task within the EU project SUS-MIRRI.IT (N. IR0000005).

Keywords: Glycerol, DMSO, Cryopreservation, Metabarcoding analysis, Metabolic profiling

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

Enhancing the ENEA Microbial Culture Collection: a valuable resource for advancing biotechnology and bioeconomy development

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The ENEA microbial collection (EMCC) is a significant resource comprising over 1,400 strains, including bacteria, fungi, yeasts, two microalgae, and a plant virus. Over the past year, as part of the PNRR SUS-MIRRI.IT project, the EMCC has expanded in both biological resources and service quality.

Operative procedures were implemented for sampling, characterization, safe storage, and cultivation. Recent isolates were obtained from environmental sources, identified via MALDI-TOF and/or 16S rRNA gene analysis, and evaluated for plant growth-promoting (PGP) traits.

Among the 281 isolates from strawberry rhizosphere soil, 8 N-fixing strains were identified, belonging to *Agrobacterium*, *Enterobacter* and *Raoultella* genera; 109 strains produced siderophores, and 42 exhibited antifungal activity, mainly from *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Bacillus*, *Enterobacter*, *Ochrobactrum*, *Peribacillus*, *Priestia*, *Roultella*, *Serratia* and *Stenotrophomonas*. Of 92 isolates from strawberry washing water and homogenates, 58 strains from 24 genera were successfully identified by MALDI-TOF, including *Bacillus*, *Listeria*, *Pseudomonas*, and *Curtobacterium*. From 49 isolates collected from a Sardinian abandoned mine (27 genera), ten with strong PGP traits were selected for phytoremediation trials to support *Helichrysum* growth. Over 300 isolates from kiwivine, grapevine, and strawberry rhizosphere soils were cryopreserved at -80 °C for future analysis.

These developments will enrich the EMCC collection and foster the design of microbial consortia for sustainable agriculture and biotechnological applications. Ongoing efforts focus on expanding the collection with strains exhibiting biodegradation and bioremediation potential, including cellulose, biopolymer, and microplastic degraders, as well as bacteria from contaminated sites for soil restoration and ecosystem recovery.

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Keywords: Microbial culture collection, Biotechnology, Bioeconomy, Sustainable agriculture, Phytoremediation, Biomarkers, Food quality and safety

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

Empowering Microbial Research: SUS-MIRRI.IT collaborative working environment platform

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The Italian Node of the Microbial Resource Research Infrastructure (MIRRI-IT), coordinated by the Joint Research Unit MIRRI-IT, plays a main role in advancing microbial research in Italy. To meet the growing needs for collaboration, data sharing, and access to services, the Italian Collaborative Working Environment (ItCWE) was developed. ItCWE aims to support the Italian microbial research community by integrating tools and services that enhance accessibility, interoperability, and scientific cooperation.

ItCWE integrates several services:

- (i) The Catalogue service provides access to the Culture Collections (~39k strains), offering a user-friendly interface for browsing microbial strains and associated metadata, with advanced features such as the automatic generation of SQL queries from natural language;
- (ii) The Data Analysis service for supporting reproducible microbiological data analysis (e.g., de novo assembly and metagenomic) on HPC infrastructure through a user-friendly web interface;
- (iii) Supporting services include infrastructure-level features such as backup and monitoring tools and a JSON Schema-based system for validating and structuring microbial metadata, as well as a data correction module to improve data quality.

The ItCWE platform provides a centralized and accessible environment for microbial researchers. It currently supports the exploration of biological resources, the execution of computational analyses, expert consulting, and access to training materials. The integration of versioning, validation, and observability mechanisms ensures data quality, system reliability, and ease of use.

ItCWE represents a significant step forward in supporting microbial research in Italy and Europe. Its flexible and modular architecture allows for future expansion and adaptation to emerging research needs, positioning ItCWE as a model for national and international research support infrastructures.

Keywords: Database, Microbiology, Workflow, Dataverse, HPC

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

The Future Sustainability of the Research Infrastructure MIRRI-IT: an overview on objectives to pursue, KPIs to reach, and strategies to adopt

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Long-term sustainability poses great challenges to the administration of Research Infrastructures (RIs). RIs are typically operational for several decades, and, therefore, they require continuous and stable support in terms of financial funding and resource management, in order to fully develop their potential in Research and Innovation and sustain top-level economic growth at national and European levels.

Some of the objectives for the effective long-term governance of a RI are: enabling and maintain scientific excellence, delivery of high education and training for the up-skilling of the human capital, enhancing collaborations in EU, facilitating economic activities through industrial partnerships and international cooperations, optimising data management and use, outreach to the public, provision of scientific advice, and managing revenues.

MIRRI-IT is the Italian RI for the conservation, characterization, distribution and valorization of microbial biodiversity and resources. To verify the long-term sustainability of such infrastructure, the strategic guidelines of the Sustainability Plan will be outlined highlighting the key actions to undertake for each objective, such as the stakeholder engagement, and the ad hoc KPIs which will be measured to assess the outputs and achievements of the RI like the usage of MIRRI-IT cutting-edge facilities or the numbers of collaborations with academia, industries, territorial bodies, and policy makers. Overall, the achievement and maintaining of scientific excellence and the attainment of key objectives that demonstrate the positive impact and benefits of the RI for the society are, indeed, associated with an increased capacity to attract funding, thereby contributing to the long-term economic and financial sustainability.

Keywords: Microbial biobanks, Sustainable bioscience, Sustainability plan, Funding strategy, Technological transfer

Biodiversity of diurnal and nocturnal Lepidoptera in different environments of South Italy

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The global decline in butterfly populations, attributed to different environmental perturbations (climate changes, agricultural intensification, and habitat loss), has a detrimental effect on biodiversity and ecosystem resilience. Due their close association with plants and their sensitivity to environmental changes, diurnal Lepidoptera are often used as bioindicators to evaluate the effects of different perturbation.

In this study, a monitoring program was carried out in the Aspromonte massif (Calabria, Italy) aimed to assess the impact of different anthropogenic pressures on the qualitative and quantitative community composition of these pollinators. Diurnal Lepidoptera were monitored on a bi-monthly basis from May to September along a total of ten 500 meters long transects located in a range of different contexts, including intensive and extensive grazing areas, cultivated fields, burned areas, and natural environments. The study also included a monthly monitoring of moths to assess the role of forest composition on the population's structure. Monitoring was carried out using UV LED light traps placed in areas of ecological or conservation interest (beech, fir, or mixed forests).

During the three years of observation, 63 species of diurnal Lepidoptera were recorded while a total of 141 moth species were sampled with the UV LED light traps. The abundance of different taxa as well as the diversity of sampled species of both diurnal and nocturnal Lepidoptera were affected by the year of sampling and the surveyed habitats.

Results highlighted the negative effects of habitat degradation, including overgrazing and wildfires, on pollinator communities. Even organic farming, despite being less invasive than conventional one, contributed to biodiversity loss, likely due to habitat simplification. Areas with minimal human disturbance showed the highest butterfly richness and diversity, emphasizing the need for conservation strategies to mitigate anthropogenic pressures.

Keywords: Moths, Butterflies, Perturbations, Bioindicators

Session: Insect genetic and functional biodiversity

How diversity of stink bugs and their parasitoids is affected by habitats and host plants

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Stink bugs (Hemiptera: Pentatomidae) pose significant challenges to crop management due to their broad host range. This study explored biodiversity patterns of stink bugs and their egg parasitoids across different habitats and altitudes in South Tyrol, Italy.

Monthly surveys were conducted over two years (2022-2023) across 27 sites including apple orchards, urban areas, and forests, in three altitude ranges (200-500 m, 501-800 m, above 801 m a.s.l.). Moreover, we examined the association between the three most abundant stink bug species (*Halyomorpha halys*, *Palomena prasina*, and *Pentatoma rufipes*) and plant families.

Results revealed that forests and urban environments hosted the highest stink bug abundance, with 21 and 20 species present, respectively. The invasive *H. halys* was dominant in urban areas, while forests showed higher prevalence of native species. Altitude significantly influenced species composition, with greater stink bug diversity recorded in the altitude range 500-800 m. Egg parasitoid species diversity was higher above 801 m and in urban environments.

Among plant families, Sapindaceae and particularly Acer species supported the highest number of stink bug and parasitoid species, representing the most biodiverse host. *Halyomorpha halys* displayed a broad host range but showed strong associations with Sapindaceae and Oleaceae.

A total of 12 parasitoid species were identified, with native parasitoids exhibiting higher parasitism rates on native bug species. The exotic parasitoid *Trissolcus japonicus* was mainly associated with *H. halys* in urban areas. Parasitism rates differed significantly among species and plant families, with *H. halys* and *P. prasina* experiencing high parasitism during reproductive peaks.

These findings suggest that maintaining habitat heterogeneity and plant diversity can create ecological reservoirs that support natural biological control mechanisms, potentially mitigating stink bug populations through enhanced parasitoid diversity.

Keywords: Hemiptera, Egg parasitoids, Species diversity, Habitat heterogeneity, Biological control

Session: Insect genetic and functional biodiversity

Increasing plant diversity into Mediterranean tomato greenhouses: benefits for natural enemies and effects on key pest management

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Functional biodiversity finds increasing application contexts in plant protection. Many success stories occurred on perennial crops in open field, while its application in greenhouse crops, including tomato, has been less explored. Current integrated management approaches targeting key tomato pests (i.e., whiteflies, moths, etc.) in Southern Europe include augmentative releases and/or conservative strategies of different biological control agents. Among them, the zoophytophagous mirid *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) is one of the most effective in reducing pest densities. Nevertheless, *N. tenuis* is also capable to develop and reproduce by feeding on plants, and in shortage of prey it can damage tomato growing tissues. In such a context, habitat management strategies aimed at increasing the functional diversity of alternative plants and natural enemies could also mitigate the negative impact of *N. tenuis* on tomato. Here, we explored the potential of two alternative plants, i.e., sesame (*Sesamum indicum* L.) and verbena (*Verbena x hybrida* Voss), when grown near tomato plants, to influence the population trends of natural enemies in commercial greenhouses. We monitored the population density of *N. tenuis*, other pests and natural enemies, and counted the number of mirid feeding-induced necrotic rings throughout the entire crop cycle. Results showed that both alternative plants increased natural enemy density and diversity (e.g., *Aphidius* spp., *Diglyphus isaea*, *Orius* spp., etc.) and supported the early establishment of *N. tenuis*, while mitigating its damage to tomato plants. However, contrasting results were observed for other natural enemies (i.e., *Necremnus tuta*, parasitoid of *Tuta absoluta*). The results suggest that alternative plants can ensure the early establishment of mirid predators and increase natural enemy populations by promoting their biological control activity, with the potential as a novel pest management tool in tomato greenhouses.

Keywords: Functional diversity, Habitat management, IPM, Natural enemies, *Tuta absoluta*.

Session: Insect genetic and functional biodiversity

Towards integrated pest and pollinator management: the role of functional biodiversity

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Pollination by bees (Hymenoptera: Apoidea) is a key ecosystem service for human welfare and environmental health. Although crop pollination can be provided by managed pollinators, such as *Apis mellifera* or *Bombus terrestris*, a greater abundance and diversity of wild pollinators can improve crop yields and quality. However, both managed and wild bees in agricultural landscapes are threatened by anthropogenic factors, including the habitat fragmentation and overuse of agrochemicals. Bees can be exposed to agrochemicals by different routes and, besides lethal effects, the sublethal exposures can cause alterations at morphological, physiological and behavioural level. Within this context, modern and sustainable agriculture relies on Integrated Pest and Pollinator Management (IPPM) to harmonize pest control with pollinator conservation, optimizing environmental and economic benefits. This research aimed to assess i) how habitat management practices (e.g. enhancing floral diversity), which underlie the IPPM pyramid, can increase wild bee diversity and activity, and ii) the effects of agrochemicals, which are at the apex of the same pyramid, in specific agricultural settings. The effects of habitat management practices are still ongoing, but preliminary results of trials in open field and protected conditions are discussed. Agrochemicals showed lethal and sublethal effects on several bee species. Specifically, commonly used agrochemicals reduced the survival of ground-nesting bees of the genus *Andrena* and altered the locomotion of managed bees. Insecticides used to protect greenhouse tomato plants reduced the survival, caused changes in feeding behavior and midgut alterations in *B. terrestris*. By contrast, essential oil-based insecticides were found selective towards the stingless bee *Melipona mondury*. The results of these studies will be useful for optimizing IPPM practices and pollinator conservation by fostering agroecosystem resilience.

Keywords: Agroecology, Companion plants, Ecotoxicology, Habitat management, Pollinators

Patterns of endemism of Italian insects: different biogeographic histories uncovered by phylogenetic and phylogeographic approaches

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Italy has a complex history due to the geological and climatic events of the Eocene and the Pleistocene, its position at the centre of the Mediterranean, and its variety of ecosystems. This explains the high number of animal species (>60,000) and endemic taxa (>15%), most of which are insects (38,000 spp., 15% endemic), and the diversified biogeographic patterns characterizing its biota. Such diversity has been scarcely investigated with a phylogeographic approach, in particular regarding endemic insect species. We explored phylogenetic and phylogeographic patterns in several Italian endemic taxa among Coleoptera (Meloidae, Oedemeridae), Dermaptera (Forficulidae), and Orthoptera (Acrididae) to elucidate their relationship with other congeneric, and to delineate their biogeographic history and population structure. Most of our results confirmed the uniqueness of the Italian genetic lineages ascribed to endemic species, and in some cases new ones were delineated (e.g. the new endemic *Meloe digiuliorum*; the two lineages, Sicilian and Maghrebian, within *Mylabris impressa*, and the two lineages, Greek and Italian, of both *My. quadripunctata* and *Lydus trimaculatus*). Different and intriguing biogeographic patterns were revealed even among closely related taxa, corroborating the correlation between the complex history of the Italian region and the evolutionary history of its biota (e.g. the three endemic species of Eurymeloe showed three different patterns of biogeographic affinities; the endemic genus *Italopodisma* hides a complex history of isolation and admixture among populations). Our results have also implications in conservation: most of the taxa investigated have a restricted range, sometimes confined to habitats threatened by climate change (e.g. *Forficula apennina*, *F. galvagni*, *F. orsinii*, *M. digiuliorum*), some are included in the IUCN red lists (e.g. *Italopodisma*, *Italohippus*), while others show a surprisingly low genetic variability (e.g. *Mylabris obsoleta*).

Keywords: Biogeography, Coleoptera, Orthoptera, Dermaptera, Conservation

Session: Insect genetic and functional biodiversity

Conservation of saproxylic beetles in the genomics era

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Genome-wide data have become essential for assessing the conservation status of endangered populations and identifying key genes involved in local adaptation, particularly for species confined to fragmented habitats.

Saproxylic insects - associated with dead or decaying wood - are especially vulnerable to habitat fragmentation, as they rely on dying trees and old-growth forests during part of their life cycle. These forest ecosystems are themselves increasingly threatened across Europe.

Among saproxylic insects, beetles play a pivotal role in forest ecosystems, contributing substantially to nutrient cycling and overall ecosystem functioning. Notably, *Rosalia alpina* (Coleoptera: Cerambycidae) and *Osmoderma eremita* (Coleoptera: Cetoniidae) are both listed on the IUCN Red List and included in Annexes II and IV of the EU Habitats Directive. While *R. alpina* in Italy comprises several evolutionary significant units (ESUs) that are yet to be clearly defined, two endemic relatives of *O. eremita* - *O. cristinae* in Sicily and *O. italicum* in Southern Italy - have been described.

As part of the initiatives of the National Biodiversity Future Center (NBFC), the phylogeographic structure of *R. alpina* was investigated using ddRAD-seq, with the aim of identifying ESUs, estimating the effective population sizes within Italian protected areas, and assessing the extent of genetic erosion over time. In parallel, a comparative genomic analysis was carried out on *Osmoderma* spp. to explore genomic and epigenomic differentiation between *O. eremita*, *O. cristinae*, and *O. italicum*, and to pinpoint genes potentially involved in local adaptation.

These genomic insights will enhance our understanding of the evolutionary history and current genetic diversity of these beetles and evaluate the effectiveness of existing conservation measures for old-growth forests. Ultimately, the results will inform the development of more targeted and effective conservation strategies for saproxylic insects.

Keywords: Genomics, saproxylic, Conservation, Evolution, Beetles

Session: Insect genetic and functional biodiversity

Pit-traps and pitfalls: reconstructing the evolutionary history of neuropteroid insects

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Neuropteroidea is an ancient insect clade that originated in the Permian and reached its peak diversity and morphological disparity during the Mesozoic. This group includes three orders: Raphidioptera (snakeflies), Megaloptera (fishflies and alderflies), and Neuroptera (lacewings, antlions, and allies). Although neuropteroids represent a minor component of modern insect communities, they are remarkable for their diverse ecologies and life histories. All neuropteroid larvae are predatory, but they exhibit striking specializations, ranging from freshwater sponge predators to social parasites and ambush hunters that construct elaborate traps, such as antlions. A key innovation driving lacewing diversity is the modification of larval mouthparts into a piercing-sucking apparatus linked to venom glands. Traditionally, larval morphology played a crucial role in inferring phylogenetic relationships and classification. However, molecular-based phylogenies have challenged this view, revealing complex patterns of convergent evolution across different lineages. Recent phylogenomic studies using genomic and transcriptomic data have profoundly reshaped our understanding of neuropteroid evolution, prompting a reassessment of key morphological traits and uncovering previously unsuspected affinities masked by ecological specializations and homoplasy. These advances provided a clearer phylogenetic framework and have identified major evolutionary radiations, including the transitions to soil and freshwater habitats.

Keywords: Evolution, Phylogeny, Lacewings, Morphology, Genomic

Breeding success of the critically endangered red-headed vulture (*Sarcogyps calvus*): an international partnership to save the species from the extinction

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Parco Natura Viva has been deeply involved in the conservation of the critically endangered red-headed vulture (*Sarcogyps calvus*), focusing on maintaining a genetic reservoir *through ex-situ* breeding. After initial success in the late 1990s, consistent hatching failures occurred for different reasons despite continued efforts. In 2013, two eggs were incubated artificially after an initial period of parental incubation. Both chicks hatched successfully and were hand reared. This marked the discovery that the species required a longer incubation period, an average of 55 days, than previously reported. Between 2013 and 2020, nine more chicks hatched via artificial incubation. In the winter of 2020, for the first time, an egg was left with the parents, resulting in successful natural hatching and parent rearing under video monitoring from a video camera placed on the nest. This event marked a significant milestone in zoo-based conservation of the Red-headed vulture. In 2021, a second chick was successfully raised by the same pair. Thanks to the experience acquired between 2013 and 2020, Parco Natura Viva developed a standardized protocol for egg incubation and chick hand rearing. In 2023, this protocol was shared with colleagues from the Korat Zoological Park in Thailand, leading to the successful hand-rearing of a chick there. A National project in Thailand to have back this vulture species in the wild was born, and in 2021 Korat Zoological Park established an aviary in the wild at Sub Fa Pa Forest Protection Unit within the Huai Kha Khaeng Wildlife Sanctuary. A breeding pair was introduced in the aviary in 2022 and, in 2024, produced their first chick, which was successfully raised by its parents. This year, the same pair has laid and is raising a second chick, again under parental care. This collaborative, multi-step effort represents a hopeful model for the long-term conservation of *S. calvus*, connecting *ex-situ* expertise with *in-situ* action.

Keywords: Asian vulture, Hand-rearing protocol, Parent rearing, in situ conservation, ex situ population

Genetic variability and differentiation of an endangered Mediterranean falcon (*Falco biarmicus feldeggii*) through a multi-marker approach: a support for conservation efforts

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The European lanner falcon *Falco biarmicus feldeggii* (subgenus *Hierofalco*) is a medium-sized raptor that is rapidly heading for global extinction following a dramatic decline mainly caused by anthropogenic impacts. Currently, the global population size counts no more than 200 breeding pairs, with Italy, historically considered a stronghold for this taxon, currently hosting around 30-40 pairs, which is 20% of the pair number counted 40 years ago. To support and strengthen the efficacy of conservation efforts, we aimed at characterising the gene pool of the European lanner, examining the extent of its variability and differentiation from other falcon taxa inhabiting the Mediterranean area, which exhibit similar morphological traits.

We used a multi-marker molecular approach to investigate variation in the mitochondrial (mt) and nuclear DNA (Short Tandem Repeats, STRs) of 146 falcons sampled from different areas. Our results show that the European lanner is genetically distinct from other Mediterranean taxa as revealed by nuclear DNA, although no significant differences were found in mtDNA. Additionally, the use of STR markers provided a deeper assessment of the genetic diversity within falcon taxa. The combined system of DNA analysis employed proved to be suitable for investigations at various hierarchical levels, ranging from (sub)species to individuals.

The findings of this study proved to be highly effective in revealing the genetic differentiation and variability of this critically endangered European bird compared to other Mediterranean hierofalcons, thus providing a valuable tool for its future management and the development of strategies aimed at preserving both the species and its habitats.

Keywords: *Falco biarmicus*, Conservation, Mediterranean, Endangered, Genetic distinctiveness.

Session: Animal biodiversity hotspots and conservation strategies for endangered species

MuVal – The future of tradition: an immersive journey through Valnerina

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MuVal is an innovative project that transforms cultural and agricultural heritage into an interactive, multisensory experience. By integrating traditional know-how and agrobiodiversity, MuVal aims to create a sustainable and resilient future. Located in the Valnerina region, known for its high-quality agricultural traditions, the project seeks to counteract depopulation and revitalize rural territories through immersive technologies and educational methodologies.

The MuVal project is structured into thematic areas utilizing immersive technologies such as virtual reality and interactive installations. The Immersive Room provides a visual tour of Valnerina's most symbolic locations, while the Interactive Room, equipped with Oculus Meta Quest 2, facilitates a deeper understanding of local products and traditional farming techniques. The core of the project is dedicated to narrating agricultural expertise and highlighting the significance of agrobiodiversity, encompassing native crops, traditional practices, and local knowledge.

MuVal effectively bridges past and present by integrating advanced technological tools with traditional agricultural knowledge. This approach fosters awareness of environmental sustainability and territorial resilience, promoting local agricultural resources as strategic assets against depopulation. By offering an engaging and educational experience, MuVal stimulates community participation and facilitates knowledge transmission across generations, ensuring the preservation of Valnerina's rich heritage while fostering innovation. MuVal represents a model for sustainable rural development, demonstrating how technology and tradition can coexist to support local communities. By enhancing agricultural and cultural heritage through interactive experiences, this project stands as a virtuous example of resilience, education, and sustainable development, providing a replicable framework for similar initiatives worldwide.

Keywords: AgriTech heritage, Immersive sustainability, Cultural regeneration, Rural innovation, Biodiversity empowerment

Characterization of heterozygosity-rich regions in Sicilian horse breeds

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Heterozygosity-rich regions (HRRs) are genomic regions of continuous heterozygosity, which may harbor functional genes related to the population's fitness response. The Sicilian horse breeds Sanfratellano (SAN), Siciliano (SIC) and Purosangue Orientale Siciliano (PSO) are known for the adaptability to a wide range of climatic conditions, sometimes even challenging. This study aimed to investigate HRRs distribution in a total of 220 horses (63 SAN; 93 SIC; 64 PSO) genotyped with the Illumina Equine 80K array. The consecutive runs method was used to detect HRRs and the top 0.1% of the SNP in RUNs were considered to define HRR islands per breed. The analysis identified 1944 HRRs in PSO (NHRR 30.38 ± 5.74), 1499 in SAN (NHRR 23.79 ± 5.59) and 2817 in SIC (NHRR 30.29 ± 7.91). The longest HRRs were observed on ECA7 across all three breeds. A total of 16, 16 and 11 HRR islands were identified in PSO, SAN and SIC, respectively. Gene annotation in HRR hotspots showed loci involved in immune response (SAN, PSO and SIC), environmental adaptation (SAN), fertility (SAN and SIC), and sports performance (PSO and SIC). The ECA7 hotspot (from 50.45 to 50.90 Mbps) was shared by the three Sicilian breeds (breeds recurrence >25%) harboring genes involved in the development and differentiation of muscle and bone tissues. QTL annotation, using markers in HRR islands, detected health traits in SAN, as well as growth and performance traits in all breeds. The investigation of the regions of the genome-wide consecutive heterozygous sites could be useful to reveal the traits linked to the balancing selection processes of Sicilian equine breeds and support their conservation plans.

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Keywords: HRRs, Heterozygosity, Horse, Molecular markers, SNPs, Animal genetics

Session: Animal biodiversity hotspots and conservation strategies for endangered species

Ecology of the lasca *Protochondrostoma genei* (Bonaparte 1939) in the Chiarò di Cialla Creek (Northeast Italy): new insights from a biodiversity hotspot affected by human impacts and alien species

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Freshwater hydrological networks can host precious biodiversity hotspots but are also among the most threatened environments and inhabiting native fish fauna can be critically endangered by human activities presence of alien species. Therefore, proper knowledge is of pivotal importance to protect/restore these species, manage their habitats and keep biodiversity hotspots safe.

In this study we investigate the ecology of the lasca *Protochondrostoma genei*, listed in the Annex II of the European Habitat Directive 92/43/EEC and among the endangered species in the Red List of Italian Vertebrates. Our study area was the Chiarò di Cialla Creek, partially included in a Site of Community importance (pSIC - IT3320041) and hosting other species listed in the Annexes of the Habitat Directive. Electrofishing sampling activities were carried out in 2024 throughout the whole creek, to analyze lasca population structure and fish community composition. Within the watercourse stretch included in the pSIC, main mesohabitats and substrate types were recorded, while values of main chemico-physical parameters were measured. The presence of anthropogenic impacts was checked. Distribution of alien species along the whole watercourse was checked, as the invasive common nase *Chondrostoma nasus* (negatively affecting the lasca population) was detected, and the brown trout *Salmo trutta* inhabits the upper watercourse stretch as consequence of introduction for fisheries.

Our results highlighted a well-structured lasca population inhabiting mainly pools and glides, while in riffles the occurrence was lower than in other habitats. Unfortunately, the lasca shares its habitats with common nase, which increased its density in the last years.

The present work provides new information about *P. genei*, that can be helpful to arrange management actions in the area and in other watercourses. Genetical investigations are now required to check potential hybridization between the lasca and common nase.

Keywords: Fish ecology, Conservation, Habitat preferences, Invasive species

Genetic characterization and conservation strategies for Mediterranean trout (*Salmo ghigii*) in Italian freshwater ecosystems

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The Mediterranean trout (*Salmo ghigii*) is a freshwater species of high conservation concern due to habitat degradation and genetic introgression from non-native Atlantic trout (*Salmo trutta*). As part of the LIFE IMAGINE project (LIFE19 IPE/IT/000015), the fish farm of Borgo Cerreto (Valnerina, Umbria, Italy) has been converted to produce Mediterranean trout, using wild breeders selected by genetic analysis. Subsequently, animals from various rivers in Central Italy were sampled to enhance the number of wild breeders. This study employed molecular markers, including nuclear LDH-C1 polymorphisms, mitochondrial control-region (mtDNA), and microsatellites (STR), to characterize wild populations across multiple Italian river basins. A total of 189 fin clips were stored in 99 % ethanol at -18°C until DNA extraction. Among these, 113 samples were obtained from Borgo Cerreto (BO) representing the breeders in the genetic center, while the others were collected from rivers in Umbria: seven samples from Argentina River (AR), 46 from Fosso delle Rote (RO), 21 from Fosso di Sensati (SEN), and 33 from Vigi River (SEL). All samples underwent LDH-C1 polymorphism genotyping using PCR-RFLP method. Only samples identified as LDH-C1*100/*100 were used for mtDNA genotyping. Ultimately, 141 samples were analysed for the mtDNA control region, and 15 samples using STRs. The results underscore the importance of reproduction center, where all animals presented the Adriatic haplotype. Conversely, all AR samples exhibited introgression with the Atlantic haplotype as well as in SEN animals, while 30% of the RO animals and the SEL population were Adriatic. These findings highlight the need for conservation measures such as genetic monitoring, and controlled breeding programs to preserve native genetic diversity. The integration of genetic tools with ecological assessments will be fundamental for the long-term viability of an ecologically and culturally valuable species such as *S. ghigii*.

Keywords: Genetic population, Biodiversity, Fish, Umbria

Genomic insights into runs of homozygosity and heterozygosity of Sicilian cattle breeds

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Genomic autozygosity (ROH) and heterozygosity (HRR) patterns were investigated in Sicilian Modicana (38 MOD) and Cinisara (26 CIN) cattle. Eighteen additional breeds (640,279 SNPs - a total of 442 animals: *B. t. taurus*, *B. t. indicus*, and admixed) framed the results within species diversity. ROH and HRR were detected using the consecutive run method. The top 0.1% of SNPs in runs formed ROH and HRR islands. 38,797 ROH segments were detected ranging from 437 (Piemontese) to 4189 (Jersey). FROH ranged from 0.018 (Piemontese) to 0.187 (Hereford); MOD showed intermediate (0.086), while CIN showed among the lowest (0.041), similar to indicine breeds. BTA5, BTA7, BTA14, and BTA21 accounted for 45% of ROH islands. Genes involved in epigenetics and reproduction were found in the most shared ROH islands on BTA21 and BTA23 across taurine, indicine, and admixed breeds, including CIN. In MOD, BTA6 harboured genes linked to milk traits and disease resistance. 771 HRR segments were detected, from 9 (Gir) to 87 (Beef Master), with admixed breeds showing the highest number and length per individual. Among taurine breeds, Hereford, MOD and CIN exhibited the highest mean of HRR per individual (2.80, 2.18, and 2.00), along with the highest mean HRR lengths (0.304, 0.263 and 0.295 Mb). BTA3, BTA7, BTA15, and BTA18 harboured 57% of HRR islands. The HRR hotspot on BTA3, shared by 11 breeds, including the Sicilian ones, highlighted genes linked to immunity and growth. An HRR island on BTA7, shared by Sicilian and three admixed breeds, harboured genes related to immunity and heat tolerance. These findings provide insights into the environmental adaptation of Sicilian cattle breeds.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)-MISSIONE 4 COMPONENTE 2 INVESTIMENTO 1.4 - D.D.1032 17/06/2022 CN00000022) and by IZSS - Project ARCAS PSR Sicilia 14/20 Op. 10.2b

Keywords: ROH, HRR, Adaptation, Sicilian cattle, Diversity

Session: Biodiversity in anthropized environments: issues and prospects

Discovering unexpected pollinators suitability in urban environment

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Bees (Apoidea) are of particular interest as they are especially vulnerable to biodiversity loss and are primary providers of pollination services, which are vital for the productivity and stability of agricultural and natural systems worldwide. Anthropogenic land-use change is a major driver of bees abundance and biodiversity decline, reducing landscape heterogeneity and ecological connectivity, and resulting in habitat fragmentation and degradation. Although many studies have addressed bees' population decline in human modified ecosystems, assessing the capacity of urban ecosystems to support functionally viable pollinator communities remains complex.

To address this knowledge gap, we developed a framework for assessing urban ecosystem suitability for pollinators integrating: i) high-resolution geospatial mapping of potential pollinator habitats, ii) a multidimensional suitability index, and iii) metrics describing spatial configuration and connectivity of habitat patches, all implemented within a GIS-based cartographic database. The methodology was applied to a case study of Brescia (northern Italy) to assess pollination suitability of this city and to evaluate the potential impacts of the new municipal policy for biodiversity conservation and climate change mitigation.

The analysis revealed a complex and heterogeneous habitat mosaic within the urban area, highlighting an unexpected potential of urban and peri-urban environments to support pollinator communities. The multi-scale approach enables assessment of both individual patch contribution and overall spatial configuration - key components of pollination suitability. Scenario analyses, based on various levels of implementation of the biodiversity conservation policy, showed how the designed strategies results in changes on habitat suitability across land-use types. The developed methodology represents a valuable tool for designing and implementing pollinator-friendly urban planning strategies.

Keywords: Bees, Urban ecosystem, Pollination service, Biodiversity conservation, Urban planning

Session: Biodiversity in anthropized environments: issues and prospects

Enrichment procedures for the selection and isolation of promising polycyclic aromatic hydrocarbons (PAHs) - degrading fungal strains: towards a Nature Based Solution (NBS) soil restoration

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Soil hosts diverse microorganisms, including fungi, whose interactions underpin important ecosystem services. Anthropogenic activities (e.g. industrial processes, traffic pollution etc.) threaten soil health by accumulating xenobiotics like PAHs. Bioaugmentation of autochthonous fungal strains capable of degrading PAHs is a promising Nature Based Solution (NBS) for soil remediation. This work led to the isolation and identification of fungal strains from enriched cultures performed on a contaminated soil.

Three consecutive enrichments were performed using four individual PAHs and a mixture of them as sole carbon source to foster the growth of specialized fungal communities. Following solid screening, isolates were purified and identified using a polyphasic approach. In parallel, metabarcoding analyses were performed to assess fungal community dynamics across enrichment steps.

Among 102 isolates (12 genera and 18 species) recovered, the most represented classes were Sordariomycetes, Eurotiomycetes, and Dothideomycetes, with Hypocreales, Cladosporiales, and Eurotiales as the dominant orders. Metabarcoding analyses yielded similar results. Biodiversity indices calculated for each sample revealed that both contaminant type and enrichment progression significantly reduced the number of OTUs, indicating a strong selection for specialised communities and, interestingly, the number of pathogenic species increased as result of the selective pressure. Additionally, strains of *Cladosporium allicinum*, *Geotrichum candidum*, *Paracremonium* sp., and *Penicillium chrysogenum* were positive to biosurfactant production assays, suggesting a potential to enhance PAHs bioavailability.

This study demonstrates effective strategies for isolating PAH-degrading fungi and underscores their potential as bioremediation agents, supporting their use in restoring contaminated soils. To this aim, the most promising strains are currently being selected through miniaturized screenings.

Keywords: Xenobiotics, Bioaugmentation, Mycoremediation, Nature Based Solutions

The effect of biodiversity on phenolic content and antioxidant activity of sprouts from fruit-tree species

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Seeds from fruit-tree species represent a relevant by-product of the juice industry and a potential source of bioactive compounds such as phenols and other antioxidants. Sprouting could be an intriguing idea to enhance the content of these compounds. This work reviews results from three experiments carried out by our group to evaluate the sprouting performances and the phytochemical and antioxidant content of seeds and sprouts from 4 genotypes of pomegranate (*Punica granatum* cv Akko, Dente di Cavallo, Mollar de Elche, and Wonderful), 3 genotypes of olive (*Olea europea* cv. Arbequina, Leccino, and Moraiolo) and 5 *Citrus* species (bitter orange; *C. aurantium*; blonde orange, *C. sinensis* cv. Biondo comune; sweet orange, *C. sinensis*; lemon, *C. limon* (L.) cv. Femminello; mandarin, *C. reticulata* cv. Tardivo di Ciaculli). Seeds of each species were separated from ripe fruit by hand, washed with tap water, and incubated in plastic trays containing sterile cotton wetted with distilled water. According to a completely randomized design with 3 replicates, the trays were incubated in a growth chamber at T>20°C in a light:dark regime of 16:8 h for olive and pomegranate and of 12:12h for *Citrus* species. For olive, a month of stratification in dark at 14°C was needed before the beginning of sprouting. Sprouts were harvested at the complete cotyledon expansion and just before the emission of the first true leaf. Pomegranate gave the most striking result, since i) germination in most cultivars was high and quite fast (one to two weeks); ii) sprouts had a good taste and texture and iii) their phenolic content and antioxidant activity were extremely higher than in seeds, with differences among cultivars. Sprouts from *Citrus* species and olive also showed high and genotype dependent phenolic and antioxidant content, but the low germination performances and the bitter taste make them not actually edible, and more suitable to the extraction of food additives, cosmetics or pharmaceuticals.

Keywords: Sprout, Fruit-tree, Germination, Phytochemical, Antioxidant activity

Session: Biodiversity in anthropized environments: issues and prospects

An updated Plant Landscape Map of the Oreto river basin (Palermo, NW Sicily)

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The Oreto River is a 12 km watercourse that flows through the municipalities of Palermo, Monreale, and Altofonte. Although there are other tributaries further upstream, its source is located to the south in the territory of Altofonte. Passing through the city of Palermo, the river flows into the Tyrrhenian Sea. Along its watercourse it crosses the so-called “Conca d’Oro”, a large plain rich in water resources and fertile soil. The Oreto River represents an important reservoir of biodiversity. In fact, its upper section is the locus classicus for *Carex panormitana* Guss. and hosts the westernmost population of *Platanus orientalis* L. However, in the post-war period, the area has undergone progressive transformation due to urban expansion of the city of Palermo and its neighbouring towns. This change in the landscape, together with widespread pollution and illegal construction, has led to the near-total loss of natural areas, particularly in the lower part of the river. In 1990, a plant landscape map of the entire Oreto River basin was published, also aimed at planning actions for the naturalistic redevelopment of the area. This survey reports the evolution of the plant landscape in the Oreto River basin over the past 35 years. An updated plant landscape map, produced through photointerpretation and supported by new field investigations, is presented. Additionally, a comparative analysis between the map provided in 1990 and the new one was carried out. It revealed a general deterioration in the quality of the area's natural state and specifically, a drastic reduction of semi-natural areas was recorded, with agricultural land giving way to urban expansion. Moreover, post-war reforestation efforts have been significantly reduced due to the numerous wildfires that have occurred in recent years. Populations of *C. panormitana* near the city of Palermo showed a decline, while those in the upper sections increased. No variations were observed in the *P. orientalis* population.

Keywords: Rivers, Land use, Urban expansion, Biodiversity, Sicily

Session: Biodiversity in anthropized environments: issues and prospects

Impact of ski slope management on soil properties and biodiversity in mountain grasslands

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Some areas of the Dolomites are subject to intensive management practices of ski resorts, which involve the use of artificial snow, snow grooming, and grazed or mowed vegetation. These factors may significantly alter soil physical and chemical characteristics, as well as micro- and mesofauna diversity.

We collected samples along an altitudinal gradient ranging from 1586 to 2066 m above sea level at five different sites. At each site, we collected 10 soil samples from the ski slopes and 10 samples from adjacent areas that were not subjected to alpine ski management practices. We measured soil pH, total organic carbon (TOC), total nitrogen (TN), texture, and aggregate stability. Additionally, we evaluated the micro- and mesofauna communities by amplifying the Cytochrome c oxidase I (COI) gene.

Most samples displayed comparable soil texture and aggregate stability values across ski slopes and adjacent areas. Nevertheless, ski slopes exhibited a higher pH compared to the surrounding area and a lower TOC and TN content. The lower TOC and TN concentrations could be related to increased surface erosion, which removes the topsoil rich in C and N, possibly due to the management and maintenance of the ski slope. Soil micro- and mesofauna alpha diversity, measured through species richness, did not show differences between slope and control areas. However, beta diversity revealed significant differences in soil micro and mesofauna communities, suggesting that while the overall number of species may remain stable, ski slope management may alter species composition. These findings highlight the impact of ski area management practices on soil health and biodiversity, with potential consequences that remain uncertain and require further investigation.

Keywords: Mountain land use, Ski slope management, Soil biodiversity, Soil properties

Novel aerobic 1,2-DCA degrading consortia from a contaminated aquifer for enhanced bioremediation purposes

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Anthropized environments are a source of unexplored microbial diversity and functions that can contribute to address emergent environmental threats in a sustainable way. 1,2-dichloroethane (1,2-DCA) is a toxic groundwater contaminant that can be aerobically and anaerobically biodegraded by specialized bacteria. The main aerobic catabolic pathway is hydrolytic dechlorination, mediated by the key enzyme DhIA. The aim of this study was to isolate and characterize novel degrading bacteria from an unexplored 1,2-DCA contaminated aquifer to exploit for enhanced bioremediation purposes.

Enrichment cultures from groundwater samples were set up on mineral medium amended with 1,2-DCA as sole carbon source. The degradation abilities of enriched consortia were tested by Cl⁻ release assay and Gas Chromatography-Mass Spectrometry. The consortia were PCR-screened for the *dhlA* gene. The consortia composition was studied by Ion Torrent 16S rRNA gene sequencing. Whole genome sequencing was performed by Nanopore technology.

Four stable 1,2-DCA-dechlorinating consortia with similar composition were obtained. All consortia consisted of known aerobic 1,2 DCA-degrading genus *Ancylobacter* and other genera with unclear role. The consortia degraded up to 1000 ppm 1,2-DCA in three days. The *dhlA* gene, identical to that of all other known aerobic 1,2-DCA degraders, and other genes involved in 1,2-DCA hydrolytic dechlorination were found and located on *Ancylobacter* chromosome in all consortia.

The consortia can efficiently degrade 1,2-DCA to a concentration that was not tested yet for other known 1,2-DCA hydrolytic degraders. The *dhlA* gene, located on plasmids in other known 1,2-DCA degraders, was located on a chromosome and it was confirmed to be highly conserved and likely acquired by horizontal gene transfer. The consortia can be exploited in bioremediation as a nature-based solution relying on the sustainable use of biodiversity.

Keywords: Dechlorinating bacteria, Aerobic dechlorination, Haloalkane dehalogenase gene, Groundwater bioremediation

Session: Biodiversity in anthropized environments: issues and prospects

A strategic tool for agrobiodiversity: the national portal of genetic resources for food and agriculture

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Agrobiodiversity is essential for enhancing the resilience of agricultural production systems and ensuring greater adaptability to climate change. However, genetic erosion and loss of biodiversity continue unabated. Among the actions supporting the conservation and enhancement of agrobiodiversity, information and its proper management plays a key role. In implementation with Article 5 of Law 194/2015, the National Portal of genetic resources for Food and Agriculture (GRFA) was created to establish an interconnected database system on GRFA; to disseminate information on GRFA and monitor their conservation status. The portal is a web platform, established at the Ministry of Agriculture, which, among other things, integrates and makes interoperable the IT systems of the National Register and the National Network of GRFA, created in implementation of Articles 3 and 4 of Law 194/2015. As a project of national interest, the Portal was developed in close coordination with relevant institutions, including regional representatives to accommodate their specific IT and organizational needs. The development process of National Register and National Network included: identifying all stakeholders involved in the registration procedure and their roles; implementing the registration workflow; and defining the system for management, updates and consultation, as well as structuring the related databases and mutual connections. Key features of the system are flexibility, allowing adaptation to different regional models; modularity, enabling the system to evolve to meet future needs; interoperability, ensuring data exchange across systems; and cooperation, supporting collaborative implementation. The Portal enables a comprehensive overview of GRFA, supports monitoring of conservation status of GRFA, enhances knowledge sharing, strengthens stakeholder cooperation, and fosters international dialogue, making it a strategic tool for national policies on GRFA conservation and enhancement.

Keywords: Agrobiodiversity, Genetic Resources for Food and Agriculture (GRFA), National portal, Information system, Conservation

Session: Biodiversity in anthropized environments: issues and prospects

The role of organic districts in nature and biodiversity protection and climate change mitigation. The example of the biodistricts in the Lazio Region

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Biodistricts in the Lazio Region are an innovative model based on organic farming and sustainability, promoted through Law no. 11/2019 to enhance sustainable agricultural production, protect the environment, and boost rural territories. The Climate Change Working Table, activated by these biodistricts, fosters synergies among institutions, farms, and research bodies, contributing to the region's sustainability efforts.

The study examines 5 biodistricts in Lazio, focusing on the Climate Change Working Table's role in assessing the effectiveness of actions for biodiversity, climate change mitigation, and sustainable water management. It also explores the challenges posed by climate change, particularly in relation to water resource use and biodiversity protection in these biodistricts. Lazio's biodistricts serve as effective tools for protecting biodiversity and water resources while mitigating climate change. Their activities have reinforced territorial governance and promoted practices with lower environmental impacts. The Climate Change Working Table has been key in fostering cooperation among stakeholders, encouraging resilient agricultural practices and the protection of natural resources.

The biodistricts in Lazio contribute significantly to ecological transition and climate resilience. The Climate Change Working Table represents an innovative governance model, fostering collaboration. Key discussions include restoring degraded ecosystems, creating corporate water reservoirs to address the water crisis, and developing financial tools like water credits. Challenges include the need for sufficient funding and better institutional coordination. Moving forward, increasing financial support and cooperation will be vital to sustaining these strategies over time.

Keywords: Biodiversity, Sustainability, Climate change, Water resources

Session: Biodiversity in cropping systems

From diversity to multifunctionality: Integrating marginal area and cover crops for enhanced agroecosystem performance

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Biodiversity can contribute to the multifunctionality of the system. A possible technique that can be used for enhancing ecosystem functions is the use of cover crops and spontaneous species, as diversification not only increases but also harnesses the functional biodiversity of the system. Since wild or sown species are an interface between the above and below ground environments, if managed correctly, we can enhance specific ecosystem services and boost the multifunctionality of agroecosystems. This requires careful consideration of the complementarity among various functional traits.

In specialised fruit-growing sector, it can also be achieved through the inclusion of marginal areas, which, despite not being productive, can be included for the sustainability of the systems; the areas are represented by the inter-row and field edges. The first area has the most important surface in the orchards; it is represented by 2.5 and 4.5 meters in width and multiplied by the tree rows.

The case studies analysed show the evolution of 3- and 2-year cover crop mixtures sown in specialised apple orchards in south-western Piedmont. The choice of species was based on an analysis of relevant functional traits for the targeted agroecosystem.

The study aims to optimize the self-assessed design indicator named Vegetation-based Ecological Functions Sustainability Index (VEFSI), which is used to assess the development of these species in relation to the ecological functions they perform.

The index evaluates five ecological functions: nitrogen fixation, soil coverage, pollination, attraction of pollinators and enhancement of soil structure. For index calculation we considered the number of species and both relative and total coverage for each species.

Our goal is to highlight how cover crops with varying degrees of diversity perform and the ecological functions they provide to improve the agroecosystem condition.

Keywords: Fruit growing sector, Functional traits, Multifunctionality, Ecological functions, Cover crops

Session: Biodiversity in cropping systems

Physiology and endophytic communities of *Xylella*-resistant and susceptible olive tree cultivars

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Xylella fastidiosa subsp. *pauca* (Xfp) causes significant physiological changes in olive trees culminating in symptoms such as leaf scorch, wilting, and dieback. The bacterium colonizes the xylem vessels, disrupting water transport and causing hydraulic failure.

Olive trees of cultivars Leccino (resistant) and Cellina di Nardò (susceptible) were sampled in productive orchards close to Lecce. We measured physiological parameters, stress markers (both metabolites and enzymes), and the microbial community composition by Next Generation Sequencing (NGS).

Results indicated differences among cultivars, with a general decrease of pigment content and relative water content and a general increase in stress markers and carotenoids in presence of infection. Cellina microbiome appeared to fluctuate depending on Xfp titer, while Leccino microbiome showed greater refractoriness to the pathogen, and a higher abundance and diversity of putative beneficial endophytes.

Xfp alters the organization of the olive tree microorganism community determining changes in the plant physiology. Healthy plants harbor a more diverse and abundant array of microorganisms, whereas infected plants exhibit a shift towards less varied communities, potentially dominated by pathogenic microorganisms. Identifying beneficial microorganisms associated with the resistant Leccino cultivar (e.g from genus *Enterobacter*), underscores the importance of cultivar characteristics in disease resistance and highlights the possibility for effective biocontrol agents in sustainable agricultural practices.

Keywords: *Xylella fastidiosa*, Olive tree, Resistant and susceptible cultivar, Microbiome

Session: Biodiversity in cropping systems

Structural and metabolic changes in *Xylella fastidiosa* resistant and susceptible olives

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Xylella fastidiosa subsp. *pauca* (Xfp) is regarded as one of the most serious plant pathogens, affecting a variety of agriculturally important host species, including olive trees. The research aim is to examine both olive cultivars and wild olives as sources of knowledge on disease resistance.

Olive trees of the cultivars Leccino (resistant) and Cellina di Nardò (susceptible), together with oleaster genotypes were sampled in different localities of Salento Apulia, Italy. Structural and metabolic changes in wood-forming processes will be investigated to detect histological and structural anomalies in xylem tissue mainly hybridizing thin xylem sections (45 µm) of one-year-old olive or wild olive branches with the FISH (Fluorescence In Situ Hybridization) KO210 probe specific for *X. fastidiosa* (Xf).

In the representative images on the right, oleaster vessels are largely single or in pairs in non-infected (-Xfp). In contrast, in infected oleaster (+Xfp), the vessels are clustered together and positioned adjacent to each other, aligned along the section's ideal radii; in this scenario, numerous xylem vessel occlusions by Xf cells are visible, as evidenced by the probe's red signal emission. Comparably images were obtained by analyzing sections of branches of the varieties Leccino and Cellina di Nardò.

The dominance of single vessels permits the bacterium to remain limited to only one vessel, slowing disease progression and potentially making the host plant more tolerant/resistant to the pathogen. In any case, it is possible that the production of more xylem vessels close to each other is a response to the infection, and thus a way of conveying more water to the leaves, with an effort that paradoxically may aggravate the infection because *Xylella* can colonize nearby vessels. Subsequent investigations will allow us to determine whether 'single' xylem vessels represent a stretch of resistance.

Keywords: Olive, *Xylella fastidiosa*, Xylem structure, Resistant and susceptible cultivars

Session: Biodiversity in cropping systems

***Solanum aethiopicum* gr. gilo accessions and their potential use as eggplant rootstock**

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Herbaceous grafting is an agamic plant propagation technique involving the union of two plants, the rootstock, selected for its tolerance to biotic and/or abiotic distresses, and the scion selected for yield and quality traits. *Solanum torvum* represents the most widely used rootstock for eggplant. Nevertheless, the employment of other rootstock genotypes and their interspecific hybrid could be a useful alternative to improve eggplant performances and to valorise the biodiversity. In our study we tested six different grafting combinations [ungrafted, self-grafted, grafted onto: *S. torvum*, *S. melongena* gr. gilo $\times$ *S. aethiopicum*, *S. aethiopicum* gr. gilo (accession 1) or *S. aethiopicum* gr. gilo (accession 2)] using the cultivar “Scarlatti” as scion. All genotypes tested as potential rootstocks did not adversely affect fruit quality characteristics. Additionally, fruit glycoalkaloids content was always below the recommended limit in all grafting combinations. The study underlined that the use of interspecific hybrid rootstocks secured high yield performance. The results also indicated that the use of *S. melongena* $\times$ *S. aethiopicum* gr. gilo interspecific hybrid could be an encouraging option to the most used *S. torvum*. Herbaceous grafting is a key tool to ensure or increase fruit yield and quality under optimal or stressed conditions. Grafting success is a further important aspect when testing new combinations of scions and rootstocks. In our study, grafting success was over 89% in all rootstock-graft combinations with a peak of 99% when using *S. torvum* and *S. melongena* $\times$ *S. aethiopicum* gr. gilo rootstocks. From these results, it is evident that the genotype choice is an important step in identifying the appropriate rootstock for fruiting vegetables. This choice may be improved by exploiting the variation among the accessions of a specific species.

Keywords: *Solanum melongena*, Herbaceous grafting, Biodiversity use, Biodiversity valorisation, Accessions

Multilevel assessment of soil biodiversity after application of biostimulant based on arbuscular mycorrhizal fungi in three cropping systems

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Complex and multilayered communities of soil biota underpin ecosystem services in healthy soils. Agricultural practices are key drivers of soil biological diversity; they can lead to its degradation but also have the potential for inducing its enhancement. Microbial-based plant biostimulants represent a promising alternative solution to chemical fertilization with great potential of positive impacts on soil biodiversity. Biostimulants containing arbuscular mycorrhizal fungi (AMF) can improve nutrient uptake and soil structure. To better understand their effect and evaluate in situ application outcomes, we applied a multilevel assessment of their impact on soil biota for three cropping systems covering apples, strawberries and tomatoes.

AMF-based biostimulants were compared with untreated controls in field trials located in the northeastern Emilia-Romagna region in Italy, in northern Mediterranean agricultural environmental zone. We assessed the soil microbial communities and activity by way of the 16S rRNA gene bacterial diversity, metabolic activity (Biolog Ecoplates), gene abundance (qPCR), as well as soil nematode and microarthropod communities, hydrolytic enzyme activity, and potential CO₂ and N₂O production.

The three cropping systems differed in most measured variables, with apple orchards showing the highest values for most of them, followed by strawberry. PCA ordination indicated that plant-parasitic nematodes characterized tomato, while N-cycle genes characterized strawberry. The AMF treatment did not increase yields for any of the crops, nor induced its decrease; but a marked increase of several biodiversity indices and functional biological parameters, especially in the strawberry cropping system, was identified.

The integrated assessment suggests that microbial biostimulants can support sustainable soil management by enhancing biodiversity and ecosystem functioning, relevant for long-term improvement of cropping systems.

Keywords: Soil biodiversity, Microbial-based plant biostimulants, Multilevel biota assessment

Session: Insect biodiversity in the modern era

Insect taxonomic and functional diversity: opportunities from genetic approaches

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Characterizing insect biodiversity is essential for understanding ecosystem functionality and resilience, including the impact of climate change, agricultural practices, and land-use intensification. DNA-based methods, particularly DNA metabarcoding, provide efficient and high-throughput solutions for insect biodiversity assessment, enabling accurate taxonomic and functional characterization. This approach relies on well-curated reference databases of genetic markers (e.g., COI, 12S rRNA) built from morphologically identified specimens, thus integrating taxonomic and molecular biology expertise. Properly formatted databases with high-quality reference sequences ensure seamless integration into bioinformatic pipelines and accurate taxonomic assignment, strengthening biodiversity monitoring efforts. A key advantage of DNA metabarcoding is its broad applicability to various environmental samples, including bulk insect, soil, water, and air samples, allowing large-scale biodiversity assessments across different ecosystems. Although these approaches rely on short genetic fragments (a few hundred nucleotides), recent advancements demonstrate their potential to also retrieve preliminary population genetics information. Additionally, DNA-based approaches, combined with functional trait databases, represent an efficient way to study the ecosystem services provided by insects, such as pollination and pest control. However, challenges remain in protocol standardization, database completeness, and methodological biases. Addressing these limitations will further enhance the reliability of DNA-based approaches, supporting their adoption in biodiversity conservation and ecosystem management strategies.

Keywords: Molecular taxonomy, DNA metabarcoding, Environmental DNA, DNA-barcoding databases

Session: Insect biodiversity in the modern era

Hoverfly (Diptera, Syrphidae) biodiversity in Italian National Parks

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The Italian fauna of Syrphidae (Diptera) is the second richest in Europe, yet our understanding of hoverfly distribution remains fragmented. Currently, 21.4% of Italy's terrestrial area is under protection, with 5.3% within 25 National Parks. Despite the critical role of Syrphidae in ecosystem services like pollination and biological control, research on their presence in National Parks is still insufficient. In recent years, some National Parks have initiated projects to enhance knowledge of hoverfly fauna, revealing notable differences between parks. While certain parks have limited species records, others show remarkable diversity. For instance, the Dolomiti Bellunesi National Park (DBNP) boasts 211 species, thanks to five years of focused research.

These efforts have significantly expanded the Italian hoverfly fauna, with species such as *Anasimyia lunulata*, *Syrphus rectus*, and *Helophilus hybridus* recorded for the first time in the DBNP, and *Eumerus narcissi* identified in the Arcipelago Toscano National Park. Standardized sampling protocols allow comparisons of conservation status among parks, as demonstrated by DBNP and Val Grande National Parks. In Alpine Parks hoverflies have been included in research focusing on pollinator distribution along altitudinal gradients, revealing a unimodal distribution of hoverflies with peak at middle altitude (1400-1600 m). Molecular approaches have advanced the understanding of population differentiation in species like *Merodon cinereus*, making it a promising model for studying climate-induced changes.

This research highlights the urgent need to expand knowledge of hoverflies in all Italian National Parks, which are crucial biodiversity hotspots and key to conserving this important group of insects.

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Keywords: Syrphidae, Italian National Parks, Pollinators, Climatic change, Altitudinal gradients

Session: Insect biodiversity in the modern era

Using biosolutions in fruit orchards against the invasive *Halyomorpha halys* to reduce pest control impact on biodiversity

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Biodiversity plays a crucial role in maintaining ecosystem resilience, including natural pest regulation. However, the introduction of invasive species, such as the brown marmorated stink bug (*Halyomorpha halys*), can disrupt ecological balance by altering native species dynamics and reducing biodiversity. Native to East Asia, *H. halys* has rapidly spread across North America and Europe, feeding on over 300 cultivated plant species and causing significant economic losses. In Italy, this pest has particularly affected fruit orchards, including pears. Although *H. halys* is commonly controlled with synthetic insecticides, increasing concerns over environmental impact have encouraged the development of more sustainable approaches, such as the use of natural compounds and biological control agents. In laboratory trials, various organically registered products (e.g., azadirachtin, sulphur, calcium polysulfide) showed significant lethality against *H. halys* nymphs. Diatomaceous earth, either alone or alternated with sulphur or calcium polysulfide, provided comparable control in field trials when compared to conventional strategies based on neonicotinoids (e.g., acetamiprid), with reduced effects on non-target beneficials like the egg parasitoid *Trissolcus japonicus*. Further research evaluated the repellency of three compounds against *H. halys*. Repellent activity was confirmed under laboratory conditions. Field experiments conducted in pear orchards showed that repellent-based dispensers effectively reduced *H. halys* presence in treated plots and mitigated fruit damage. These findings support integrated pest management (IPM) strategies that limit chemical inputs while enhancing biodiversity conservation and ecological sustainability in agroecosystems.

Keywords: Brown marmorated stink bug, Integrated pest management, Hemiptera, Natural compounds

Session: Insect biodiversity in the modern era

Funerary archaeoentomology: a discipline to investigate biodiversity, local extinction and new arrivals

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Archaeoentomology is a branch of environmental archaeology that focuses on insects and other arthropods, contributing alongside other disciplines to reconstruct a more complete view of past events, climate, and landscape. Funerary archaeoentomology, as defined by Huchet in 1996, focuses on the arthropods associated with human remains of archaeological interest or with elements involved in funerary rituals, such as offerings.

In recent years, several studies have been conducted on Italian archaeological sites covering around 5,000 years and various contexts. Sardinian necropolises, Egyptian mummies stored in Italian museums, and offerings in public collections are among the oldest samples from which insects have been isolated. Mummified and skeletonized bodies of kings, saints, and common people also represent an important source of insects. It is worth mentioning that more than 80% of the studies deal with burials that occurred in the modern era, especially in crypts under churches or related to the remains of World War I soldiers.

In all cases, the majority of the findings are insects in the orders Diptera and Coleoptera, while among the Arachnida, mites represent the most important taxon. Cases of local extinction (e.g., *Phormia regina*, Diptera: Calliphoridae), new potential species introduction (e.g., *Hermetia illucens*, Diptera: Stratiomyidae), ectoparasites associated with humans and other animals (Siphonaptera and Psocodea), food and wood pests (Coleoptera: Curculionidae, Ptinidae, Dermestidae, Cleridae, and Tenebrionidae), and the distribution of several flies (Diptera: Phoridae, Muscidae, Fanniidae, Calliphoridae, and Sarcophagidae) are reported and discussed. This study, having a historical perspective based on well documented and reliable dating, allows a temporal analysis of the entomofauna evolution also depending on human activities, trades and travels.

Keywords: Insects, Funerary archaeoentomology, Past reconstruction, Diptera, Coleoptera

Session: Insect biodiversity in the modern era

The use of archaeoentomology and cartography to investigate the landscape transformation in the modern era

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Archaeoentomology is an interdisciplinary discipline that focuses on insect remains from archaeological contexts, providing deeper insights into human-environment interactions from a historical perspective. This study aims to analyze variations in land use by examining insect assemblages from archaeological contexts and comparing them with contemporary species records, based on historical (e.g., Catasto Chiesa, 1727-1734) and modern land-use cartography. Archaeological contexts offer well-dated or easily datable material, providing a reliable chronological framework.

Since 2022, this research has been conducted at the Pieve di San Cristoforo church in Pian di Marte (Umbria, Central Italy), employing pitfall and Malaise traps alongside direct hand collections. Five hypogean crypts (18th-19th century) containing human remains were analyzed as part of a multidisciplinary project, with a particular focus on the entomofauna associated with the bodies. Two chambers, located on the northeastern side of the church, have drainage channels connecting them to the external environment. However, these channels were sealed around the end of the 18th century. Before their closure, they likely allowed access to predatory and opportunistic insect species unrelated to cadaveric fauna. Ground beetles (Carabidae: *Carabus*, *Pterostichus*, *Nebria*, *Trechus*, etc.) and leaf beetles (Chrysomelidae) were sampled. These taxa, with their specific habitat and food preferences, are recognized as reliable bioindicators of soil conditions and vegetation.

The results indicate that, despite significant variations in land use near the church, the ground beetle fauna has not been strongly affected. This could be explained by the survival of small wooded patches that served as refuge areas for Carabids. On the other hand, leaf beetles reveal the past abundance of elm trees, which no longer exist in the area. However, some local toponyms testify to the historical presence of these trees.

Keywords: Insects, Carabidae, Chrysomelidae, Land-use, Past reconstruction

Session: Insect biodiversity in the modern era

Small creatures, big impact: unveiling the power of nematode diversity in the Mediterranean ecoregion

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Studying the biodiversity of nematode communities in Mediterranean regions is crucial for several reasons, especially given the unique ecological and agricultural characteristics of these areas. The Mediterranean climate supports a wide variety of habitats that harbor rich assemblages of nematode species. This biodiversity plays a key role in maintaining ecological balance and ensuring the functionality of both terrestrial and marine ecosystems.

Examples from natural and agricultural terrestrial habitats will be provided, where nematodes are known to contribute significantly to nutrient cycling and soil health. In Mediterranean orchards, vineyards, and olive groves, beneficial nematodes can help regulate pest populations, reducing the reliance on chemical pesticides. By integrating nematological knowledge into land management practices, farmers can improve soil structure and fertility, supporting a more sustainable form of agriculture with reduced environmental impact.

Furthermore, the study of nematode communities extends beyond land into the meiofauna of the Mediterranean Sea, where they are essential components of benthic food webs. Their biodiversity reflects the health of marine ecosystems, making them valuable indicators of environmental change. Understanding the dynamics of marine nematode communities can inform conservation strategies and the sustainable management of marine resources, particularly in the face of anthropogenic stressors such as overfishing and pollution.

In conclusion, evidence suggests that the integrated study of nematode biodiversity in Mediterranean areas is vital for advancing sustainable agriculture and preserving the ecological integrity of both terrestrial and marine environments. By recognizing the diverse roles nematodes play, we can enhance ecosystem resilience, support food security, and promote environmental sustainability goals that align with broader biodiversity conservation and ecosystem management efforts.

Keywords: Sustainable agriculture, Nematode communities, Meiofauna, Biological control, Ecological indices

Phenotypic and genomic assessment of sugar assimilation in the pomegranate yeast *Hanseniaspora valbyensis*

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Among non-conventional yeasts, *Hanseniaspora* is known for its high capacity to produce aroma compounds during fermentation. Its apparent reduced ability to completely utilise sugars in some ecosystems (e.g., grape must) may be explained by growth impairment due to sensitivity to ethanol or antimicrobials produced by other yeasts. Although the capacity of *Hanseniaspora* species (e.g. *Hanseniaspora valbyensis*) to grow on fructose was previously reported, no fructose-specific transporters were identified. This study aimed to characterize fermentation behavior and scout potential fructose transporters of *H. valbyensis* biotypes, isolated from pomegranate.

The capacity of *H. valbyensis* to assimilate diverse sugars was first assessed using agar minimal medium (MM) supplemented (at 2%) with glucose, fructose, lactose, xylose, maltose, or sucrose. Subsequently, growth, sugar consumption and metabolite production were investigated during liquid cultivation with three different carbon sources: glucose, fructose, glucose and fructose. Well characterised strains of *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* were used as references. Whole genome analysis was performed to identify potential sugar transporters of *H. valbyensis*, and a phylogenetic tree was built.

H. valbyensis grew at highest level on MM plates containing glucose or fructose. Among the yeast species under investigation, it was the only one able to consume fructose and glucose after cultivation on MM broth, thus suggesting potential fructophilic behavior. The analysis of the phylogenetic tree showed that some sugar transporters of *H. valbyensis* were close to Ffz1 fructose facilitator of *Zygosaccharomyces bailii*. Ffz transporters are phylogenetically distinct from all the other previously characterized hexose transporters and more similar to drug transporters. Further research will focus on the role of this candidate Ffz1-like transporter in *H. valbyensis*.

Keywords: *Hanseniaspora valbyensis*, Sugar

Session: Microbial biodiversity in foods: evolving insights and uncharted horizons

From by-product to innovation: exploiting biodiversity of *Yarrowia lipolytica* strains to valorize cheese whey and develop adjunct cultures for improved cheese ripening and quality

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Cheese whey represents an important dairy by-product which contains valuable components that can be recovered or used as microbial growth substrates. This study explores the potential of using cheese whey to cultivate *Yarrowia lipolytica* biomasses for application as adjunct cultures in cheesemaking. *Y. lipolytica* strains were screened on three types of cheese whey for growth, lipolytic activity, and proteolysis. The best-performing strains, RO3. and Y3, were scaled up in a 20L fermenter and applied to the cheese surface. RO3. was further incorporated into both milk and cheese surface in an industrial-scale (500L) cheesemaking process. The cheeses were stored at 6°C for 35 days and analyzed for microbiological (plate counts), chemical (water activity, pH, color), proteolytic (SDS-PAGE), lipolytic (Folch method with GC), and volatile molecule profiles (SPME-GC-MS), alongside sensory evaluations. The tested strains exhibited strain-dependent behavior in hydrolytic profiles and growth capabilities reaching up to 8.77 log CFU/mL in caciotta whey after 72 h. When scaled up in a 20L fermenter, RO3. and Y3 reached 7.97 and 7.68 log CFU/mL, respectively, after 48 h. These biomasses were applied to the cheese surface and showed a ripening acceleration by enhancing proteolysis and increasing unsaturated fatty acid content (25% for RO3. vs. 19% in the control). Moreover, they stimulated the development of ripened cheese aroma compounds (e.g., butanoic, hexanoic, and decanoic acids). RO3. was further tested in cheesemaking by incorporating it into both milk and cheese surface. This approach reinforced and enhanced the results observed with surface application alone, differentiating the product from the benchmark in less than 12 days of ripening. This study highlights that the selection of specific *Y. lipolytica* strains and development of tailored protocols for production represent a promising approach for upcycling a dairy by-product into innovative ingredients and foods.

Keywords: *Yarrowia lipolytica*, Cheese whey, Innovative cheese, Adjunct culture, by-product valorization

Yeast biodiversity in brewing: a comprehensive screening of 30 strains of *Saccharomyces cerevisiae* isolated from different ecological niches

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Yeasts biodiversity significantly influences the quality of fermented beverages, including beer, wine, and spirits. This study investigates different *Saccharomyces cerevisiae* strains isolated from diverse ecological niches (natural and industrial environments) to identify novel yeasts that can enhance flavour, aroma, and overall beer quality.

Thirty *S. cerevisiae* strains were evaluated for brewing capacity using standardized 12 Plato barley malt wort in micro-fermentation trials. The fermentative abilities were evaluated on a micro fermentation scale by analysing the main qualitative parameters (e.g. alcohol, attenuation, pH), the volatile profile by GC-MS (high alcohols, esters, aldehydes), and the sugar profile by HPLC (sucrose, glucose, maltose, maltotriose, dextrins).

The screening work demonstrated remarkable diversity among yeast strains of the same species but originated from different habitats. Alcohol content analysis enabled the identification of weakly fermenting strains (0.7 % v/v alcohol) isolated from natural and oligotrophic habitats, medium fermenting strains (2.5-4 % v/v alcohol), highly fermenting strains (up to 5.5 % v/v alcohol) mainly derived from industrial and nutrient-rich niches. Strains exhibiting low alcohol production demonstrated limited maltose utilization, making them promising candidates to produce low-alcoholic beers. Other strains fermented maltose but not maltotriose, suitable for full-body beers. Unexpectedly, three strains fermented maltotriose but not maltose, indicating an original metabolic trait compared to brewing yeasts currently available on the market.

GC-MS analysis confirmed substantial heterogeneity in volatile aromatic profiles among the strains, with esters ranging from 0.5 to 21 mg/l, higher alcohols from 25 to 218 mg/l, diacetyl from 0 to 400 µg/l, and aldehydes from 14 to 87 mg/l. Future work will focus on selecting the most performant strains for scale-up to a 25-liter pilot scale.

Keywords: Yeast biodiversity, Beer, Low-alcohol beer

Session: Microbial biodiversity in foods: evolving insights and uncharted horizons

Microbial resources for sustainable meat productions in the green deal era

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Nitrate and nitrite salts are widely used for improving the quality and safety in fermented meat products. They are effective against both cells and spores of *Clostridium botulinum*, prevent lipid oxidation, and promote the red colour of meat products. However, the association between this additives and health-related issues and the consequent awareness for foods without synthetic additives has led to explore new ways to ensure the safety and extending shelf-life.

Lactic acid bacteria (LAB) and Coagulase-Negative Catalase-Positive Cocci (CNCPC) are known for their safety and fermentation capacity and are promising due to their in vitro antioxidant activity and the production of enzymes such as Nitric Oxide Synthase (NOS), which converts L-arginine into Nitric Oxide (NO). NO binds to myoglobin, producing nitroso-myoglobin (NO-Mb) responsible for meat products red colouration.

Several strains of *Staphylococcus* spp. and Lactic Acid Bacteria were characterized for their ability to produce Nitric Oxide via Nitric Oxide Synthase (NOS) pathway or NOS-like enzymes. The strains that showed higher NOS activity were used for the production of low-nitrite fermented sausages, evaluating their ability to stabilize the red colour of meat through the production of nitrosomyoglobin and for their proteolytic and antioxidant activity. The effect on physico-chemical parameters, microbial population, volatiles as well as sensory characteristics was evaluated.

The results suggest that using starter cultures with NOS activity could be a promising strategy for producing clean-label products without compromising the safety and sensory properties, representing a step forward in the search for sustainable solutions for meat preservation, opening new perspectives for the food industry.

Development of a new brewing strain with improved aroma through hybridization and UV mutagenesis

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Yeasts are fundamental in beer production, influencing fermentation, flavor, and stability. To meet consumer demand, we developed a novel, GMO-free yeast hybrid between a sourdough strain of *Saccharomyces cerevisiae* and a cryotolerant *S. eubayanus* x *S. bayanus* via spore-to-spore hybridization. Microfermentations showed improved brewing traits in one hybrid but also the undesired Phenolic Off-Flavor (POF+) phenotype, caused by the conversion of ferulic acid into 4-vinyl guaiacol (4VG). To eliminate this trait, we applied UV mutagenesis, optimizing irradiation conditions (50 cm distance, 30 s exposure) to achieve 8-22% mortality. Nystatin treatment, which selects slow-growing mutants, reduced the mutation success rate (13.3% without vs. 0.4% with nystatin). Mutants were screened on YPDA with 4 mM ferulic acid and analyzed via Ferulic Acid Conversion Index (FACI) and Cinnamic Acid Sensitivity Index (CASI). POF- candidates exhibited low FACI and high CASI values, confirming reduced 4VG production. Further microfermentations confirmed that selected mutants retained improved brewing traits without the POF+ phenotype. This study demonstrates that hybridization combined with UV mutagenesis is an effective, GMO-free strategy for developing superior brewing yeasts.

Keywords: Yeast hybridization, Phenolic off-Flavor, UV mutagenesis, Brewing yeast improvement

Session: Plant biodiversity: environmental preservation to face global change

Climate changes, habitat transformation and germination behavior. A complex story from three Mediterranean plants of conservation interest: *Muscari gussonei*, *Petagnaea gussonei* and *Poterium spinosum*

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Knowing the optimum germination temperatures of endangered plants, as well as the magnitude of climate changes (e.g., rising temperatures) across the spatial range of these species, is essential to assess the level of threat for such species and, consequently, to implement tailored and enduring conservation programs. These analyses should be also integrated by the study of land-cover changes, which can act as an indicator of landscape fragmentation and, therefore, as a proxy of gene flow across metapopulations of species of conservation interest. This study, in particular, investigated both the germination behavior of *Muscari gussonei*, *Petagnaea gussonei* and *Poterium spinosum*, and the trends of climate and land-cover changes occurred in the distributional areas of these species. *M. gussonei* and *P. gussonei* are two endangered and narrowly distributed endemic plants from Sicily (Italy), whereas *P. spinosum* is another biogeographically important species with a highly scattered range across south-eastern Sicily. All the studied plants showed the same narrow germination optimum of 10-15 °C, but different final germination percentage (FGP): 82-98% in *M. gussonei*, 10-38% in *P. gussonei*, 5-61% in fruits and 21-68% in seeds of *P. spinosum*. These optimum germination temperatures are low and limited and, therefore, make the three species much more vulnerable to ever-rising temperatures, which specifically increased by up to 2 °C in the period 1931-2020. Similarly, the analysis of CORINE Land Cover classes showed highly fragmented landscapes, where forest and seminatural areas (class 3) declined, and agricultural areas (class 2) increased. The ongoing climate changes will make the suitable germination temperatures harder to be reached by plants with low and narrow germination optimum, such as *M. gussonei*, *P. gussonei* and *P. spinosum*, whose survival is further threatened by a complex fragmented territory with declining natural areas.

Keywords: Mediterranean endemic plants, Global warming, CORINE Land Cover, Germination performance, in situ and ex situ conservation, Sicily

Session: Plant biodiversity: environmental preservation to face global change

Shading lights on *Silybum* genus biodiversity: chemodiversity, genetic and genomic characterization of a wide germplasm collection

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Silybum marianum (L.) Gaertn., also known as milk thistle, is an herbaceous medicinal plant of the Asteraceae family native to the Mediterranean basin. The species is a weed but is cultivated since past for silymarin production, a high-value complex of flavonolignans accumulating in achenes with hepato-protective and anti-inflammatory properties. The *Silybum* genus also includes *S. eburneum*, but its classification is often misreported in the literature and knowledge about it is limited. Thanks to the NBFC-spoke 3 project, the *S. marianum* germplasm collection available at CREA-CI Bologna was implemented with 83 wild *Silybum* accessions of diverse geographical origins, by sampling campaigns or from seed GeneBanks, to characterize the genus biodiversity at biochemical, morphological, and genetic levels. Targeted metabolomics revealed that *S. marianum* is characterized by three diverse chemotypes A, B, and C, while *S. eburneum* accessions exhibit a unique chemotype (D) with isosilychristin as the major flavonolignan component. Flower and leaf morphology were identified as distinctive traits and combined with DNA barcoding based on the ITS2 sequence allowed us to clearly differentiate the two *Silybum* species. To investigate the genetics of silymarin biosynthesis, the genome of chemotype B accession was de novo assembled into 17 chromosomes using a combination of Oxford Nanopore long and ultra-long reads. The approach yielded a final assembly of ~ 721 Mb of which 6 chromosomes were telomere-to-telomere, with the longest sequence of 62.1 Mb. The genome completeness was assessed with BUSCO which identified a high proportion of complete single-copy orthologs (> 99%) indicating a highly complete and contiguous genome. Genome annotation using RNAseq data of different tissues is still ongoing. Our work paved new knowledge on *Silybum* genus biodiversity and resources that will facilitate its exploitation in nutrition and human health sciences.

Keywords: *Silybum*, silymarin, Chemotypes, Genetic resources, Genome assembly

The potential of the terpene profile of Mediterranean fir species in the chemotaxonomic study and the conservation prospects

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Some fir species in the Mediterranean have restricted geographical distributions and are often endemic and endangered, typically found in relict areas, except for *Abies alba*. Among these threatened species, *Abies pinsapo* (Spanish fir) is classified as endangered by the International Union for Conservation of Nature (IUCN), and *A. nebrodensis* (Sicilian fir) is included in the Red List of IUCN as one of the most critically endangered conifers in the Mediterranean region. Conifers have evolved various physical and chemical defences against biotic and abiotic stress; for instance, the variation of terpenes significantly affects insect behaviour and herbivory. The relationships between the quantity and composition of terpenes have been extensively studied in different parts of coniferous plants, except seeds, where there has been less focus. Therefore, this study aimed to examine the features and terpene profiles of seeds collected from the critically endangered Sicilian and Spanish fir and the common European silver fir species. After X-ray analysis, only the empty seeds were used for morphometric measurements and terpene isolation. The results of GC-MS analysis reported the dominance of monoterpenes in all the species, while *A. nebrodensis* revealed the considerable presence of sesquiterpenes. Statistically significant differences were found in most of the detected terpenes, and only 3 (α -pinene, limonene, and bornyl acetate) out of 25 compounds showed no statistical differences between species. For a clear discrimination, the linear discriminant analysis was more effective in separating the *Abies* species. The current findings confirmed the potential and suitability of the terpene profile in the chemotaxonomic studies between species from the same family. Moreover, the identified compounds will enable future research on plant defense against biotic stress to reduce the risk of species extinction caused by pests and diseases.

Keywords: Sicilian fir, European silver fir, Spanish fir, Threatened species, Conifers, Chemotaxonomic markers, Empty seeds

Conservation status of Annex I habitats in Italy: first overview from the Fifth National Report under Article 17 of the Habitats Directive

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Under Article 17 of the "Habitats" Directive (92/43/EEC), Member States periodically have to assess and report on the conservation status of Annex I habitats. The reports include data on habitat conservation status, trends, pressures, and threats. The results of reporting address policy evaluation and biodiversity strategies. Italy's V national report (period 2019-2024), coordinated by the Italian Society of Vegetation Science (SISV) and the University of Sassari, with co-coordination by the Italian Institute for Environmental Protection and Research (ISPRA) on behalf of the Ministry of the Environment and Energy Security (MASE), is nearing completion. A multidisciplinary team of regional and thematic experts validated and integrated data on habitat distribution and conservation status submitted by Italian Regions and Autonomous Provinces. Distribution maps, based on the ETRS89-LAEA5210 10x10 km grid, were created and made available via a dedicated WebGIS developed by ISPRA for data entry, validation, and management. Additional datasets, including the Natura 2000 database released by MASE in Dec 2024, were also utilized. The assessment covers 124 terrestrial and inland water habitats across Alpine, Continental, and Mediterranean biogeographical regions. Analytical efforts address data gaps and inconsistencies through robust methodologies and scientific dialogue. Emphasis is placed on traceability and integration of multiple sources, such as peer-reviewed literature, phytosociological surveys, and unpublished expert materials. This extensive knowledge base underpins a long-term strategy to ensure continuity in future reporting cycles. Following European Commission validation, maps, datasets, and associated documentation will be publicly accessible on ISPRA's national reporting portal. This forward-looking process marks a crucial step toward a science-based monitoring system for conserving Annex I habitats in Italy.

Keywords: Biodiversity, European Commission, Monitoring, Vegetation

Plant biodiversity and conservation of endemic species of the Dolomites and Southeastern Alps

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The Southeastern Alps are not only a biodiversity hotspot but also host the highest proportion of endemic plants in the Eastern Alps. During the climatic changes of the Pleistocene, long-term stable habitats - so-called refugia - probably facilitated the emergence of endemic biodiversity in the area. In two projects, we investigated, first, the evolutionary history of nine key plant species endemic to the Southeastern Alps by sequencing large parts of their genomes using RADseq data. We infer how recurrent climatic changes have influenced the evolutionary history of these target species, how glacial cycles have generated unique evolutionary lineages, and how this unique genetic diversity has evolved and is distributed in time and space. Our results show that, depending on the respective ecology, the species found peripheral refugia in the southern and southeastern areas, showed patterns of nunatak, i.e. unglaciated mountain tops, survival or a combination thereof. Our results also allow the identification of evolutionarily unique populations and lineages within each species to inform conservation efforts. Second, we assessed potential future climatic refugia of these key species, using high-resolution (50 m) downscaled climate data and topographic factors to identify areas of climatic stability under different climate change scenarios. Future climate projections suggest moderate to severe range contractions, with lower elevations being most affected. Stable and newly suitable habitats were primarily at higher elevations, but the Dolomites' high-altitude plateaus exhibited rapid climate change velocity, potentially limiting long-term persistence. Integrating all our results advances our knowledge of why areas such as the Southeastern Alps are hotspots of endemism, how the endemic key species might develop under ongoing climate change and how both aspects should be combined for conservation efforts in mountain landscapes.

Keywords: Endemic alpine plants, Phylogeography, Intraspecific diversity, Climate change, Species distribution models

Occurrence and distribution of yeast communities related to honeybees system: an ecological overview

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Pollination is one of the most important ecosystem services, and honeybees (*Apis mellifera*) play a crucial role in this process. They are essential for biodiversity and food production and, due to their complex society, serve as a great model of social behavior. The aim of the study was to increase the knowledge regarding to the yeasts related to the honeybee system. For this reason, flowers, bee gut and hive products such as bee pollen, bee bread and propolis were sampled and analyzed.

A total of 27 yeasts genera and 51 species were identified. Basidiomycetes were predominant in flowers, while *Aureobasidium* sp., *Filobasidium* sp., *Meyerozyma* sp., and *Metschnikowia* sp., were found in all environments. Fermenting yeasts, such as *Debaryomyces* sp., *Saccharomyces* sp., *Starmerella* sp., *Pichia* sp., and *Lachancea* sp., were mainly found in the gut, whereas most species identified in bee products were absent in gut mycobiota. Moreover, the yeast species *Meyerozyma guilliermondii*, *Debaryomyces hansenii*, *Hanseniaspora uvarum*, *Hanseniaspora guilliermondii*, and *Starmerella roseus* were detected in the bee gut during both summer and winter sampling, suggesting their role as a stable eukaryotic component of the core gut microbiota. 3 strains of *Meyerozyma guilliermondii* and 4 strains of *Meyerozyma caribbica* exhibited probiotic traits. These results contribute to understanding the yeast community as a component of the bee gut microbiota and its relationship with the environment. Moreover, honeybee ecosystem represents a valuable tool for a source of probiotic and functional yeasts.

Keywords: Yeasts, Honeybee, Honeybee gut microbiota, Bee bread, Bee pollen

Enhancing soil bacterial community profiling: PMA treatment and long-read sequencing across biogeographic regions and land uses

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In the context of the BIOServicES project, climate chambers are used to simulate future climate conditions and study their impact on soil health. A climate change field experiment began in July 2024 in European semi-natural areas of the Alpine, Atlantic, Boreal, and Mediterranean regions. These four experimental areas are continuously monitored to link multi-level soil biodiversity and land-use types to soil ecosystem services over three years. In recent decades, total DNA extraction and sequencing for the assessment of environmental microbial communities has been performed ignoring relic DNA from non-viable cells. In this scenario, propidium monoazide (PMA), a DNA-intercalating agent that selectively penetrates membrane-compromised or dead bacterial cells, enables the targeted removal of relic DNA before downstream analyses. This approach facilitates the differentiation between viable and total bacterial communities within samples. The present work provides a preliminary evaluation of PMA's potential in studying soil bacterial communities, combining PMA staining with Oxford Nanopore Technology to analyze the full-length bacterial 16S rRNA gene.

DNA was extracted from 120 soil samples, subjected to PMA treatment and untreated control, and sequenced. The findings revealed region-specific variations in the correlation between viable and total bacterial community structures, suggesting differential capacities of soils to preserve relic DNA. Land-use intensity significantly influenced both viable and total bacterial communities, with a slightly reduced effect on total communities. Further in-depth analyses of sequencing data, coupled with quantitative PCR assays, could provide valuable insights into the functional roles of the viable soil bacterial community compared to the total community. Additionally, incorporating future soil sampling data will enable a more comprehensive exploration of the impacts of climate change and land-use practices on soil bacterial dynamics.

Keywords: Vital bacterial community, Oxford Nanopore Technology, Environmental microbiology, Soil health

Session: Agro-environmental microbiology: emerging perspectives and new frontiers

Plant Growth-Promoting Yeasts (PGPYs) as a sustainable solution to mitigate salt-induced stress on zucchini plant growth

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Among the long-term sustainable solutions to mitigate saline stress on plants, the use of plant growth promoting microorganisms (PGP) is considered very promising. While most of the efforts have been devoted to the selection and use of bacterial PGPs, little has been proposed with yeast PGP (PGPYs). In this study, three PGPY strains belonging to *Naganishia uzbekistanensis*, *Papiliotrema terrestris* and *Solicoccozyma phenolica* were employed singularly and in a consortium to mitigate salt stress of zucchini (*Cucurbita pepo*). The results demonstrated that these yeasts, when applied to salt-amended soil, mitigated the growth inhibition caused by NaCl. Among the three species, *N. uzbekistanensis* and *P. terrestris* showed the most significant improvements in plant performance, with *N. uzbekistanensis* exhibiting hormetic effects under salt stress by improving root length and dry plant biomass. In general, the root system was the most affected part of the plants due to the presence of the yeasts. The entire rhizosphere bacterial microbiota was significantly influenced by the addition of PGPYs, while the mycobiota was dominated by the introduced yeasts. Metabolomic fingerprinting using FTIR revealed modifications in hemicellulose and silica content, indicating that PGPY inoculation impacts not only the plant but also the soil and rhizosphere microorganisms.

Keywords: PGPYs, Salt-stress, Zucchini, Rhizosphere microbiome, Rhizosphere mycobiome

Cascade effect of microplastic aerial deposition affects tomato growth, root metabolome and exudome, and rhizosphere microbiome

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Here, we evaluated the impact of microplastics (MPs) aerial deposition on the physiology of tomato plants (*Solanum lycopersicum* L.) and rhizosphere microbial communities. Tomato plants were grown in soil and hydroponics for 31 days. After 15 and 21 days, leaves were sprayed with polyethylene microspheres (PEMS; 790-4999µm; 10-100-1000 mg L⁻¹) or MilliQ water (Control), avoiding direct contact of MPs with the growing media. After 31 days, tomato plants, soil, and root exudates were sampled. Fresh and dry biomass, elemental composition, and root metabolome were assessed on soil-grown plants, while root exudates were collected from hydroponically grown plants. The concentration of nutrients in the shoots was determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES). Gas chromatography-mass spectrometry (GC-MS) was used to evaluate root metabolome and exudome, while rhizosphere microbial diversity was investigated via DNA metabarcoding of the bacterial 16S rRNA gene and fungal ITS2 region. In shoots, PEMS aerial application significantly reduced the water content and increased most of the element concentrations ($p < 0.05$). In addition, PEMS caused leaves' ultrastructure deformation. In roots, PEMS significantly decreased the content of serine and tryptophan, 5,6-Dihydrouracil, lactic and tartaric acid, and palmitic and stearic acid. Similarly, in root exudates, the relative abundance of organic acids (i.e., malic, succinic, and citric acid), amino acids (i.e., aspartic acid and isoleucine), and carbohydrates (i.e., gluconic and threonic acid) decreased in PEMS-treated plants. Consistently, PEMS had a profound impact on rhizobacterial diversity (i.e., alpha and beta diversity) but not rhizofungal diversity. These findings highlight the potential impact of airborne PEMS on plant physiology and the rhizosphere bacterial community, underscoring the need for further research in this area.

Keywords: Microplastics, Root exudates, Microbial diversity, Rhizosphere

Characterisation of *Pseudomonas syringae* pv. *syringae* Italian strains causing severe wilting in eggplants

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The study of bacterial plant pathogen diversity is a crucial aspect for understanding how bacterial populations evolve in response to changes in agricultural systems, global trade and climate. *Pseudomonas syringae* is an evolutionarily diverse bacterial species complex and a preeminent model for studying plant pathogen interactions due to its remarkably broad host range. We focused on two bacterial strains isolated in Italy from eggplants (*Solanum melongena* L.) showing unusual and severe wilting symptoms. Phylogenetic analysis based on the housekeeping genes *gapA*, *rpoA* and *recA* revealed that the two strains are identical and clustered together with 22 *Pseudomonas syringae* pv. *syringae* strains infecting herbaceous plants. The genome of one of the strains, named DAPP-PG 773, was sequenced on an Illumina MiSeq platform using indexed paired-end 250-nucleotide v2 chemistry. A total of 7.907.342 pairs of reads were obtained, representing approximately 182-fold coverage of the genome and comprising 142 contigs. The assembled genome size was 6.06 Mb and the G/C content was 59.4%. Annotation of the DAPP-PG 773 draft genome sequence was carried out using Bakta yielding 5262 CDS. Phenotypic tests revealed that our strains produced exopolysaccharides, biofilm and N-acyl-homoserine lactones and very low level of indole-3-acetic acid; they had motility by swimming and swarming. Pathogenicity tests conducted on Solanaceae species highlighted the ability of the DAPP-PG 773 strain to infect exclusively eggplant plants, colonizing the xylematic vessels. Since genome analysis using EDGAR 3.2 platform revealed that the strain clustered with other *P. syringae* pv. *syringae* strains belonging to the 2b-a clade causing disease on Cucurbitaceae species, we conducted additional pathogenicity tests on squash plants, which confirmed the ability of the DAPP-PG 773 strain to systemically infect this plant species as well.

Keywords: Pseudomonas, Eggplant, Squash, Systemic disease, Genome analysis

The environmental context of honey bee gut microbiota: insights from the endemic Maltese subspecies, *Apis mellifera ruttneri*

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Understanding how microbial communities connect across ecological compartments is key to preserving biodiversity and enhancing ecosystem resilience. This study explores the link between soil, plant, and pollinator microbiomes by investigating the gut microbiota of *Apis mellifera ruttneri*, the endemic Maltese honey bee, in relation to environmental microbial sources.

Previous work indicated that *A. m. ruttneri* harbours a greater proportion of environmental microbes compared to *A. m. ligustica*, but the drivers of this pattern remain unclear. To disentangle genetic and environmental influences, colonies of both subspecies were co-located in experimental apiaries in Malta and Italy, representing Mediterranean and continental climates.

Monthly samples of worker bees and pollen were collected over a year, and 16S rRNA gene sequencing was used to characterize the microbial communities. Comparative analyses revealed that *A. m. ruttneri* exhibited a higher prevalence of environmental bacteria, including taxa typically associated with soil, such as Bartonellaceae, *Apilactobacillus*, and *Pseudomonas*. In contrast, *A. m. ligustica* displayed a microbiota dominated by host-associated *Lactobacillus* and *Bombilactobacillus*. Despite similar core taxa, their relative abundances differed markedly, indicating that environmental factors are a major influence on honey bee gut microbiota composition.

These findings suggest that *A. m. ruttneri* hosts a microbiota shaped by long-term exposure to the ecological conditions of the Maltese landscape, highlighting the importance of the environment in pollinator-microbe interactions. By demonstrating the microbial distinctiveness of local subspecies, we highlight the need to consider microbial ecology in pollinator conservation strategies, particularly in the face of climate and land-use change.

Research carried out in the European Union Next-Generation Framework (PNRR mission 4, component 2, investment 1.4 D.D. 1032 17/06/2022, CN00000022).

Keywords: Honey bee microbiota, Pollinator conservation, Environment pollinator interactions, Microbiome adaptation, Endemic species

Microbial drivers of nectar flows: the case study of sunflower

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Over the past decade, beekeepers in southern Europe have faced mounting challenges due to declining honey yields. Despite being a major nectar source for beekeeping, sunflower (*Helianthus annuus*) has shown declining nectar production, likely driven by increasing climate pressures such as drought and heatwaves. Compounding the issue, declining soil microbial diversity in agricultural lands may disrupt plant microbiome interactions essential for nectar production. Soil microorganisms play a key role in mobilising micronutrients like manganese and magnesium, making them crucial for nectar synthesis. Naturally occurring in soils, Plant Growth-Promoting Bacteria (PGPB) are commonly used as biofertilisers to promote plant growth and stress tolerance, yet their role in nectar-related physiological processes remains unclear.

To address this, a field trial in Italy was conducted using hybrid and non-hybrid sunflower cultivars. Selected PGPB from the bacterial genus *Bacillus* and family Lactobacillaceae were inoculated into experimental plots. Two hundred sunflowers were sampled across treatments. Gene expression of SWEET sugar transporters was determined via RT-qPCR. Simultaneously, root samples were collected and subjected to high-throughput sequencing to assess epiphytic microbial communities.

Results revealed that sunflower genotype significantly influenced rhizosphere microbial colonisation. Some PGPB strains also affected expression levels of genes encoding SWEET sugar transporters. These findings offer preliminary insights into how PGPB may regulate nectar production and, by extension, plant pollinator interactions. Overall, this research highlights how climate change can impact ecological interactions not directly related to honey bee biology, yet still influencing its productiveness and health.

This research was funded by the EU within the context of the Next-Generation EU (PNRR Mission 4, Component 2, Investment 1.4 D.D. 1032 17/06/2022, CN00000022).

Keywords: Sunflower, Microbiome, Bio-fertilizers, PGPB, Nectar-flow

Session: Challenges and opportunities for the implementation of the EU nature restoration law

The challenges and opportunities of the EU Nature Restoration Law and the role of research. The national project for the development of a catalogue and planning support tool for Nature-Based Solutions

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The adoption of the Nature Restoration Regulation (EU) No 2024/1991 represents a decisive step in Europe's strategy to protect biodiversity and strengthen ecosystem resilience. The legislation sets binding targets for the restoration of degraded terrestrial and marine ecosystems, requiring Member States to accelerate conservation policies and the integration of Nature-Based Solutions (NbS) into spatial and sectoral planning processes.

In this scenario, research assumes a fundamental role not only as a monitoring and evaluation tool, but as a stimulus to guide planning, support innovation in practices and strengthen the capacity of public institutions. The opportunities opened by the Regulation for the world of research are manifold, such as the development of ecological indicators, the modelling of impact scenarios, the experimentation of adaptive approaches, the construction of interoperable databases and integrated decision-making tools.

In this regard and within the framework of the National Biodiversity Future Centre, the NbS CataTool is a multi-stakeholders Decision Support System aimed at facilitating the selection of effective, adaptive and coherent Nature-based Solutions (NbS).

The CataTool classifies NbS and real-world case studies according to ecosystems and functional criteria distinguishing them across urban, terrestrial and marine domains. It is structured around two interlinked databases: the 'NbS Database' and the 'Case Study Database'. The first is a catalogue of over 100 theoretical NbS derived from existing catalogues and scientific literature. The second hosts more than 100 georeferenced NbS case studies from across Italy, sourced from European repositories and NBFC demonstration sites. Both databases use expert defined Key Performance Indicators to evaluate and compare solutions offering measurable insights to support informed decisions and develop concrete guidelines for evidence-based implementation of Regulation (EU) No 2024/1991.

Keywords: Nature, Restoration, Biodiversity, Research, Nature-based solutions

Session: Challenges and opportunities for the implementation of the EU nature restoration law

The role of integrated LIFE programmes in restoring ecosystems: a case study from the “Integrated management and actions for a green and Innovative nature” Project

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The Nature Restoration Act is a major legislative effort supporting the Global Biodiversity Framework, the European Green Deal, and the EU Biodiversity Strategy. It aims to restore at least 20% of the EU's land and sea areas by 2030, and all ecosystems needing restoration by 2050. Member States must submit National Restoration Plans to the European Commission by mid-2026, detailing how they will meet these goals.

Restoration of ecosystems is not new in Europe. These efforts often rely on Payments for Ecosystem Services (PES), where public funds compensate landowners for ecological improvements. PES schemes create incentives for sustainable practices by assigning economic value to ecosystem services, that are the benefits which ecosystems provide to humans (e.g., clean water, biodiversity, recreation). These services are central for integrating ecological and economic policies, moving beyond the traditional view of environmental protection versus development.

PES address the market failures caused by externalities (unpriced social or environmental impacts) by aligning private and public interests. PES are voluntary transactions between a service provider and a beneficiary, where compensation is given for maintaining or enhancing a defined ecosystem service. Two configurations are possible:

1. PES involving direct payments between local beneficiaries and service providers.
2. PES-like, funded by public institutions, without direct market exchanges

This study presents the PES-like scheme, aimed at preserving and restoring a rare habitat in Umbria (alkaline fens - Natura 2000 habitat type 7230). Interventions include mowing and clearing of invading vegetation; removal of the surface layer of the soil, including reed litter; interventions aimed at maintaining the surface water table; planting of species typical of habitat 7230. Local community has been involved to make it part of the restoration process.

Keywords: Ecosystem services, Payments for ecosystem services, Ecosystem restoration

Session: Challenges and opportunities for the implementation of the EU nature restoration law

The LIFE IMAGINE UMBRIA project for the conservation of insect species of community importance in Umbria

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Life Imagine Umbria project (LIFE19 IPE/IT/000015, 2020-2027) supports the development of a management strategy for the Natura 2000 network in the Umbria region (Italy). Its main objective is to maintain and improve the conservation status of habitats and species protected under the Habitats and Birds Directives, in specific target Natura 2000 Sites. To address this challenge, effective ecological restoration projects have been carried out, focusing on selected species of Odonata (*Coenagrion castellani* and *Lindenia tetraphylla*), Coleoptera (*Cerambyx cerdo*, *Lucanus cervus*, *Osmoderma eremita*, and *Rosalia alpina*) and Lepidoptera (*Eriogaster catax*, *Euphydryas provincialis*, and *Melanargia arge*) listed in the annexes of the Habitat Directive. For *C. castellani*, the project focused on maintaining the riparian vegetation of small watercourses (e.g. channels and ditches) and implementing best practices for their management. For *L. tetraphylla*, it involved actions to preserve and promote the growth of *Phragmites australis* and the typical lake vegetation formation known as reedbeds, utilizing technologies such as artificial floating islands. Best practices for enhancing the habitats of *C. cerdo*, *L. cervus*, and *R. alpina* focused on measures aimed at increasing the presence of senescent trees and associated forest necromass. For *O. eremita*, the main approach involved creating and installing artificial cavities to increase the presence of microhabitats suitable for larval development. For the lepidopteran species *E. catax*, best practices involved creating artificial ecotonal strips rich in shrubs, predominantly *Prunus spinosa*. Finally, best practices for *E. provincialis* and *M. arge* focused on safeguarding grasslands by limiting the settlement of shrubs, trees, and alien or synanthropic vegetation. The project aims to implement demonstrative actions for ecological management and habitat restoration to counteract the decline of these species and improve their conservation status.

Keywords: LIFE Programme, Habitat directive, Conservation, Best practices, Insects

Session: Challenges and opportunities for the implementation of the EU nature restoration law

Tipping points of biodiversity and sustainability of South Tyrol traditional pasture systems (BIOPAS)

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Traditional alpine pastures are high nature value ecosystems shaped by extensive livestock grazing dating back to pre-roman times. However, recent changes in grazing management have altered biodiversity, soil properties, and ecosystem functions, threatening long-term sustainability. While intensification has occurred in lowland areas, less accessible pastures face abandonment, leading to shrub and tree encroachment and biodiversity loss. In sheep farming, management has shifted from flocking and accompanied grazing to mainly free-range grazing, creating a more heterogeneous impact on pasture habitats. To anticipate and mitigate these effects, it is crucial to identify potential ecological tipping points and their indicators.

This study examines the impact of different grazing management types - (I) homogeneous rotation grazing, (II) heterogeneous free-range grazing, and (III) pasture abandonment - on vegetation, soil biodiversity, and sheep grazing behaviour in South Tyrol. We select three plots per management type across six sites, assessing plant indicator species of reduced management, vegetation composition, and soil biodiversity (bacteria, fungi, protozoa, micro- and mesofauna) alongside physico-chemical soil properties. At the site scale, we track sheep movement using GPS sensors to analyse grazing patterns and their link to vegetation changes. We hypothesize that reduced grazing management alters biodiversity in South Tyrol's pasture systems, leading to a stepwise shift in vegetation and soil communities. We expect to identify threshold values for biological and ecological parameters connected to soil and plant diversity that signal tipping points in pasture conditions. Our findings will contribute to sustainable management recommendations for mountain pasture ecosystems in South Tyrol and facilitate their application by sharing insights with farmers and stakeholders.

Keywords: Traditional pasture systems, Grazing management, Alpine biodiversity, Ecological tipping points, South Tyrol

POSTER ABSTRACTS

Session: Impact of climate change on biodiversity

MultiForDiv: a trait-based, multi-taxon analysis of forest understory biodiversity

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Forest ecosystems provide essential ecosystem services but face increasing threats from global changes. Although conservation of forest biodiversity is prioritized in international and national policies, many components, especially those in the understory (vascular plants, fungi, lichens) remain overlooked. These organisms are crucial for forest functioning and sensitive to environmental change.

The MultiForDiv project investigates spatio-temporal patterns and drivers of forest understory biodiversity using a multi-taxon, trait-based approach. This research integrates long-term data collected in the Italian permanent plots of the ICP Forests LII network distributed across all the national territory.

Analyses on the vascular plants reveals significant species richness declines over the last 25 years. Using linear mixed models, forest structural variables, soil pH, and climate features are identified as key drivers of diversity, with biome-specific responses. Preliminary results on epiphytic lichens suggest temporal stability in species richness. Functional traits of epiphytic non-vascular communities - including lichens and bryophytes - are explored. The relationships among air, bark and cryptogams thermal and hydric regimes under different environmental conditions are disentangled based on IR and NIR spectrum signatures and measured photosynthetic parameters. This allows to better understand their energy balance and their contribution to forest microclimate regulation and ecosystem functioning. Soil microfungi diversity is investigated through functional traits (including xerotolerance, thermotolerance, and solubilization of phosphates) to explore their responses to environmental variables.

This trait-based, multi-taxon approach reveals group-specific responses to environmental conditions, highlighting the need to integrate diverse biological components for a better understanding of forest biodiversity dynamics and effective forests management.

Keywords: Multi-taxon, Trait-based, Forest monitoring, Permanent plot

Session: The challenges of crop biodiversity in 21st Century

Evaluation of gastrointestinal motility and sensory preference characteristics of cucumber and fruits of “Barattiere” and “Scopatizzo”

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The Puglia region (Southern Italy) is a treasure trove of horticultural agrobiodiversity, renowned for its melon production (*Cucumis melo* L.), particularly unripe melons, such as Scopatizzo and Barattiere, consumed as an alternative to cucumbers (*Cucumis sativus* L.) for their sensory traits and better digestibility. Although consumers often misuse the term “digestibility”, literature lacks comparative data on the digestibility of cucumbers versus unripe melons. This study investigated the gastrointestinal motility by simultaneous combined measurements of gastric, gallbladder emptying, and orocecal transit time in response to the ingestion of cucumber, Scopatizzo, and Barattiere fruits for two hours following the meal ingestion. Moreover, the Visual Analogue Scales were used to quantify homeostatic (hunger, satiety) and rewarding (wanting liking, and learning) control of food intake and gastrointestinal symptoms (i.e. nausea, fullness, epigastralgia, meteorism). Finally, a cross-sectional population-based survey was conducted to assess demographic, socioeconomic, and sensory preference characteristics related to the consumption of cucumber, Scopatizzo, and Barattiere. Cucumber showed the highest reports of mild digestive discomfort (16.3%) and complete maldigestion (11.5%), whereas Barattiere and Scopatizzo were better tolerated. In the clinical study, Scopatizzo and Barattiere scored higher in rewarding food control compared to cucumber. No significant differences were observed for homeostatic control (i.e. appetite and satiety). All fruits produced similar agreeability, mild gastric emptying (max antral area ~7 cm²), and mild gallbladder emptying (ejection volume ~ 20%), without gastrointestinal symptoms and microbial fermentation. In conclusion, the promotion of Scopatizzo and Barattiere could provide consumers with highly palatable and digestively favorable alternatives to common cucumbers.

Keywords: Agrobiodiversity, Cucumis, Digestibility, Local varieties, Puglia region

Session: The challenges of crop biodiversity in 21st Century

Fermentation of carob juice for the production of innovative low-alcohol beverages with high health benefits

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Fermentation of fruit juices from minor species, such as carob (*Ceratonia siliqua*), is an attractive proposition for the development of innovative products. This fruit has a unique composition that includes high levels of bioactive compounds, sugars and polyphenols. This study describes a biotechnological approach to enhance this species, with great potential for food innovation in the fermented beverage market.

Carob juice was fermented with the two starter strains of wine-derived *Saccharomyces cerevisiae*. At the end of the fermentation process in the obtained juice, the following were quantified: 1) the content of total polyphenols, 2) the antioxidant capacity, 3) the content of sugars and organic acids, and 4) the presence of volatile compounds. The juice was also analyzed with untargeted analytical technologies using an 80 MHz NMR and an E-nose GC-FID for liquid and VOCs components, respectively.

The results obtained indicate that the fermentation process enhances the functional properties of carob juice particularly in total polyphenol content and antioxidant capacity. Chemical analysis showed specific metabolic differences between the two strains, which may influence the organoleptic profile of fermented carob juice.

The high concentrations of esters, alcohols, acids and phenols in the fermented juices revealed the complex aroma and sensory profile of the final product suitable for specific food applications.

Fermentation of carob juice has been shown as an optimal approach to develop functional low-alcohol beverages enriched with prebiotic and antioxidant compounds. This bio-technological approach might lead to the enhancement of local biodiversity to create typical regional products using sustainable technologies to enhance these minor species and expand offerings in the fermented beverage market.

Keywords: *Ceratonia siliqua*, Fermented beverage, Local biodiversity

Session: The challenges of crop biodiversity in 21st Century

Future of crop biodiversity: the case of the germplasm bank preserved by CREA-CI in Bologna and Rovigo (Italy)

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The exploitation of industrial crops at the Cereal and Industrial Crop Centre of Bologna and Rovigo dates to the beginning of the last century and has seen the development of a germplasm bank consisting of 2237 accessions. This collection reflects the multidisciplinary approach of geneticists, agronomists, biologists and chemists to the study of wild relatives and landraces collected in Italy or Europe, breeding lines and populations, as well as ancient or modern varieties, many of which were developed by our breeders.

Main crops are grain legumes (different species with 1250 accessions), sugar beet (*Beta vulgaris* L., 381 accessions), flax (*Linum usitatissimum* L., 283 accessions), sunflower (*Helianthus annuus* L., 95 accessions), hemp (*Cannabis sativa* L., 90 accessions), several species of the Brassicales order (75 accessions), potato (*Solanum tuberosum* L., 45 accessions) and castor bean (*Ricinus communis* L., 18 accessions).

Each of these crops has been studied with different approaches: genetic and genomic studies enabled the explanation of qualitative and quantitative traits as well as structure and diversity; chemical analyses allowed the characterization of primary and secondary metabolites important for the pharmaceutical, food and chemical industries.

With species-specific insights, we clarify how an *ex-situ* germplasm bank, characterised by physiological, agronomic, chemical, genetic data is the *conditio sine qua non* to face the future challenges of a modern agriculture. Able, therefore, to propose effective solutions for: 1. Fighting genetic erosion and biodiversity loss concerns (i.e. over-reliance on a restricted number of commercial varieties, climate change and habitat destruction endangers the genetic diversity of wild species, ecotypes and landraces, which is essential for breeding resilient crops); 2. Ensuring a concrete and active resource to address the challenges of quality, health and environmental sustainability in terms of global food security.

Keywords: Germplasm, ex-situ conservation, Industrial crops, Breeding, Diversity loss

Session: The challenges of crop biodiversity in 21st Century

Genetic and metabolic diversity of Broccoli-Rabe Landraces (*'Brassica rapa'* L. subsp. *'sylvestris'*) with a focus on glucosinolate pathways

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'Brassica rapa' L. is a diploid (2n=20, genomic formula = AA, haploid genome size ca. 425 Mb) and mostly allogamous species including broccoli-raab (*'B. rapa'* subsp. *'sylvestris'*), known in Italy as "cima di rapa" or "friariello". Due to limited investments, no broccoli-raab official cultivars are registered in Italy or the EU. Broccoli-raab breeding and seed propagation are traditionally managed by farmers, who selected improved populations often classified by cultivation cycle length (short, medium, long, e.g. 40-90-120 days). A CNR-UNIBA collaboration is developing the first broccoli-raab reference genome together with morphological, genomic and metabolic characterization of several broccoli-raab populations. We investigated the genetic diversity of 48 populations (41 of Apulian origin, conserved at the CNR Mediterranean Germplasm Genebank, and 7 from seed industries) by whole genome resequencing of DNA pools (each consisting of 5 plants) and read mapping against the *'B. rapa'* cv *'Chiifu'* genome (field mustard), while awaiting the specific broccoli-raab genome. Overall, polymorphism distribution varied along chromosomes, with A2 and A6 showing regions of highest variation. The number of SNPs ranged from 1,048,015 to 1,380,244, with the lowest in population '3202' (Putignano, BA) and the highest in '113' (Monopoli, BA). Remarkably, Principal Component Analysis and structural genetic diversity surveys separated populations from the province of Lecce from those of other Apulian provinces, which clustered together. Finally, an in-depth analysis of the glucosinolate pathway genes identified polymorphisms potentially relevant in GWAS analyses targeting various flavour traits. NMR profiling of populations grown in Apulia allowed quantification of over 40 metabolites in florets associated with pungency, sweetness and acidity.

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Keywords: Broccoli-raab population diversity, Fingerprinting by whole genome resequencing, Glucosinolates, NMR metabolic profiling

Session: The challenges of crop biodiversity in 21st Century

Genetic diversity and phylogenetic relationships of ancient and monumental olive trees of Crete Island: implications for sustainability and breeding purposes

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Olive (*Olea europaea* L.) possesses a rich genetic heritage, with its diversity closely linked to its geographical distribution. Monumental olive trees hold significant cultural and ecological value and serve as a vital genetic reservoir for future advancements in oliviculture. An in-depth exploration of the island of Crete led to the collection of over 300 samples from olive trees located in provinces of Chania, Rethymno, Heraklion and Lasithi. Sample included 57 monumental, 35 ancient and 47 feral plants. To distinguish between grafted and non-grafted individuals, leaves were collected from both the canopies and rootstocks. Genetic relationships were investigated using 17 well-known Greek cultivars from ELGO-DIMITRA collection, along with 400 olive cultivars from 20 Countries worldwide.

SSR and cp-SSR analysis of all samples enabled the identification of 156 genotypes most of which were traceable to the olive cultivars Throumbolia, Koroneiki and Mastoidis. The application of plastid markers allowed the identification of six distinct lineages among Cretan genotypes, with the majority belonging to the E1.1 chlorotype. Ten monumental and uncultivated olive trees showed the E1.N4 chlorotype, a lineage recently discovered in the Cretan germplasm. The phylogenetic study, which included 400 olive cultivars from across the Mediterranean Basin, revealed that a large group of rootstocks from monumental olive trees formed a distinct cluster, separate from the rest of the germplasm.

Our research highlights that the uniqueness of the Cretan olive germplasm is primarily associated with its monumental trees. Given the high risk of extinction due to natural and human factors, the conservation and propagation of this germplasm are essential. This study offers tangible outcomes and directly contributes to conservation strategies for the Cretan germplasm, both ecologically and productively, supporting the future of regional olive cultivation and potential genetic improvement efforts.

Keywords: Ancient olive trees, Breeding for Climate Changes, Genetic biodiversity

Session: The challenges of crop biodiversity in 21st Century

Integrative genomic approach unravels genotypic diversity in the potato-leaf phenotype of Italian tomato landraces

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Italy is a secondary diversity centre for tomato (*Solanum lycopersicum* L.). The country hosts cultivation areas characterized by diverse environmental conditions and likewise different use and storage practices. These factors, linked with the selection activity, have led to the formation of an important genetic reservoir of tomato landraces, carrying peculiar plant and fruit traits. This study aimed to investigate the genetic origin of the potato leaf (PL) phenotype found in four Italian landraces: "Spagnoletta" (SPA) from Lazio, "Giagiù" (GIA) and "Patanara" (PTN) from Campania and "Pomodoro di Mola" (MOL) from Apulia. First, an allelism test was carried out by crossing the PL genotypes to assess the involvement of the Potato leaf (C) gene in the phenotypic expression. Then, both SCAR, dCAPS marker and Sanger sequencing analyses on C gene were performed. Two American PL accessions, (LA2374 and PC711571) carrying the traditional rider mutant allele of the C gene on chr6, as well as a Red Setter PL mutant line (affected in the Aux/IAA9 gene), were used as controls for the PL trait. The cv Nagcarlang (NAG) was used as the wild-type leaf control. The allelism test revealed that a mutation in the C gene is responsible for the PL phenotype in the four Italian landraces. SCAR marker analysis permitted to distinguish the Italian tomato landraces from the two American C gene rider mutant lines. On sequencing data publicly available, a private SNP mutation was found in SPA. dCAPS marker analysis confirmed the identity of the SNP in SPA. Sanger sequencing of C revealed the presence of two further mutations not previously reported in tomato in this gene within Italian landraces. These results highlight the potential utility of the C alleles in agri-food traceability and genetic distinctiveness. Such efforts could further enhance our understanding of biodiversity while strengthening connections between genetic traits and their land of origin.

Keywords: Tomato, Italian agrobiodiversity, Qualitative traits, Molecular markers, Landraces

Session: The challenges of crop biodiversity in 21st Century

SAVEGRAIN-CER a project for the collection, characterization and safeguard of agricultural diversity of cereal species in the Apulia region

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Germplasm conservation is crucial for preserving knowledge of plant species. Human intervention, favoring some genes and eliminating others, has eroded genetic diversity.

Cereal landraces, traditionally selected by local farmers over time and well adapted to the climatic conditions of the Apulia region (southern Italy), are currently threatened by genetic erosion/extinction, because of the gradual replacement with modern, high-yielding cultivars.

To address this issue, within the framework of the Rural Development Programme of Apulia 2014-2020, Measure 10.2.1, the Apulia Region is funding the project titled "Biodiversità dei cereali antichi pugliesi per la sostenibilità e la qualità (SAVEGRAIN-CER)". The project, coordinated by IBBR-CNR, is being carried out in collaboration with public research institutions, universities, and private companies operating in the Apulia Region.

The main objectives of SAVEGRAIN-CER are: i) the collection of historical data; ii) the classification and conservation of Apulian cereal landraces; iii) their morphological, genetic and bio-agronomic characterization; iv) the assessment of qualitative traits.

Launched in 2023, the initiative has already recovered over 100 accessions of cereals, including bread wheat, durum wheat, barley, oats, and hulled wheat. These materials originate from diverse Apulian agroecosystems, including biodiversity hotspots and traditional farming landscapes. Collected germplasm is duplicated for ex-situ conservation at the IBBR-CNR Mediterranean Germplasm Bank (<https://www.ibbr.cnr.it/mgd/>) and maintained on-farm by custodian farmers to ensure dynamic in-situ preservation.

To date, over 20 landraces have been proposed for their inclusion in the Apulia Regional Register of Plant Genetic Resources.

Preliminary findings underscore the agroecological resilience and cultural value of Apulian landraces, positioning them as cornerstones for sustainable agriculture and regional identity.

Keywords: Agricultural diversity, Plant genetic resources, Landraces, ex-situ and on farm conservation, Cereals

Session: The challenges of crop biodiversity in 21st Century

The BiodiverSO project as a model for preserving the biodiversity of vegetable crops in Puglia (Southern Italy)

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The Puglia region, rich in agro-biodiversity, demonstrates how local vegetable varieties integrate with modern horticulture. However, its crop genetic diversity has declined due to rural abandonment, an aging farming population, and lost generational knowledge. This contribution summarizes the objectives, methodologies, and outcomes of the “Biodiversity of Puglia 's Vegetable Crops (BiodiverSO)” projects, funded by the Puglia Regional Administration under the Rural Development Program, aim to recover, characterize, conserve, and enhance local vegetable varieties.

In the first project edition (2013-2018), eight work packages were implemented. Among key achievements we highlight recognition and promotion of “Carota di Polignano” (*Daucus carota* L.), a local carrot variety with yellow, purple, and orange roots, and sanitization of eight globe artichoke varieties from some viruses (AILV, ArLV, TICV) via meristem-tip culture and in vitro thermotherapy.

In the second edition (2022-2025), activities expanded to ten work packages with two extra actions for dissemination. Notable progress was made for two landraces of unripe melons (*Cucumis melo* L.) used like cucumbers: “Scopatizzo” yielded 5.3 kg/plant (26.2 fruits) in hydroponics within 71 days; “Barattiere” showed resistance to Tomato leaf curl New Delhi virus making it a promising rootstock for susceptible cucurbits.

For both project editions, a computerized database “the BiodiverSO Management System (BMS)” was developed to ensure accurate and efficient management and processing of project data.

The results underscore that while some local varieties were replaced by modern ones before reaching their full potential, their genetic value remains critical for future crop improvement programs.

In conclusion, the BiodiverSO project serves as a model initiative for the protection and recovery of vegetable varieties at risk of genetic erosion, facilitating the identification and valorization of Puglia's diverse plant germplasm.

Keywords: Genetic erosion, Local varieties, Protection, Recovery, Valorization

Session: The challenges of crop biodiversity in 21st Century

The relationship between biodiversity and a cooperative of floriculture producers: a pilot case study in Italy

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The European Union is the world 's largest floriculture market, with a production valued at around 9.4 billion and over 33,000 companies operating on 55,600 hectares. This economic sector has a relationship with environment and biodiversity characterized by inner contradictions, since it generates both a positive and a negative impact on it. We selected and wrote a case study investigating the characteristics and implications of the relationship between biodiversity and business, focusing on “Flora Toscana”, an Italian cooperative of floriculture producers.

The initiative was carried out within the framework of the European National Biodiversity Future Center, task 7.5.5. Interviews and site visits were conducted in order to write the case study.

The negative and positive impacts of Flora Toscana on environment and biodiversity, together with the dependence relationship of the cooperative on biodiversity, were analysed and described, outlining their indirect and direct effects. Based on the data provided by the cooperative and the conducted interviews, the path taken over time by the cooperative members towards biodiversity management was described, as well as the results of the initiatives taken so far to reduce the environmental impact of the production activities.

The study showed that Flora Toscana consistently draws on the extensive repertoire of flowering plants provided by biodiversity to meet the pressing demand of new plant varieties characteristic of the floriculture sector. Nevertheless, the company has shown to understand the value of biodiversity through the environmental certifications achieved, as well as the growing number of initiatives undertaken to reduce the environmental impact and to guarantee a sustainable use of biodiversity.

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Keywords: Ornamental plants, Business, environmental impact, Agro-biodiversity, Green economy

Session: The challenges of crop biodiversity in 21st Century

Unveiling intra-varietal genetic diversity in olive cultivars through whole genome resequencing and assembly

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The Italian olive germplasm represents the largest basin of species variability, with hundreds of varieties preserved in traditional olive groves and in thousands-year-old trees. Despite extensive research on inter-varietal diversity, intra-varietal variation remains poorly explored, as olive cultivars are commonly assumed to be genetically uniform due to vegetative propagation. However, standard markers, such as simple sequence repeats (SSR), are unable to detect subtle intra-varietal differences, as they cover limited genomic regions. This issue also affects other clonally propagated crops such as grapevine.

To address this, two Italian olive cultivars were selected as models: “Frantoio”, widely spread under various synonyms across Italian regions, and “Bosana”, predominant in Sardinia and also present elsewhere under different names.

In order to detect somatic mutations, the genomes of 17 clones (9 from cv. Bosana and 8 from cv. Frantoio) were sequenced using Illumina 150PE technology at ~100X depth and aligned to the cv. Arbequina reference genome. Somatic mutations were identified using FreeBayes to infer genetic relationships among clones of each group. In the meantime, high-quality de novo genome assemblies of both cultivars are under generation using PacBio HiFi long reads and HiC-based scaffolding, in order to capture full genomic variability.

A first overview of the genome somatic differences among clones of the two cultivars has been obtained.

These findings provide insight into the origin and differentiation of clones within each cultivar, offering a basis for understanding intra-varietal diversity in clonally propagated trees.

This study highlights the genetic distinctiveness of territorial clones within the same cultivar, shedding light on their local adaptation and evolutionary trajectories. Such insights may support the valorization and conservation of region-specific olive germplasm.

Keywords: Biodiversity, Olive, Clonal diversity, de novo assembly, Somatic mutations

Session: Innovation in vine and wine microbiology

Biodiversity of culturable yeast population isolated for two consecutive years from hypogean wine cellars located in Southern Italy

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The hypogean wine cellars can be considered production and aging sites of traditional wines. These ecosystems could also be the keepers of yeasts responsible for transformation of grape must into wine, which in the past were certainly entrusted to the naturally present yeasts and not to the use of commercial starters. The yeasts resident in these habitats can be defined “ancient yeasts”, selected over the time in each traditional cellar; this population might be a source of indigenous yeasts, poorly explored until now. In this study, the cultivable yeast population sampled over two consecutive years from hypogean cellars located in Southern Italy, mainly in Basilicata region, was studied. The yeasts were identified by restriction analysis of ITS region, followed by sequencing of D1/D2 domain of 26S rDNA. The genetic diversity among *Saccharomyces cerevisiae* strains were evaluated by amplification of inter-delta region, while all wine yeasts were submitted to a technological screening, such as the resistance to antimicrobial compounds present during vinification (i.e. sulphur dioxide, ethanol, copper sulphate), production level of hydrogen sulphide. The cultivable yeast population isolated from this habitat is characterized by different species, which also varied depending on the analyzed site. The majority of yeasts was composed by wine-associated species, such as *S. cerevisiae*, *Hanseniaspora* spp., *Wickerhamomyces anomalus*, *Pichia* spp., *Candida* spp. High genetic polymorphism among *S. cerevisiae* isolates and high variability for technological traits was found. The preliminary results obtained confirmed the suitability of this approach to capture and preserve the microbial biodiversity associated to these ancient sites.

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Keywords: Traditional wine cellars, Wine yeasts, New starters

Session: Innovation in vine and wine microbiology

Investigating the microbiome of a Barbera vineyard by a shotgun metagenomic approach

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Vineyard microbiome is deeply interconnected with the plant and serves as a reservoir of genes and functions, to the extent that it is considered part of the holobiont. In this study, the dynamics of fungal and bacterial communities were characterized in a conventionally managed *Vitis vinifera* cv. Barbera vineyard using shotgun sequencing. Samples of rhizosphere soil, leaves, and grapes were collected at different time points, from fruits development to harvest. A clear distinction between the phyllosphere and rhizosphere microbiota was observed for both bacterial and fungal communities. The specificity of certain species in different compartments was confirmed, as well as the presence of a core microbiome. Regarding the bacterial community, *Stenotrophomonas maltophilia* was the only core species, while LEfSe analysis revealed an enrichment of genera known for their plant growth-promoting (PGP) properties or their roles in carbon and nitrogen fixation. The fungal core consisted of 21 species with both beneficial and detrimental effects in the vineyard. Additionally, an enrichment of saprobic fungi rich in carbohydrate- and biomass-degrading enzymes was observed across all compartments. Functional analysis revealed that the Barbera vineyard microbiome was primarily involved in carbohydrate and amino acid metabolism. KEGG pathway analysis highlighted the enrichment of genes related to valine, leucine, and isoleucine biosynthesis in all compartments. The rhizosphere microbiome exhibited a wide array of enriched pathways associated with organic matter decomposition, carbon, lipid, and nitrogen recycling, as well as energy metabolism. In fungi, an enrichment of pathways involved in xenobiotic degradation was also observed. This study expands our knowledge of the Barbera vineyard microbiome. These findings may be useful for designing a tailored microbiome to enhance plant health and promote productivity and wine quality.

Keywords: Barbera vineyard metagenome, Rhizosphere and phyllosphere microbiota, Functional genes, Metabolic pathway

Session: Innovation in vine and wine microbiology

Low-Temperature active dry yeast rehydration: nutrients for enhanced fermentation and membrane fluidity

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Active dry yeasts (ADY) are vital in winemaking due to their durability, but successful rehydration-relying on optimal trehalose levels, time, temperature, pH, nutrients, and ergosterol-is essential for restoring function. Low rehydration temperatures, however, reduce membrane fluidity and may impair yeast performance. This study evaluated the effects of suboptimal rehydration temperatures on selected yeast strains of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*, preserved at the Unimore Microbial Culture Collection (UMCC), and assessed the efficacy of two rehydration nutrients-FERMOPLUS® Energy Glu 3.0 and FERMOPLUS® Energy Glu 4.0 (AEB S.p.A)-focusing on improving fermentation performance and stabilizing membrane fluidity. All tested strains, except for *T. delbrueckii*, exhibited enhanced initial fermentation performance upon treatment with both nutrients. Analyses of membrane fluidity revealed that yeast cells rehydrated at 20°C displayed reduced fluidity compared to those cultivated at 25°C for 24 hours, confirming a temperature-induced stiffening effect. Notably, cells treated with FERMOPLUS® Energy Glu 4.0 demonstrated less variation in membrane fluidity, suggesting a more effective stabilization of membrane properties under suboptimal conditions. Overall, this work provides valuable insights into optimizing ADY rehydration at low temperatures-conditions employed in the wine industry to save time and costs-and offers practical strategies to enhance fermentation performance, ultimately improving wine quality.

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Keywords: Active dry yeast, Rehydration, Yeast strains, Membrane fluidity, Fermentation performance

Session: Innovation in vine and wine microbiology

Microbial biodiversity as a source of new yeast strains for winemaking

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In the past 25 years, IRVO has studied microbial biodiversity in grapes and musts from various areas of Sicily and selected 7 yeast strains belonging to 4 genera, now on the market for the benefit of wineries.

Grape juice or spontaneously fermenting musts were plated, single colonies were isolated, their genera were determined by microbiological (colony morphology on WL Nutrient agar) and then by molecular methods (PCR-RFLP of the ITS region; amplification and sequencing of the D1/D2 region of the 26S rDNA). Laboratory tests were performed to determine fermentative vigor without and with sulfites and the production of ethanol, glycerol, volatile acidity, β -glucosidase, and H₂S. Strains with the best characteristics were used as starters in vinifications of 100 L, and the wines were analyzed for chemical-physical parameters and VOC (by GC-MS) and by sensorial analyses. Best strains were finally used in industrial vinifications.

From late spontaneous fermentations made in southeastern Sicily, 918 *Saccharomyces* colonies were isolated, and two strains were selected for red wines, one for white wines, and one for bottle fermentations. From grape musts collected in western Sicily through the analysis of over 2,500 yeast colonies, one *Starmerella bacillaris* (syn. *Candida zemplinina*) strain, in sequential fermentation with *S. cerevisiae*, was able to produce red wines with higher glycerol concentrations. One *Lachancea thermotolerans* strain produced lactic acid and wines with higher total acidity. From grapes harvested on the small island of Linosa, south of Sicily, through the analysis of over 3,800 yeast colonies, one *Kluyveromyces marxianus* strain was identified for its ability to produce beta-glucosidase activity, as well as for the production of greater quantities of esters.

Yeast populations present in Sicily have proven to be an excellent source of strains usable in the wine industry to improve the quality of wines.

Keywords: Wine, Fermentation, Yeast, *Saccharomyces*, non-*Saccharomyces*

Session: Parallel session: Fungal diversity and bioprospecting

Characterization of yeast strains isolated from agricultural soils for their potential in biodegradation of glyphosate

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Glyphosate (GP) is the most used herbicide in agriculture. Its prolonged use has led to the frequent occurrence of GP and its main metabolite, AMPA, in the environment, posing risks to ecosystems and human health. Yeasts, due to their metabolic plasticity, represent promising candidates for soil bioremediation. This study aimed to isolate and characterize yeasts able to degrade GP and AMPA using them as nutritional source. Soil samples were collected from a wheat field with a history of GP use in Marsciano (PG), and isolation in enrichment condition with GP and AMPA as sole P source was carried out. Yeast strains were identified through molecular approach and will be deposited in the Culture Collection DBVPG of the University of Perugia. Out of 25 isolates, 17 were identified as the black yeast *Exophiala alcalophila* Goto & Sugiy (Herpotrichiellaceae). Screening in 96-wells plates were performed for all the strains in P-free mineral medium with GP or AMPA at 2.5, 5 and 10mM and growth was monitored by measuring OD600 twice daily for 7 days. Preliminary results showed different growth patterns among treatments. Most of *E. alcalophila* strains showed increased growth values at the highest AMPA concentration compared to the negative control (C-), which suggests their ability to use AMPA as P source. Moreover, since the growth value at 10 mM exceeded twice that of the positive control (C+), this may even stimulate yeast growth. However, the presence of GP reduced the performance of these strains. Instead, other strains not yet identified showed higher growth values with GP compared to C-, and reduced growth with AMPA. In conclusion, our results suggest that yeasts may degrade GP and AMPA, highlighting their potential for the bioremediation of GP- and AMPA-contaminated agricultural soils.

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Keywords: Glyphosate, Bioresources, Bioremediation, Yeasts, Agricultural soils

Session: Parallel session: Fungal diversity and bioprospecting

Exploring the cultivable mycobiota associated with Mediterranean *Cymodocea nodosa* for ecological and biotechnological applications

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Marine fungi occupy a broad spectrum of ecological niches and are widespread across diverse marine habitats. Analogously to their terrestrial counterparts, marine taxa associate with both abiotic substrates such as sediments and the water column and biotic hosts across all trophic levels, functioning context-dependently as parasites, pathogens, saprotrophs, or mutualistic symbionts. Despite reports of their presence are increasing, comprehensive understanding of their abundance, diversity, and functional roles in marine ecosystems remains limited, raising questions about their ecological significance and potential as reservoirs of biomolecules with biotechnological applications.

To address these questions and gain deeper insights into their abundance, diversity, and ecological functions, we isolated and identified the cultivable mycobiota associated with *Cymodocea nodosa*, a seagrass species that plays a crucial role as an ecosystem engineer in upper sublittoral areas, littoral zones and coastal lagoons. Samples were collected from two meadows along the southern Spanish Mediterranean coast: one located in a littoral area and the other in a coastal lagoon, each with distinct mean annual salinities (37.5 psu and 43.5 psu, respectively) and characterized by a different degree of anthropogenic disturbance. The epi- and endophytic communities isolated from both roots and leaves were then compared. In total, more than 60 taxa (mainly Ascomycota) were identified. Notably, different species from strictly marine genera, such as *Paralulworthia* and *Cumulospora*, were the only endophytes isolated exclusively associated with the roots of both meadows. The results provide a framework for understanding the role of seagrasses-associated marine fungi that could be a source of novel molecules of biotechnological interest.

Keywords: Marine fungi, Seagrasses, Ecological applications, Biotechnological potential

Session: Parallel session: Fungal diversity and bioprospecting

Fungi in Multi-Taxon analysis: updates from the PRIN MultiForDiv Project

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Temperate forests in Europe provide essential ecosystem services, such as carbon storage, water regulation, and habitat provision. However, they face significant threats from climate change and human disturbances. Biodiversity conservation is a priority of national and international policies, yet many aspects of forest diversity, particularly understorey plants, and fungi (including lichens) remain poorly understood. This project investigates the patterns and drivers of multi-taxon forest diversity in Italian forests using sites from the International Co-operative Programme on Forests LII network. The study focuses on soil fungal diversity, key players in nutrient cycling and forest resilience. In particular, we studied the vital soil microfungal component. Soil samples from ten Italian plots were analyzed using dilution plating to quantify and identify fungal strains. Functional traits such as xerotolerance and thermotolerance were assessed to explore their ecological roles and responses to environmental variables. Five plots were analyzed, revealing 1289 colony-forming units (CFUs) and 140 morphotypes. Molecular analysis refined genus identification. The most represented genera were *Penicillium* (55%), *Trichoderma* (9%), and *Aspergillus* (6%). Significant differences in fungal distribution among plots suggest the influence of soil type, forest composition, and organic matter. For instance, the TOS2 plot (Tuscany), dominated by holm oaks and sandy colluvial soil, showed higher fungal presence (248 CFUs) than BOL1 (Bolzano), dominated by Norway spruce and podzolic soil on drift (168 CFUs). The dominant genus also varied: *Penicillium* in TOS2 and *Umbelopsis* in BOL1. Culture-based tests on *Penicillium* and *Aspergillus* strains were conducted to characterize their functional traits. These findings will provide insights into their contributions to organic matter decomposition, symbiosis, and vegetation interactions, enhancing our understanding of forest ecosystem dynamics.

Keywords: Microfungi, Soil, Fungal biodiversity, Global change

Session: Parallel session: Fungal diversity and bioprospecting

Genetic biodiversity of summer truffle (*Tuber aestivum* Vitt.) from ten regions of Italy

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Truffles are hypogeous ascomycetes belonging to the order Pezizales. They form symbiotic relationships with the roots of various plants, contributing to plant health. Even though some truffles are highly appreciated for their aroma and flavour, the yields of natural truffle grounds are decreasing, and their life cycle is not completely understood.

Tuber aestivum Vitt. is widespread in Italy and interest in this species is growing because climate change is threatening its natural truffle grounds with a severe risk of biodiversity loss. Studying the DNA of *T. aestivum* is pivotal for understanding summer truffle biodiversity, deepening our knowledge of its life cycle and characterising the genetic variability of wild populations locally adapted to different environmental conditions. Genome sequencing can also help to understand the plant-truffle symbiosis, the formation of the fruiting body and the aroma formation, thus providing data that could help to preserve the biodiversity of summer truffle. Moreover, shotgun sequencing can be used to enhance insight into the role of bacterial biodiversity in the development of the distinctive flavour of *T. aestivum* by identifying the bacterial species associated with the fruiting body of summer truffles and the bacterial genes involved in the development of truffle aromatic and sensory characteristics.

One hundred DNA samples were extracted from the gleba of summer truffles coming from ten Italian regions (Apulia, Basilicata, Calabria, Campania, Emilia Romagna, Lazio, Marche, Piedmont, Tuscany, and Umbria). Shotgun sequencing is being employed to analyse the genetic diversity of truffles and the biodiversity of the bacteria associated to the gleba. By analysing and comparing a heterogeneous population of truffles we aim to highlight the implications of our findings for truffles biodiversity and spreading.

Keywords: Truffle, *Tuber aestivum* Vitt., Biodiversity, Whole genome sequencing, Aroma

Session: Parallel session: Fungal diversity and bioprospecting

Genome-based exploration of *Mrakia*: sequencing all type strains to unlock fermentative traits of this promising non-conventional yeast (NCY) genus

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In recent years, the fast progress of next-generation sequencing (NGS) and integrated omics has transformed the study of microbial diversity. This has sparked new interest in non-conventional yeasts (NCYs) and sped up the development of their genetic and functional tools for biotechnological use. Among these, the genus *Mrakia* - comprising 20 psychrophilic and psychrotolerant species in the Basidiomycota phylum and typically isolated from cold and extreme environments - has gained attention for its unexplored biotechnological potential. Some strains have shown promising fermentative abilities, and *Mrakia gelida* has been successfully used in the production of a low-alcohol beer prototype, supporting the relevance of this genus in the development of fermented products.

In this study, we generated high-quality genome and transcriptome data for 20 *Mrakia* type-strains, preserved in different international Culture Collections. We used a hybrid sequencing method with Oxford Nanopore long reads and Illumina short reads to get high-contiguity genome assemblies. Average nucleotide identity (ANI) was used to perform a phylogenomic analysis and explore genome-based relationships among the 20 *Mrakia* type strains. The ANI analysis identified two clusters: *M. gelida* is closely related to *M. robertii* and *M. sp.1* (ANI 86%), forming a subcluster with *M. psychrophila*, *M. blollopis*, and *M. frigida* (ANI 82%). This group is less related to *M. stelviica* and *M. montana* (ANI 87%).

Gene prediction and functional annotation were carried out with the support of transcriptomic data, allowing the identification of confidently supported coding sequences. We focused on genes linked to alcoholic fermentation, aiming to understand the genetic base of fermentative traits in *Mrakia*.

The genomic framework developed here offers the most complete resource currently available for this genus and provides a basis for future research into the biotechnological application of NCYs in sustainable food systems.

Keywords: non-conventional yeast, Genomics, Food biotechnology, Bioprospecting

Session: Parallel session: Fungal diversity and bioprospecting

Microbiome of *Zelkova sicula*, a critically endangered relict tree of Sicily, Italy

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Zelkova sicula is a bioclimatic relict and a critically endangered tree, incapable of sexual reproduction: today only two small populations are known in the Iblei Mountains (Siracusa, Italy), Pisano (ZS1) and Ciranna (ZS2). We investigated the microbial interactions of *Z. sicula* to characterize its microbiome and to identify key beneficial symbionts to improve the conservation strategies in progress. We investigated bacterial and fungal endophytes and focused on arbuscular mycorrhizal fungal (AMF) symbionts as they are recognized as beneficial for most terrestrial plants and helpful in reproduction processes.

Fine roots, bulk and rhizosphere soil were sampled from the two populations ZS1 and ZS2. Physical-chemical analysis and metabarcoding sequencing of Bacteria, Fungi and Microarthropods communities were conducted on bulk soil; AMF spores were counted after wet-sieving extraction from rhizosphere soils and molecularly identified. Root samples were stained in trypan blue to assess endomycorrhizal status and DNA was extracted from roots for metabarcoding analysis of endophytes.

ZS2 site revealed higher soil pH, lower organic carbon content and higher spore density in respect to ZS1. Bulk soil Bacteria communities differed between the two sites. Higher AMF colonization rate and higher AMF biodiversity were detected in rhizosphere soil and individuals of ZS2; this result was confirmed by the greater number of AMF's OTUs detected by metabarcoding. Among AMF we identified five genera and two species: *Funneliformis geosporum* in the root of *Z. sicula* and *Claroideoglomus etunicatum* as the most abundant in soil of both populations.

We report for the first time AMF symbiosis in *Z. sicula*. Considering the importance of AMF for the surviving of this rare and endemic tree, the management of these symbionts could be exploited for conservational purposes, improving both in vivo and in vitro propagation.

Keywords: *Zelkova sicula*, Arbuscular mycorrhizal fungi, Microbiome, Endangered species

Session: Parallel session: New frontiers in the studies of animal biodiversity

Comparative analysis reveals a shared core gut microbiota in livestock with genus-specific modulation by dietary polyphenols

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Despite differences between monogastric and ruminant species, studies confirm the presence of a shared gut core microbiota, which modulates digestion, development, immunity, and host-microbe symbiosis. Genera such as *Prevotella*, *Lactobacillus*, *Faecalibacterium*, *Bifidobacterium*, and *Clostridium* consistently appear across species. Dietary components, such as polyphenols, can shape microbiota profiles. In this study, four experiments were set up to evaluate gut microbiota diversity and modulation in the presence of dietary polyphenols from hazelnut skins (HSP) in chickens, pigs, lambs and cattle. For each species, animals were divided into two groups: a control group fed a conventional diet and a treated group receiving the same diet supplemented with HSP.

Rumen, small intestine and cecum contents were collected and stored at -80 °C. The microbiota was assessed through 16S rRNA gene sequencing, targeting the V3-V4 hypervariable regions, and bioinformatic analysis using the QIIME2 pipeline and custom R scripts.

Comparative analysis revealed 16 bacterial genera common across all studied species. *Prevotella* and *Faecousia* were the most abundant genera across species and gut compartments. This shared core microbial profile provided a reference framework to assess the modulatory effects of HSP. *Prevotella* was markedly influenced by HSP presence, especially in the ruminants' caecum, which was the anatomical district showing the highest degree of microbial variations. The rumen and the monogastric small intestine core microbiota were composed of 44 and 35 genera, respectively. Among them, *Cryptobacteroides* and *Ruminococcus* in the rumen, and *Alloprevotella* and *Rothia* in the small intestine, were the genera most affected by HSP. Certain microbial populations exhibited higher resilience, ensuring stability and functionality despite dietary changes. These resilient microbes are known to play a crucial role in digestion, immune modulation, and overall gut health.

Session: Parallel session: New frontiers in the studies of animal biodiversity

Small basin, big data: eDNA metabarcoding tracks vertebrate diversity and distribution in the Serchio river basin

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Effective biodiversity monitoring is vital for detecting species loss and early incursions of alien species, especially in freshwater systems under increasing pressure. Traditional monitoring methods in flowing water are often invasive, require taxonomic expertise, and involve handling organisms, which can cause mortality. Environmental DNA (eDNA) metabarcoding offers a non-invasive alternative by detecting genetic material that organisms release into their environment.

In this study, we applied eDNA metabarcoding at five sites within the Serchio River basin, an understudied watershed in Tuscany, central Italy. Water samples were filtered, and eDNA was extracted from rivers with varying flow regimes. This method identified 64 taxa, including protected native and invasive alien species, across major vertebrate groups. Species distributions matched ecological expectations.

We compared eDNA results with traditional monitoring techniques, including transect surveys (March 2021- October 2023) and electrofishing. eDNA metabarcoding detected higher overall species richness, though some taxa were uniquely identified by each method. Richness was typically greater in the river 's mainstem than in its tributaries, likely due to eDNA accumulation, suggesting that main channels may be strategic points for efficient biodiversity monitoring.

Overall, eDNA metabarcoding enabled a rapid, non-invasive assessment of vertebrate diversity in the Serchio River basin, capturing both aquatic and terrestrial species. This approach revealed valuable insights into regional gamma diversity and proved more time-efficient than conventional methods.

Keywords: Environmental DNA, River, Monitoring, Vertebrates

Session: Parallel session: New frontiers in the studies of animal biodiversity

The DNA bank of five Italian beef cattle breeds

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A DNA bank is a collection of biological animal samples from which DNA extraction is possible. The advantages of preserving genetic material are several: implementation of selective breeding programs with genomic selection, preservation of genetic diversity, products molecular traceability. Genomic selection is useful for young animals and for sex-limited traits, traits with low heritability or expensive to measure. Since almost 20 years ANABIC (National Association of Italian Beef Cattle Breeders) has set up a DNA bank for the five Italian beef cattle breeds: Marchigiana (M), Chianina (C), Romagnola (R), Maremmana (MM), and Podolica (P). The DNA bank plays an important role in selection and valorization of these breeds, it is used for parentage testing, estimation of inbreeding to protect the biodiversity, molecular traceability of meat products and for genetic and genomic analyses to diagnose genetic diseases. The samples are catalogued and they are stored in different ways depending on conservation needs: hair roots, dried ear cartilage and nasal swabs are stored in boxes at room temperature, blood, meat, or ear cartilage with liquid preservative in a freezer at -20° C, extracted and purified DNA in a freezer at -80°. ANABIC has also a semen bank with around 38,000 doses as a germplasm reserve to use for planned mating aimed to recover particular lines and genotypes, or for scientific research. The total samples collected at the present time are 802,722: 291,818 M, 309,762 C, 76,378 R, 35,352 MM, and 89,412 P. Regarding sample type: 3,562 DNA samples, 18,753 whole blood samples, 8,949 FTA card, 314,772 hair roots, 454,796 ear tissue samples and 1,890 nasal swabs. The continuous maintenance and implementation of this DNA bank constitute a pillar of genetic characterization of these breeds and represent an important biodiversity tool for Italian beef cattle.

Keywords: Italian beef cattle, DNA bank

Session: Biodiversity, invasive species and multitrophic interactions

Pesticides and biodiversity conservation: how low pesticide doses affect foraging behaviour of two Scelionidae and Coccinellidae biocontrol agents

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Biological control is a pest control strategy helping to reduce chemical pesticides and conserving invertebrate biodiversity. In agricultural systems, the susceptibility of natural enemies to pesticide applications may preclude successful control. Besides lethal effects, non-lethal effects due to exposition of the beneficial insects to chemicals are critical and can impair foraging behaviour. Here we present data on the behavioural and cognitive effects of low concentrations of an acetamiprid-based neonicotinoid insecticide on two biocontrol agents: the egg parasitoid *Trissolcus japonicus* (Hymenoptera: Scelionidae) and the predatory ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae). In open arena bioassays, *T. japonicus* females exhibited positive responses to host chemical traces and reduced foraging time following associative learning. However, parasitoids exposed to the insecticide showed altered walking behaviour and prolonged memory retention, despite no immediate effect on learning ability. *Harmonia axyridis* males demonstrated the ability to associate plant-host cues with mating experience, but exposure to sublethal insecticide doses disrupted this behaviour and affected decision-making during foraging. These behavioural impairments, observed in both species, suggest that even low pesticide residues can interfere with foraging efficiency and reproductive performance. Such effects may reduce the establishment and effectiveness of natural enemies, particularly exotic species, such as *T. japonicus* introduced for the control of invasive brown marmorated stink bug, *Halyomorpha halys*. Therefore, sublethal impact of pesticides should be carefully considered in biological control strategies aimed at promoting biodiversity conservation in agricultural systems.

Keywords: *Halyomorpha halys*, *Harmonia axyridis*, Neonicotinoid, *Trissolcus japonicus*

Session: Biodiversity, invasive species and multitrophic interactions

Phenological and germination insights for managing the spread of invasive *Baccharis halimifolia* in the Venetian Lagoon

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The impact of invasive species on biodiversity deserves to be analysed, as they affect agriculture, forestry, and natural ecosystems.

Baccharis halimifolia L. is a dioecious shrub belonging to the Asteraceae family. It is listed among the invasive species targeted for eradication under European Regulation No.1141/2016. This species has now naturalized in six European countries and has been recorded in Italy along the coastal areas of the Veneto region and Tuscany. In recent years, it has rapidly expanded, especially invading conserved native coastal communities.

The main objective of this study is to investigate potential strategies for controlling this highly invasive species in the Venetian Lagoon, a natural environment strongly influenced by temperature variations and by both the quantity and quality of freshwater, brackish, and seawater. Analyses of both the phenology and germination responses of *B. halimifolia* are therefore essential for developing an effective control strategy for this species.

The study was conducted in the WWF Oasis of Valle Averte, a wetland nature reserve located in the Venetian Lagoon. Five different sites were selected within this area, and ten individuals of *B. halimifolia* were identified at each site. The development of phenological phases was monitored through weekly surveys and visual assessments. In addition, the growth and phenological responses of the species to different cutting heights were evaluated to support practical management strategies. This helps clarify how mechanical control methods affect its life cycle, facilitating the identification of the optimal intervention.

Seeds collected from the five sites are being tested in Petri dishes to assess their germination response to constant and fluctuating temperatures and different salinity levels.

Preliminary results suggest that a combination of tactics is necessary to ensure long-term management and to predict its current and potential distribution in the Venetian Lagoon.

Keywords: Invasive species, Phenological phases, Salinity, Germination

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

Biodiversity of fructophilic lactic acid bacteria isolated from raw honey sampled in Abruzzo region, Italy

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Raw honey, collected directly from the comb, may host beneficial microorganisms such as fructophilic lactic acid bacteria (FLAB), which can have a positive impact on bee health.

The aim of the present study was to investigate the biodiversity of FLAB isolated from honey samples collected from two apiaries located in the Abruzzo Apennines, in central Italy, as possible source of new biotechnological properties to enrich VUCC-DBT (Verona University Culture Collection - Dept. Biotechnology) catalogue.

Eight honey samples were collected after 2-3 days or after 10 days in comb, both multifloral or derived from *Sulla coronaria* (L.) B. H. Choi & H. Ohashi.

Six different culture conditions were used, with either fructose or glucose as carbon sources and addition of tetracycline and ampicillin as selective agents. FLAB were present at up to 10⁵ CFU/mL, and isolation was focused on bacilli compatible with FLAB morphology.

Overall, 133 isolates were obtained, which were dereplicated by RAPD-PCR and REP-PCR, and identified by partial 16S rRNA gene and pheS gene sequencing, which revealed they mainly belong to genera *Apilactobacillus*, and *Fructobacillus*.

Genetically unique isolates, as revealed by the combination of RAPD and REP-PCR profiles, deriving from the same honey sample were considered single strains and were further analyzed for antibiotic resistance to ampicillin and tetracycline through MIC assays and PCR detection of antimicrobial resistance genes. Remarkably, some *Apilactobacillus* strains were characterized by phenotypic tetracycline resistance and presence of tet resistance genes.

This study provides the first characterization of FLAB from raw *Sulla coronaria* honey, and further strain characterization will be focused on possible applications in beekeeping as well as in food fermentation.

Keywords: Fructophilic Lactic Acid Bacteria, Raw honey, *Apilactobacillus*, *Fructobacillus*

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

Implementation of a common Quality Management System for the Italian Microbial Resource Research Infrastructure (MIRRI-IT)

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Collaborations between different Microbial Resource Centres (mBRCs) and ethical sourcing practices are mandatory to guarantee biodiversity conservation, successful preservation and fair share of benefits arising from the exploitation of genetic resources (ABS). In this context, the European and Italian Infrastructures, MIRRI-ERIC and MIRRI-IT respectively, inter-operate actively in the design of standard guidelines to certify their biological materials and guarantee viability, purity, and identity according to internal standard operating procedures (SOPs). A common Quality Management System (QMS) is a challenging goal of the ongoing SUS-MIRRI.IT project (n. IR0000005), which aims to establish quality criteria and build a valid national network of mBRCs facing the needs of public and private actors in the fields of research and bioeconomy. Three working groups dealing with (i) Common Collections Procedures, (ii) Compliance with the Nagoya protocol, and (iii) Code of Conduct on Biosafety and Biosecurity, have joined forces to define a general and harmonized management system. The general flow chart of the QMS has been defined with guidelines and common harmonised procedures for accepting microbial cultures, their quality check, and the different protocols for microorganisms' (fungi, bacteria, virus, microalgae etc.) preservation and distribution. Regarding the legal compliance with the Nagoya protocol, starting from available Material Transfer Agreement (MTA) and Material Deposit Agreement (MDA) models, a common template and guidelines for the Italian mBRCs have been developed. Moreover, training and capacity-building activities have been carried out to ensure due diligence regarding ABS within the sensitive processes of mBRCs. Finally, a working group on Biosafety and Biosecurity, with experts on human, animal, and plant diseases, is implementing guidelines for handling microorganisms based on the risks they may pose to humans, animals, plants and the environment.

Keywords: mBRCs, Quality control, Nagoya protocol, Biosafety, Biosecurity

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

Managing and exploiting microbial diversity within the ITEM Culture Collection

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Culture Collections (CCs) and microbial Biological Resource Centres (mBRCs) have a vital role in preserving, characterizing, managing and exploiting microbial diversity. mBRCs are essential not only as ex situ conservatories for biodiversity but also to assure research purposes and promote appropriate exploitation of biological resources in different fields such as food, industry, medicine and environment. Within the Italian scenario of BRCs, ITEM is an internationally recognized and ISO 9001-certified culture collection hosted at ISPA-CNR (Bari, Italy).

ITEM holds more than 14,000 eukaryotic and prokaryotic strains of agricultural and food interest, including resources of interest for topics ranging from plant pathology to fermentations, from the study of microbiomes to food safety, all relevant in supporting the sustainable transition of agro-food production systems. Most strains belong to filamentous fungi with phytopathological and toxicological significance but also with beneficial traits of interest to develop bio-based applications. These fungi are related to the production of an enormous number of metabolites with biological interest. Yeasts and lactic acid bacteria are useful as starter cultures for fermented products, and as probiotic and biocontrol agents.

ITEM strengthened the potential of collection and scientific services to customers and stakeholders due to the support of the EU Project “Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy” (SUS-MIRRI.IT- N. IR0000005), which provided the acquisition of new specialized equipment for high-throughput strains identification and characterization, and the implementation of their long-term preservation. In addition, this resource centre implemented the new public and interoperable database BioloMICS (BioAware, catalogue online item.bio-aware.com), in which biological, molecular, chemical and structural information about microbial strains are available.

Keywords: Biological Resource Centres, Biodiversity, Microbial resources, Exploitation, SUS-MIRRI.IT

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

Microbial sourdough type I biodiversity preservation as label of MBDS-UNISSCC culture collection

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No patents or guidelines exist for spontaneous food-microbiome preservation methods. The SUS-MIRRI project addresses this by developing methodologies to preserve microbial biodiversity and microbiome of sourdough. For this reason, this research aims i) to preserve microbial communities associated to Sardinian sourdough through culture dependent and culture independent approaches; ii) to optimise microbiome preservation methods; iii) to implement the MBDS-UNISSCC collection (University of Sassari) by developing specific databases and methods to preserve microbiome samples. Each sourdough shows unique microbial profiles with specific physicochemical and microbiological parameters including pH, total titratable acidity, and microbial counts. 105 Sourdough samples collected from 64 small villages are preserved in the MBDS-UNISSCC collection at -80°C after backslipping. Descriptive records exist for 78 samples, regarding original preparation methods, provenance, time and temperature of fermentation and bakery production, that is mainly focused on traditional breads and baked goods. The microbial characterisation and optimisation of protocol for sourdough microbiome preservation could be extended to other sourdough type I samples, collected in a special section of MBDS-UNISSCC.

The preservation tests involved the entire matrix (4°C) and the microbiome extracted from the matrix (by extruding the flour) with the addition of glycerol at -80°C. Data analyses of the various storage times (0, 6 months, one year) including culture-dependent, culture-independent, phenotypic and technological methods are currently in progress. A dedicated database section is being developed for sourdough microbiomes within the MBDS-UNISSCC catalogue to comprehensively document this valuable biocultural heritage, ensuring traditional sourdough knowledge and microbial diversity are preserved for future generations.

Keywords: Sourdough, Microbial biodiversity, Microbiome preservation, Database, Microbial culture collection

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

Preserved microbiomes: a resource for biotechnological applications

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Fermented food microbiome refers to microbial communities, namely mixed cultures carrying out the transformation of suitable substrates into fermented products. The study of fermented foods and microbiomes allows to expand the knowledge of the microbial diversity, which inhabit the fermented ecosystems, to collect and characterize the microbial composition and to use the information for assembling controlled microbiomes. Long-term preservation of these communities is crucial. In this study, microbiomes from vinegar and Kombucha tea were preserved using ultra-freezing and freeze-drying for 12 months, and the microbial communities were evaluated via phenotypic and genotypic characterization. Microbiomes preserved by both methods exhibited a reduction of 1-2 logarithmic units in viable counts compared to the initial community; however, after a propagation period, counts recovered to levels comparable to the original samples. Chemical analyses showed that ultra-freezing did not affect the ability of microbiomes to ferment as titratable acidity levels were similar to those prior to preservation in both vinegar and Kombucha tea samples, whereas freeze-drying significantly reduced the capability of microbiomes to metabolize sugars and acidify the substrate. Furthermore, 30 yeast and 66 bacterial isolates (42 Gram-positive and 24 Gram-negative) were characterized. First, focusing on bacterial isolates, they were grouped into different clusters by molecular fingerprinting analysis, and 16S rRNA gene sequencing provided species-level identification. These findings highlight the possibility of preserving the food microbiome, thus safeguarding its biodiversity, deepening its characterization and enhancing its potential in biotechnological applications.

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Keywords: Fermented beverages microbiome, Cryopreservation, Freeze-drying, Microbial communities, Microbial biodiversity

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

Strengthening the UNICA-DSMSP culture collection through the SUS-MIRRI.IT project

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In the last decades, biodiversity preservation has gained growing attention, and many strategies, laws and regulations have been enacted with this purpose. CC and mBRCs are depositories of microorganisms that study and protect biodiversity and make microbial strains and associated knowledge more accessible to the scientific and industrial communities. UNICA-DSMSP is an internationally recognized microbial collection hosted at the Dept. of Medical Sciences and Public Health of the University of Cagliari.

Since the year 2022, UNICA-DSMSP collection has been involved in the project SUS-MIRRI.IT, which aims at improving civil infrastructure and acquiring state-of-the-art laboratory equipment in order to identify and characterize microbial strains, implementing quality management system according to international standards, creating the Italian integrated catalogue of microorganisms, developing new services and training activities aimed at the public and private needs at territory level.

Thanks to the SUS-MIRRI.IT project (granted by NextGenerationEU, National Recovery and Resilience Plan, Code IR0000005), the collection has been able to: acquire cutting-edge laboratory equipment pivotal for characterizing and preserving so far about 600 strains of the collection, mainly represented by LAB and yeasts isolated from various food products; achieve the ISO 9001:2015 certification; implement new services and training activities; set up a disaster plan by storing a copy of the strains in a facility at about 200 km from Cagliari; improve the online accessibility of data through harmonization of the local database with the national one.

Taking part to the SUS-MIRRI.it project has allowed to improve the management and sustainability of UNICA collection, therefore contributing to the preservation of resources to be exploited for both research purposes and biotechnological applications.

Keywords: Culture collection, SUS-MIRRI.IT, Biodiversity preservation

Session: SUS-MIRRI.IT Strengthening the MIRRI Italian research infrastructure for sustainable bioscience and bioeconomy

The SUS-MIRRI.IT project: research, services, and training for sustainable bioscience and bioeconomy by the MIRRI-IT Research Infrastructure

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SUS-MIRRI.IT, funded by the NextGenerationEU programme with a total budget of about 17,000,000, is a project coordinated by the University of Turin and involves 24 Italian Operative Units. The mission is to strengthen the Italian Microbial Resource Research Infrastructure (MIRRI-IT) by the acquisition of cutting-edge equipment, infrastructural improvements, recruitment of high-qualified staff, to valorize microbial resources with associated (meta) data, and to implement top-level services for academia and industry. The project consists of 6 Work Packages (WPs): WP1 drives management, governance, and sustainability; WP2 is involved in microbial Culture Collections consolidation; WP3 focuses on digital activities for the creation of the single-entry point to the National Database of microbial resources and services; WP4 aims to study the biotechnological valorization of microbiomes; WP5 deals with the implementation of offered services and training courses; WP6 is dedicated to communication and dissemination actions. The strategic impact of SUS-MIRRI.IT is to promote partnerships, to encourage aggregation of skills, structures, and bioresources, promoting the transition towards the green economy, and contributing to the sustainability and safety of the environment.

To date, 50 researchers, PhD students, and technicians have been hired; about 80% of the funds for innovative technologies have been used; the online platform with the catalogue of Italian microbial resources holding 37,000 strains of bacteria, filamentous fungi, yeasts, microalgae and viruses has been launched; 29 advanced Training courses have been delivered; 56 new services ranging from microbiological analyses to user-tailored consultancies have been set up; 55 scientific papers have been published. All project updates are communicated by SUS-MIRRI.IT website and social media. An important recently achieved goal is the creation of Italian National Node of the European consortium MIRRI-ERIC, MIRRI-IT.

Keywords: Bioresources, Culture collections, Microbial Resource Research Infrastructure, MIRRI-IT, SUS-MIRRI.IT

Session: Animal biodiversity hotspots and conservation strategies for endangered species

Analysis of the genetic variability among Italian local pig breeds

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Within the SUI2 project, the Italian pig breeders association (ANAS) conducted a study on Italian native pig breeds that are currently managed with genetic conservation programs: Sarda (SR), Casertana (CT), Mora Romagnola (MR), Cinta Senese (CS), Apulo Calabrese (AC), and Nero Siciliano (NS). The aim of the study was to evaluate the genetic variability of these breeds and, in particular, to analyze their pedigree to investigate the genetic contributions of the founders to the current populations. Using PEDIG software, the following parameters were calculated: pedigree completeness, number of equivalent founders that equally contribute to the genetic variability, and effective number of ancestors (the minimum number of ancestors able to explain the whole genetic variability).

The numbers of animals in the pedigree (rate of pedigree completeness) were: 7,718 (93.02%) for SR, 15,535 (98.72%) for CT, 35,583 (99.86%) for MR, 154,535 (99.9%) for CS, 60,758 (98.06%) for AC, and 75,121 (97.19%) for NS. The number of founders ranged from 21 in MR to 1367 in NS; the number of ancestors ranged from 22 in MR to 1391 in NS. When looking at the effective founders and ancestors, the numbers were lower as expected: effective founders ranged from 3 (MR) to 27 (CT), whereas effective ancestors ranged from 3 (MR) to 25 (CT). It is interesting to note that CS had the largest number of animals in the pedigree, but it had only 66 (11 effective) founders and 77 (10 effective) ancestors. The number of ancestors able to explain 50% of the total variability was very low (<10) for all breeds, except NS (49).

Results of the present study demonstrate that the Italian autochthonous breeds derive from a low number of ancestors and, thus, are characterized by low genetic variability and high levels of inbreeding. ANAS activity is focused on the management of the mating schemes to maintain good genetic variability in the populations.

Keywords: Italian native pig breeds, Genetic conservation

Session: Animal biodiversity hotspots and conservation strategies for endangered species

“BERE A SAPERSI”: a journey through knowledge, flavors, and Umbrian biodiversity

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Launched in April 2023, “Bere a Sapersi” promotes Umbrian agri-food biodiversity by blending taste culture, scientific dissemination, and sustainability. Through events in wineries, restaurants, and farms, the initiative explores regional products, highlighting the deep connection between territory, traditions, and innovation. Umbria’s extraordinary biodiversity is reflected in ancient legumes like lenticchia di Castelluccio, traditional cereals (farro di Monteleone di Spoleto), and unique vegetables like sedano nero di Trevi and aglione del Trasimeno. Native livestock breeds, including Sopravissana sheep and Suino Nero Cinghiato, enrich this heritage. Since 2019, the Ape ligustica has been listed in the Regional Register, playing a vital role in pollination and ecosystem balance. In the wine sector, rare native grape varieties such as Tostolello, Dolciame, Grero, Cornetta, Vaiano, and Lacrima have been rediscovered and reintroduced. Completing the regional ecosystem are microorganisms responsible for traditional food transformations, among Italy’s first officially registered microbial genetic resources. During the events, producers and researchers showcase biodiversity’s role in sustainable agriculture and food production. Guided tastings reveal how biological diversity enhances gastronomy, economy, and local development. Since 2023, many participating restaurants have introduced newly discovered native products into their menus, expanding market opportunities and raising awareness of Umbrian excellence. The growing network of producers, chefs, and consumers proves that biodiversity is a resource to protect and promote, not only for its environmental value, but also for its cultural and economic impact. More than an event, “Bere a Sapersi” is an invitation to awareness, where BENE and SAPERE intertwine, giving voice to Umbrian traditions and encouraging responsible consumption, conscious choices, and appreciation of authentic local products.

Keywords: Event, Territory, Traditions, Innovation, Agri-food biodiversity

Session: Animal biodiversity hotspots and conservation strategies for endangered species

Conservation genomics tools for biodiversity monitoring in three Italian freshwater fish species: Italian chub (*Squalius squalus*), Italian barbels (*Barbus* spp.) and vairone (*Telestes muticellus*)

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The genomic variation of three Italian freshwater fish species, i.e. the Italian chub (*Squalius squalus*), the Italian barbels (*Barbus* spp.) and the vairone (*Telestes muticellus*), is currently being investigated as part of the biodiversity monitoring and conservation activities of the National Biodiversity Future Centre NBFC funded by the PNRR (Piano Nazionale di Ripresa e Resilienza).

Freshwater fish, in fact, play an important role as environmental bioindicators in freshwater ecosystems. To ensure long-term survival of these species, a thorough evaluation of their status is fundamental to monitor the levels of molecular variation and identify populations/species with decreasing variability which might impair their adaptive potential. Furthermore, for some of the investigated taxa, e.g. Italian barbels, additional clarifications regarding their phylogenetic relationships and taxonomic status are needed.

To assess the degree of isolation/interconnection between populations and the distribution of genetic variability for the three species across the country, samples were collected from North-western, Central and Southern Italy. The extent of molecular variation will be estimated by both i) direct sequencing of an informative fragment of the mitochondrial cytochrome oxidase I gene (COXI) and ii) scoring nuclear DNA ddRAD (double digest restriction associated DNA) markers. So far, the sampling encompassed 394 Italian chub samples from 39 populations, 228 Italian barbels from 14 populations and, 222 vairone samples from 14 populations. The data production is in progress for both mitochondrial and nuclear markers. The genetic data will be analysed with multiple approaches to provide useful information consistent with the goals of the NBFC, i.e. the monitoring, conservation, restoration, and valorisation of biodiversity in the Italian and Mediterranean landscapes.

Keywords: Conservation genomics, Biodiversity, Italian chub, Italian barbels, Vairone

Session: Animal biodiversity hotspots and conservation strategies for endangered species

From morphological traits to milk: evaluation of Teramana goats for conservation strategies

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The protection and characterization of indigenous breeds is essential to establishing new breeding policies that are sustainable and adapted to climate change. The aim of this study was to monitor the population and characterize the milk from Teramana goats. We used a multi-stage strategy, first, we conducted a census of the animals and compared their morphological characteristics. It revealed a total of 188 Teramana goats, which were assessed individually for six qualitative and six quantitative morphological traits. For the quantitative traits, the PCA analysis did not reveal distinct clusters among animals from different breeders. For qualitative attributes the chi-square test did not show differences between groups of animals of different breeders, except for qualitative trait "shape of horns". In the second step, we focused on milk characterization using biochemical techniques (Western blot analysis) to examine β -casein and κ -casein, compared with Saanen goats (chosen as a comparison as a more cosmopolitan breed) followed by in silico methods (STRING and Gene Ontology analysis). The results indicated no significant difference in β -casein levels, while a higher abundance of κ -casein was found in Teramana goat milk. Then, we conducted an enrichment analysis using the STRING database, which identified relevant molecular function and biological pathways associated with κ -casein, including positive regulation of lactation, cytolysis and defense response to bacterium. Additionally, the analysis revealed molecular functions such as lysozyme activity.

In conclusion, this study highlights the importance of monitoring indigenous breeds like the Teramana goat to develop breeding policies for their preservation. The findings emphasize the unique biochemical properties of their milk which might have significant implications for dairy production and breed conservation.

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Keywords: Teramana goat, Milk, Biodiversity, Indigenous breeds, Casein

Session: Animal biodiversity hotspots and conservation strategies for endangered species

Genetic characterization of local sheep and goat populations in Apulia within the frame of the regional project L.O.C.A.L.

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Sheep and goat farming under semi-extensive systems represents a sustainable livestock model, exploiting available natural resources in marginal areas and playing a crucial role in providing income to rural communities. Nevertheless, the sector faces significant challenges, including low generational turnover due to the limited attractiveness and profitability of the profession, thus leading some farmers to favor selected high-yield breeds over local genotypes. While this leads to increased production, it also demands higher input costs, often without proportional market returns.

In this context, local breeds, having adapted over time to specific environmental conditions, assure high-quality animal products at competitive prices, yet their economic potential is often hindered by weak market positioning strategies. As a result, the decline in local populations' farming leads to a significant loss of genetic diversity, a concerning issue in the face of climate change. To contribute to the conservation of local sheep and goat populations at risk of genetic erosion, the Apulia Region funded L.O.C.A.L., a project aimed at surveying and genetically characterizing local genetic resources. The focus is on the Grigia del Subappennino Dauno, Antica della Murgia, and Capestrina goat populations, as well as the Gentile Moretta sheep population, which are integral part of the Apulia's ovi-caprine genetic heritage. Overall, more than 1,600 goat and sheep samples were genotyped using several medium-density SNP arrays (i.e., the Goat Genome Consortium 60K array, the Illumina Ovine SNP50 array and the Axiom Ovine Genotyping Array 50K). Preliminary analyses to assess genetic diversity and genetic originality are currently ongoing. In parallel, *ex-situ* conservation efforts are being conducted at the Apulian Regional Livestock Germplasm Bank to preserve genetic material and secure these valuable breeds for future use.

Keywords: Sheep, Goat, Local breeds, Genetic diversity and SNP genotyping

Session: Animal biodiversity hotspots and conservation strategies for endangered species

Interesting insights on wild egg hatchability and chick survival in artificial and semi-natural settings: preliminary observations from a rock partridge (*Alectoris graeca*) ex-situ conservation program

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The rock partridge (*Alectoris graeca*) is classified as Near Threatened in Europe and Vulnerable in Italy. In 2017, the Sibillini Mountains National Park, the Umbria Region, and the University of Tuscia initiated an ex situ conservation program through the captive rearing of individuals from wild-collected eggs (ISPRA auth. 28382/T-A31) aimed at reinforcing residual populations in the Apennines. However, standardized protocols for successfully transferring and incubating wild eggs of unknown incubation stage are still lacking.

Between June and July from 2017 to 2022, nine nests were located via VHF telemetry from radio-tagged bird. A total of 78 eggs were collected, stored in thermal bag (36-37 °C) and transferred to two breeding centers (La Starniana and Torre Certalda). Egg candling confirmed the fertility of 72 eggs.

In 2017-2018, 37 eggs were fully incubated under controlled conditions (37.6 °C, 80-83% RH) and hatched artificially. Chicks were reared in a heated room, under a heat lamp, with temperatures gradually reduced from 35 °C to 22 °C over 25 days. They were kept inside a circular fence for the first two weeks and then reared in open pens until 60 days post hatching (dph). In 2021-2022, 35 eggs were incubated artificially for 7 days, then transferred to hens (11-12 eggs/hen) for semi-natural hatching and parental rearing until 60 dph. All juveniles were later moved to outdoor aviaries.

A power surge during artificial incubation caused irreversible damage to 20 eggs. Of the 17 remaining, 6 hatched (35.3%). In contrast, semi-natural incubation yielded 31 chicks from 35 eggs (88.6%). Survival at 90 days post-hatching was 83.3% for those raised without hen and 90.3% for hen-reared chicks.

Preliminary results suggest that semi-natural incubation and rearing represent a more effective and reliable approach, supporting both high hatching success and early chick survival. Further specifically designed studies are recommended to optimize the protocols.

Keywords: Galliformes, Incubation techniques, Rearing methods, Captive breeding, Conservation management

Session: Animal biodiversity hotspots and conservation strategies for endangered species

Monitoring and managing of animal genetic resources at risk of extinction in Italy

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Within the frame of FAO's Global Plan of Action for Animal Genetic Resources (GPA-AnGR), the Italian livestock production system has planned and implemented a powerful intervention in the management and preservation of animal genetic variability. The initiative involved National and Regional Governments, Breeder Associations and Universities and it was co-funded by the European Agricultural Fund for Rural Development (EAFRD), and the National Rural Development Program (NRDP 2014-2022).

The plan strategic objectives were outlined according to the four GPA priority areas (characterization and monitoring; sustainable use and development; conservation; and policies). The ultimate goal of the program was to preserve farm animal biodiversity while improving animal welfare and the environmental impact of animal farming.

Projects covering 9 livestock sectors were funded (Dairy cattle, Beef cattle, Dual purpose cattle, Buffaloes, Horses and donkeys, Sheep and Goats, Swine, Rabbits and Poultry). Overall, 18 Breeder Associations and 8 University worked on 213 animal breeds. A total of over 690,000 phenotypic characterizations and 234,000 genetic/genomic characterizations were carried out on 200 breeds. About 70 indexes were developed and published: 41% related to animal welfare, 29% to reproductive efficiency, 21% to environmental impact and 9% to biodiversity evaluation. Germplasm collections have been established for 112 breeds, which will implement the National Germplasm System to be set up. A total of 113 descriptive sheets of autochthonous breeds were published on the institutional breeder association websites.

The results obtained allowed for a more precise assessment of genetic erosion, for preparing rational mating plans, for estimating genetic distances among breeds, for the identification of genetic variants indicators of disease resistance, development of new welfare indicators, reduction of GHG emissions and the improvement of reproductive and feed efficiency.

Keywords: Characterization, Conservation, Genetic erosion, Policies

Session: Animal biodiversity hotspots and conservation strategies for endangered species

Seeds of Resilience: Safeguarding Umbrian Agro-Biodiversity for Future Generations

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Umbria boasts a rich heritage of agro-biodiversity, essential for ecosystem resilience and sustainable development. Since 2013, the Regional Commission has safeguarded this genetic wealth by registering 18 animal breeds in the Regional Register of Autochthonous Genetic Resources, each documented with a dedicated descriptive form containing characteristics, distribution, and conservation strategies. The registered breeds include 2 sheep breeds (Appenninica, Sopravissana), 1 goat breed (Facciuta della Valnerina), 3 equine breeds (CAITPR, Del Catria, Maremmano), 3 asinine breeds (Sardo, Amiata, Bianco dell'Asinara), 5 poultry breeds (Livorno Argentata, Livorno Bianca, Livorno Dorata, Livorno Nera, Ancona) along with the Trota Fario, Carpa del Trasimeno, Luccio del Trasimeno, and Ape ligustica. A key conservation effort focuses on reconstituting the ancient Suino Nero Cinghiato breed, known only from historical frescoes. This back-breeding program seeks to restore lost genetic diversity, promote sustainable farming, and enhance traditional Umbrian products. Registration in the Regional Register plays a crucial role in preventing extinction and also enhancing breed value through incentives, short supply chain projects, and agro-food promotion initiatives. Despite the efforts significant challenges persist, including climate change, rural depopulation, and the declining profitability of traditional farming. Recent surveys show population variability among breeds, ranging from a few hundred to several thousand individuals, underscoring the urgency of conservation. Addressing these issues requires integrated strategies and represents an opportunity to develop a sustainable agricultural model that balances environmental conservation, economic growth, and cultural heritage. The future of Umbrian agro-biodiversity depends on a strong synergy between institutions, breeders, and scientific research, ensuring that these genetic resources are preserved for future generations.

Keywords: Animal breed, Regional law, Recovery, Safeguard

Session: Biodiversity in anthropized environments: issues and prospects

Characterization of goat milk from three local breeds in the focus of biodiversity preservation: chemical composition and antioxidant properties

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In Italy, there are a large number of native breeds well adapted to the territorial realities, which deserve particular attention for their productive valorization and protection in terms of maintaining biodiversity and territorial identity. The consumption of goat milk and its derivatives is taking ever greater interest, due to its peculiar nutritional and biofunctional qualities. Much attention is to be pay to the antioxidant capacity for its potential positive effects on human health by protecting the body against damage caused by oxidative stress and preventing many diseases. Antioxidant properties of milk is affected by different factors. The aim of this study was to determine the chemical composition and antioxidant properties of milk of three local goat breeds reared in Apulia region (southern Italy). Milk samples of Capestrina, Grigia del Sub-Apennino Dauno (Grigia-SAD) and Garganica breeds were collected during the same lactation period (spring, middle lactation) and analyzed for fat, protein, casein and lactose contents, and antioxidant capacity based on total phenol content (TPC), ABTS and DPPH assays. No differences were found among the breeds for the gross composition parameters ($P>0.05$) while antioxidant properties of milk showed significant differences ($P<0.05$). Compared to Garganica breed, the highest ($P<0.05$) total phenol content and DPPH values were found in Capestrina breed, while the highest ABTS value ($P<0.05$) in Grigia-SAD. In conclusion, these findings give a first knowledge on the antioxidant properties of the local Capestrina, Grigia-SAD and Garganica goat breeds, and provide useful information for their productive valorization, in the focus of biodiversity preservation.

Keywords: Goat milk, Local breeds, Chemical composition, Antioxidant properties, Biodiversity preservation

Session: Biodiversity in anthropized environments: issues and prospects

GreenTraINT - Green Training INternational Program for agriculture and livestock farming and conservation

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The total land area of Seychelles is 455 km² spread across 115 islands, of which 50% of the land mass is under protection. Seychelles still depends on imported food items, with an estimated 80% of food consumed locally imported into the country. The GreenTraINT, a two-year ERASMUS+ project, aims to improve the educational offerings in agriculture, livestock farming, and conservation in the Seychelles. Started in November 2024, this project, coordinated by the University of Bologna, joins partners from Italy, Germany, and the Seychelles to build a valuable platform for exchanging ideas and perspectives among international partners. Integrating academic knowledge with hands-on expertise is a unique opportunity to bridge academic know-how with practical fieldwork to tackle sustainability challenges effectively through international cooperation in shaping innovative and practical solutions for agricultural and environmental management.

Based on the needs identified in Seychelles through a survey conducted on-site, the key objectives of the project are enhancing educational opportunities in agriculture, livestock farming, and conservation through international cooperation, developing innovative teaching methodologies that integrate field-based experiences with theoretical knowledge and promoting sustainability and biodiversity conservation through interdisciplinary approaches. The first steps of cooperation reinforced the project's commitment to fostering a dynamic and applied learning environment for students and professionals in the field.

The results provided valuable insights into the existing educational gaps and the specific areas where GreenTraINT can make a tangible impact from multiple perspectives -educational, economic, and environmental- highlighting its relevance for agricultural development, biodiversity conservation, and policymaking to ensure its long-term success.

Keywords: Biodiversity, Higher education, Environment, Need analysis, Partnership

Session: Biodiversity in anthropized environments: issues and prospects

The PRO-GRACE (Promoting a Plant Genetic Resource Community for Europe) project

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Plants are the basis for all food, feed and renewable bioenergy production. They are essential for the transition from a fossil-based to a bio-based economy. Plant genetic resources (PGR) play a key role in ensuring this transition, as well as food security and climate change mitigation. As reported in the EURISCO database, more than 2 million plant accessions are conserved ex situ in 410 institutions in Europe and associated countries. In addition, even more diversity is found in situ in Europe's farmlands and wild habitats. There, it contributes significantly to agricultural resilience and climate change mitigation. Detailed information on ex situ collections is fragmentary at best and almost non-existent for in situ collections. Due to limitations in ex situ infrastructure and management, climate change, habitat loss and invasive/alien species, a significant proportion of these resources could be lost in the next decade. The 2016 ESFRI (European Strategy Forum on Research Infrastructure) Roadmap identifies a clear gap 'Plant facilities - unlocking green power', namely the lack of dedicated plant genetic resources (PGR) research facilities. The PRO-GRACE project (grace-ri.eu/pro-grace) coordinated by Italy, involving 31 partners from 17 countries, aims to fill this gap by developing the visionary concept of a European research infrastructure that brings together genebanks and other European institutions working on PGRs, connects them to global genebank organisations and other ESFRI infrastructures, and facilitates their transformation from mere plant or seed repositories into a networked system of dynamic research centres. The ultimate aim of this infrastructure will be to catalogue, describe, conserve and enhance the agrobiodiversity of European plants and to translate the results into conservation practices and agricultural innovation through 7 work packages: WP1 - Plant genetic resources inventory and information systems; WP2 - Quality certified ex situ and in situ management; WP3 - Research infrastructure; WP4 - Plant genetic resources for food; WP5 - Research Infrastructure concept, social and regulatory aspects, governance and financial plan; WP6 - Communication, dissemination, exploitation and training and WP7- Scientific coordination and management.

Keywords: Agrobiodiversity, Research infrastuctures, Conservation, Collections management, Climate change

Session: Biodiversity in anthropized environments: issues and prospects

Promotion of agrobiodiversity: diffused plantation of orchards for biodiversity in urban and non agricultural areas for local communities

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The forty-year work of research, conservation and promotion of Archeologia Arborea, created an orchard of old local varieties, as reservoir of genes and culture, to share with the new generations. Foundation Archeologia Arborea is created for conservation of memory and knowledge but also for the diffusion of agrobiodiversity.

The proposal of orchards of biodiversity has emerged as a contribution to the maintenance of agrobiodiversity in non-agricultural areas or urban areas, to keep our cultural and cultural roots.

The orchards we propose will be made up of ancient local varieties, so each place will be different and unique. The basic orchard module includes different species and all the measures for a sustainable agro-ecosystem, including intercropping with herbaceous and shrubby plants to enrich the soil with organic matter and to favor beneficial insects as much as possible. They are proposed for different situation and different sizes: municipalities or private companies, therapy areas, historical gardens.

Collaborations have already been activated with the Umbria Chamber of Commerce, the Municipality of Perugia, and L'Abbondanza Group.

The orchards created could have different functions:

Educational; good nutrition and health passes through knowledge and respect for seasonality; children and adults will be able to see and taste fruits that we are no longer used to eating.

Evocative; fruit trees were strong symbols, present often in art. Furthermore, their beauty in all seasons must be emphasized.

Inclusive; orchards will be a meeting point between different generations; the elderly with their knowledge will be able to communicate to the new generations. The places will be easily accessible to everyone.

Productive; these orchards will be cultivated to encourage community harvests. They will be managed with organic farming systems and will guarantee different fruit in different seasons.

Keywords: Local fruit varieties, urban orchards, Community orchards, Agrobiodiversity

Session: Biodiversity in anthropized environments: issues and prospects

The BIO-MASTER project: development of a standardized protocol for bioaerosol monitoring and classification

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The BIO-MASTER (BIOaerosol Monitoring And classification: development of a Standardized procedure by integrating different data analysis Techniques and Experimental Research methodologies) is a PRIN2022 project aiming to establish a standardized protocol for bioaerosol monitoring and classification, focusing on bacteria, fungi, and pollen. It explores relationships with air pollution levels (PM10 concentrations), seasonality, human health, and environmental factors. This study seeks to address the variability in experimental designs and inefficiencies in current methodologies. Given the low environmental concentration of bioaerosols, obtaining sufficient DNA for molecular characterization is crucial, and the choice of collection substrate influences DNA recovery efficiency. To address this, standardized procedures for collecting both biogenic and non-biogenic particles were developed, starting with tests on commonly used filter types, including quartz and Teflon, whose effects on DNA recovery are not well understood.

Two intensive monitoring campaigns were conducted at the University of Salento in Lecce across two seasons: winter (Dec 2023-Mar 2024) and summer (Jun-Oct 2024). Air samples were collected using a Leland Legacy Personal Sample Pump at a flow rate of 10 L/min for 48 hours on weekdays and weekends with two filter types. DNA was extracted, quantified, and sequenced on the MiSeq platform for species-level resolution of eukaryotic and prokaryotic communities.

The BIO-MASTER project provided guidelines for standardizes bioaerosol monitoring and classification. It improves our understanding of aerosol and bioaerosol characteristics, seasonal variations, and their environmental and health impacts. The comparison of filter efficiency aims to optimize sampling methods for: 1) reconstructing microbial community diversity in relation to pollution levels, and 2) detecting and identifying potential opportunistic human and plant pathogens in particulate matter.

Keywords: Bioaerosol monitoring, Microbial community, Air pollution, Human health, Sampling standardization

Session: Biodiversity in cropping systems

A multi-year study on soil biological quality: key insights for sustainable vineyard management

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The importance of soil biodiversity in viticulture has received growing attention in recent years. Edaphic arthropod communities, which represent over 85% of soil fauna species richness, play a pivotal role in maintaining the biodiversity and ecosystem functioning of vineyard soils. Their responses to soil characteristics and management practices make them effective bioindicators of soil quality. Understanding the diversity of soil arthropods also contributes to enhancing ecosystem services they provide, offering valuable insights to guide wine producers toward more sustainable vineyard management.

This study presents the findings of a multi-year research investigating the biological quality of Italian vineyard soils and the influence of environmental variables and management practices on soil arthropod communities. Since 2014, a comprehensive survey has been carried out across several important Italian wine regions, resulting in a substantial dataset of 654 records by 2023. To quantify soil biodiversity, the QBS-ar Index (Soil biological quality Index for arthropods) was used. This index serves as a key tool for assessing soil health, which includes physical, chemical, and biological properties. This research specifically explores the relationship between the QBS-ar Index, environmental factors, and vineyard management practices.

This extensive data collection offers a valuable case study demonstrating how biological data can provide insights into ecosystem responses to soil conditions changes. The large dataset and geographical variability of the observations allow to reveal the impacts of soil abiotic conditions and vineyard management practices on soil arthropod communities. This research can help to provide the scientific basis for developing supports for decision in vineyard soil management, promoting ecological transition, and advancing sustainability in viticulture.

Keywords: Biodiversity, Viticulture, Soil biological quality, Soil arthropods, Vineyard management

Session: Biodiversity in cropping systems

Agronomic and qualitative evaluation of four cauliflower landraces in Puglia region (Southern Italy)

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Cauliflower (*Brassica oleracea* var. *botrytis*) is one of the most widely cultivated brassicaceous crops in the Puglia region (Southern Italy). This region boasts significant agrobiodiversity, including several promising cauliflower landraces. “Cima di Cola” (CDC), “Agostana” (AGO), “Settembrina” (SET), and “San Martino” (MAR) are highly valued at the local level for their distinctive organoleptic properties. To evaluate their agronomic, commercial, and nutritional properties, these landraces were compared to the commercial hybrid variety “Emeraude” (IBR), which is cultivated in the same region. All the varieties produce light-green closed inflorescence (heads). The plants were grown in an open field in Mola di Bari (BA) from late July (transplanting) to mid-February 2024 (30 weeks), at a density of 2.6 plants/m². Drip irrigation was applied during the early growth stages, and cultivation followed local agronomic practices. All aboveground plant organs and heads were described using the morpho-physiological standard descriptors of GIBA (Gruppo di lavoro Biodiversità Agricola) and the International Union for the Protection of New Varieties of Plants (UPOV). Yield, firmness, colour and phytochemical composition of the cauliflower heads were also analysed. SET, AGO, and MAR began producing at 13 weeks after transplanting (WAT), followed by IBR at 19 WAT, and CDC at 28 WAT. On average, the landraces produced 1.17 kg/m² of inflorescences, with a dry matter content of 8.3 g/100 g of fresh weight, which were 41.3% and 20.3% lower compared to IBR, respectively. While head height, diameter, and chroma did not differ significantly among the varieties, IBR exhibited the highest firmness, while CDC the highest colour lightness. The ongoing phytochemical analysis will complement these preliminary findings to explore the nutritional potential of the abovementioned landraces.

Keywords: *Brassica oleracea* var. *botrytis*, Agrobiodiversity, Descriptors, Yield, Nutritional profile

Session: Biodiversity in cropping systems

Bambina: a minor Apulian olive cultivar combining nutraceutical value and agroecosystem resilience

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In Italy, the Apulia region is renowned for its extensive olive biodiversity, encompassing a vast array of autochthonous germplasm that plays a crucial role in the regional economy and cultural heritage. The preservation and valorization of this biodiversity are essential for sustaining healthy agro-ecosystems, promoting local olive varieties, and enhancing the marketability of their high-quality food products. This study emphasizes the importance of characterizing minor olive genotypes, such as the cultivar Bambina, which demonstrates rusticity and remarkable adaptability to diverse climatic conditions.

The drupes of cv Bambina were processed using two milling strategies: conventional and nutraceutical. The latter involves the application of low temperatures throughout all stages of drupe processing and oil extraction. Genetic and chemical analyses of the resulting olive pastes and oils revealed a high content of bioactive compounds, such as total phenols and tocopherols, in particular in nutraceutical milling. These compounds contribute to the oxidative stability of the oil and provide significant benefits to human health. The expression levels of genes involved in both vitamin E biosynthesis (VTE1, VTE2, VTE3, etc) and oxidative status regulation (POD and LOX) were significantly higher in cv Bambina compared to cv Coratina.

The qualitative properties of Bambina oil make it comparable to that of the renowned cv. Coratina, underscoring its potential as a valuable resource for nutraceutical applications. By focusing on genetic characterization and the valorization of local cultivars, this research aims to ensure the long-term sustainability of olive cultivation in Apulia while contributing to global efforts to mitigate the impacts of climate change on agriculture.

Keywords: *Olea europaea*, Biodiversity, Nutraceutical milling, Bioactive compounds, Oxidative stability

Session: Biodiversity in cropping systems

Biodiversity as a measure of sustainability: impact of agricultural management on soil prokaryotic diversity of rice fields

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Rice paddies are unique agroecosystems where soil bacterial communities are crucial for nutrient cycling, soil fertility, and ecological balance. However, the effects of agricultural practices on these communities are not fully understood. This study explores the composition, diversity, and functional potential of soil bacterial communities in conventionally and organically managed Italian rice fields, with a special focus on methanogenic Archaea and methanotrophic Bacteria due to their impact on climate altering gas emissions.

The study was conducted in three areas of the western Po Valley, where sediments from conventional and organic rice paddies, along with nearby natural wetlands were sampled. Total DNA extracted from sediments was quantified and related to the sediment dry weight, as a proxy of microbial biomass. The sediment microbiota was analyzed by Illumina sequencing of the 16S rRNA gene. The sediment DNAs were probed by PCR to detect the methane oxygenase-encoding gene *pmoA*.

Wetlands and organic rice fields exhibited higher microbial biomass than conventional rice fields. Greater bacterial diversity was detected in organic rice fields, indicating that organic practices support richer microbial ecosystems. The distribution of methanogenic Archaea is indicative of a clear distinction between cultivated and natural areas, where they are more abundant. In contrast, methanotrophic bacterial genera are more abundant and diverse in organic rice paddy areas although the key gene of methanotrophy was detected in all areas.

The study highlights the critical role of agricultural practices in the modeling of soil microbiota and focuses on the search for key taxa as bioindicators. By integrating biotic and environmental parameters, the research offers insights to promote sustainable management of rice fields.

Keywords: Organic rice cultivation, Conventional rice cultivation, Methane cycle, Wetlands

Session: Biodiversity in cropping systems

Biodiversity returns. From germplasm banks to cultivated fields, a targeted and participatory use of conserved germplasm

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The Umbria Region has promoted and financed, with PSR/CSR funds, multiple activities aimed at research, conservation, study of Agrobiodiversity, at the same time providing indications through a specific law (L.R. 12/2015, Capo IV). The first project dating back to 2000 led to the creation of a Germplasm Bank of herbaceous species that today collects almost 300 different local accessions. To address the ever-increasing difficulties that farmers encounter in using their own company seeds, 3A-PTA has started an experiment in collaboration with some farmers in 2022 on local legume varieties (Chickpea, Grass Pea, Lentil, Roveja).

The field trials involved the accessions of Lentil and Roveja (2023); Lentil and Chickpea (2024); Lentil, Chickpea and Grass Pea (2025). Together with the company varieties, the other Umbrian accessions available in the regional collection were evaluated and also compared with materials requested outside the region from other Banks or Companies. After the randomized block varietal comparison trials, the accessions were also subjected to field multiplication to increase the stocks and to carry out agronomic evaluations.

At the moment the data have allowed to characterize 24 accessions of Lentil, 6 of Roveja, 16 of Chickpea and 21 of Grass Pea (in progress). From the data collected so far, also discussed together with the farmers, some initial indications have emerged on the most interesting typologies and that, in the case of Lentil, have led to the creation of a population obtained by mixing 9 different local accessions.

Studies are still ongoing and the involvement of professionals in the intentions should contribute to ignite and increase interest in Agrobiodiversity. Paths are also being initiated that allow us to overcome the critical issues encountered and reintroduce, in compliance with the rules, these varieties among farmers.

Keywords: Local varieties, Legumes, Participatory selection

Session: Biodiversity in cropping systems

Chemical composition of essential oils from *Smyrniolus atrum* L. (Apiaceae) ecotypes from Salento

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Smyrniolus atrum, well known as wild celery or Alexanders, is a biennial herb belonging to the Apiaceae and used for many centuries as an edible vegetable because of the aromatic flavor of its parts. The entire plant is used for culinary purposes, either raw or cooked. In addition to its traditional culinary uses, *Smyrniolus* has also been utilized for its therapeutic properties for urinary system and prostate disorders, gynecological diseases, gastrointestinal pain, and indigestion. The family Apiaceae is well-known for its ability to produce and store volatile oils in its secretory ducts located in the vegetative and reproductive organs. In the present work the composition of essential oils obtained from different organs of *S. olus atrum* collected from March to April in five different sites of Salento in Apulia region, was investigated by gas chromatography. The chemical profiles of essential oils of *S. olus atrum* growing in Salento area were similar, although quantitative differences in marker compounds have been highlighted. The results suggest that the wild populations of *Smyrniolus* represent a rich source of isofuranodiene, a furanosesquiterpenoid, a compound found in few other plants. Isofuranodiene has potential applications as hepatoprotective, neuritogenic, anti-inflammatory and anticancer effects. Therefore, the *Smyrniolus* essential oil (EO) may have interesting pharmaceutical applications. In the framework of the project: Biodiversity of Apulian Non-Fruiting Horticultural Species - BiodiverSO Veg (CUP: B97H22003760009).

Keywords: *Smyrniolus atrum*, Apiaceae, Essential oils, Furanosesquiterpenoid

Session: Biodiversity in cropping systems

Conserving and enhancing local agrobiodiversity: exchange between Italy and the Sahel region to save local varieties and NUS and promote sustainable food value chains

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Recovery and enhancement of agrobiodiversity are recognized worldwide as a means to save local varieties and promote food security and sustainable production. BiodiverSO research projects, funded by the Rural Development Program (PSR) in the Apulia region (Italy), support the regional network of farmers and seed savers to recover and enhance local agrobiodiversity. The expertise gained in BiodiverSO projects is the basis of the exchange of knowledge between Italy and the Sahel region within the internship organized in the framework of the project "SUSTLIVES: SUSTaining and improving local crop patrimony in Burkina Faso and Niger for better LIVes and EcoSystems" (EU DeSIRA program). The main objective of the internship is to undertake training on agrobiodiversity management towards more sustainable and resilient food systems in the two countries.

BiodiverSO VEG and Karpos projects, while spreading sustainable and agroecological cultivation techniques and strengthening informal community biodiversity management systems, have in common with SUSTLIVES their focus on niche crops or neglected and underutilized species (NUS). During the last edition of the internship all the activities developed in BiodiverSO were discussed. The program will be implemented again in 2025 for eight interns from Burkina Faso and Niger.

Nutritional and functional valorisation of NUS, agroecological criteria for the agrobiodiversity management, creation of local supply chains and related income, and increase in the use of vegetables for healthier diets were central topics of the last year's exchange program, animating the discussion on several points. The new edition is expected to be more in-depth and livelier since various points of view and knowledge needs will be on the table.

Keywords: Agrobiodiversity, Neglected and Underutilized Species (NUS), BiodiverSO VEG, BiodiverSO Karpos, Sustlives

Session: Biodiversity in cropping systems

Defining soil quality indicators in vineyards under conventional and organic practices

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Intensive grape production has dramatically impacted chemical, physical, and biological soil fertility due to the significant inputs of agrochemicals and fertilizers. This work aims to identify the biotic and abiotic drivers of soil quality and the keystone species influencing it, applying an innovative agroecological network analysis in vineyards based on their management and geographic location. The sampling area is in South Tyrol (Italy), one of the highest-quality viticultural regions in Italy. One thousand eight soil samples associated with *Vitis vinifera* cv. Pinot Noir were collected from 42 vineyards (22 organic and 20 conventional) distributed along a geographic gradient from south to north over two seasons (spring and summer). The soil was analyzed to determine its chemical properties, including carbon (C), nitrogen (N), phosphorus (P), and pH. Agrochemicals were extracted using the QuEChERS method and quantified using LC-MS. Furthermore, the soil will be examined for its enzymatic activity and biodiversity, encompassing bacterial, fungal, and faunal diversity.

Preliminary results showed that conventional vineyards had significantly higher total organic C (TOC), inorganic C (IC), total C (TC), and total N (TN) compared to organic vineyards ($P < 0.03$), while the pH was significantly lower in conventional vineyards (-7.15%). However, there was variability among sampling areas and seasons. In the northern area, pH, TOC, TC, IC, and TN were significantly higher in organic vineyards ($p < 0.001$), while they were lower in the other areas. The phosphorus content did not significantly differ between management practices and geographic areas.

Finally, agrochemical residues were found to accumulate significantly more in conventional vineyards compared to organic, while seasonal variation and geographic location had minimal effects. Together with enzymatic and microbial results, the networks will provide a potential monitoring approach for sustainable agriculture.

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Keywords: Vineyards, Biological, Conventional, Soil quality, Sustainability

Session: Biodiversity in cropping systems

Dominance Vulnerability Index (DVI): a biodiversity index for early prediction of weed-induced yield losses

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Chemical weeding causes the over-simplification of the agroecosystems, leading to the establishment of dominant species that are the main cause of crop yield losses. The ability to selectively remove only these species, thus rebalancing the species equilibrium, can be an effective strategy to contextually preserve yield and biodiversity. The aim of this study was to identify a biodiversity index calculated at the early stage of weed infestation, able to predict which areas in the field would be more susceptible to weed overgrowth (i.e., high biomass), and thus where and how the weed removal is necessary to contain yield losses.

Triticum aestivum var. Rebelde was cultivated in Portici (NA) and Bellizzi (SA). Two treatments, namely weed-free and weedy, have been randomly assigned to different plots. Weed number and biomass have been measured per species at the tillering and heading stage. Wheat biomass and yield have been measured at kernels maturity. On the datasets obtained, we developed a novel biodiversity index, named Dominance Vulnerability Index (DVI).

DVI shows high and significant correlation with total weed biomass at the heading stage of wheat, and with wheat yield losses at harvest both in Portici and Bellizzi fields. Moreover, DVI allowed the identification of the dominant species, and the amount to be removed to maximize the equitability of the production. The linear equation obtained allowed us to calculate that, once the maximum equitability was reached ($DVI = 0$), the impact of weeds on yield was significantly reduced compared to conditions where dominant species were present.

DVI can be a functional approach to guide weeder robots to remove only dominant weeds, while leaving non-competitive species in the field. Restoring species balance will preserve crop yields while maintaining high levels of biodiversity in the fields for the maximization of the ecosystem services provided by agriculture.

Keywords: Dominant species, Wheat, SSWM, Ecology

Session: Biodiversity in cropping systems

Effect of cytokinin and inductive treatments on the regrowth and rooting of encapsulated microcuttings for olive conservation

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Germplasm preservation could be considered as one of the most significant applications of encapsulation. Synthetic seeds, which are the end product of this technology, can effectively support the conservation of plant material over short, medium, and long-term periods under various temperature conditions, ranging from frigoconservation to cryopreservation systems. Suitable protocols were already identified in several fruit species, but not in olive (*Olea europaea* L.). Unsatisfactory levels of regrowth and conversion of encapsulated unipolar propagules (microcuttings) seem to limit the application in this species, depending on genotype traits, different rooting ability or effectiveness of inductive treatments for the differentiation of the root primordia. This work was conducted with the aim to identify a suitable protocol for the production of synthetic seeds of “Moraiolo”, widespread in Central Italy and collected in our laboratory as a pilot olive cultivar. Compared to previous studies, these experiments allowed for highlighting the influence of the nutritive conditions prior the collection of the olive microcuttings on their sprouting (regrowth) after the encapsulation in a calcium alginate matrix and sowing in aseptic conditions. A less expensive formulation of zeatin (a natural cytokinin) added to the medium during the multiplication phase allowed the improvement of the sprouting of encapsulated propagules of olive (from 30 to 67%). In addition, the effect of two different root inductive treatments (classic and innovative) positively influenced the rhizogenic ability of synthetic seeds of olive, showing the effectiveness of the innovative protocol in terms of conversion and average length of roots. In the overall evaluation of the experiment, the well-known “genotype-dependence” should not be neglected, which may at least partly account for the reduced conversion capacity (limited to 47%) influenced by the reduced rhizogenic aptitude of the “Moraiolo” cv..

Keywords: *Olea europaea* L., Synthetic seed, Zeatin, Sprouting, Conversion

Session: Biodiversity in cropping systems

Glucosinolates profiles and antioxidant activity of *Brassica rapa* subsp. *sylvestris* from Salento area

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Brassica rapa subsp. *sylvestris* is a vegetable crop widely distributed in Southern Italy, their consumption has been related to human health due to their phytochemicals, such as glucosinolates and phenolic compounds that influence different physiological functions including antioxidant activity, enzymes regulation and apoptosis control and the cell cycle. In Apulia region many cultivated landraces are characterized by wide phenotypic variability and named according to length of time between sowing and inflorescence appearance as “cima di rapa cinquantina and sessantina” and “rapacula”. Many cultivars of this species and the resulting large metabolic variation have been obstacles for systematic research of the plant. Metabolomic analysis of different landraces of *Brassica rapa* L. subsp. *sylvestris* (L.) Janch. var. *esculenta* was performed. Inflorescences from six landraces growing in three areas of Salento were collected in April and May 2024.

The profile of glucosinolates and phenolic compounds in relation to the antioxidant activity of six *Brassica rapa* landraces were determinate and compared. The qualitative and quantitative compositions of metabolites were determined by HPLC/MS (high performance liquid chromatography/mass spectroscopy) technique.

We found 11 different glucosinolates and two main phenolics, kaempferol and quercetin in each extract analyzed.

This study provides a starting point for subsequent genetic characterization with the aim to preserve genotypes with relevant biological activities.

In the framework of the project Biodiversity of Apulian Non-Fruiting Horticultural Species - BiodiverSO Veg (CUP: B97H22003760009).

Keywords: *Brassica rapa* subsp. *sylvestris*, Glucosinolates, Phenolics, HPLC/MS

Session: Biodiversity in cropping systems

Impact of cover crops on belowground microbial diversity in a Mediterranean olive grove

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Cover crops (CCs) are temporary plants grown between main crops to improve soil health, biodiversity, and nutrient cycling while reducing erosion. In Mediterranean olive groves, where erratic rainfall limits fertility, CCs of self-reseeding annual legumes can fix nitrogen, suppress weeds, and reduce fertilizer use.

This study, performed within the project Agritech National Research Center, funded by the European Union Next-Generation EU (PNRR, MISSION 4 COMPONENT 2, INVESTIMENT 1.4, D.D. 1032 17/06/2022, CN00000022), assessed the impact of different CC mixtures on soil microbial communities and on mycorrhizal root colonization in a traditional olive grove in northern Sardinia. Two CC treatments, Commercial Mixture (CM) and Innovative Mixture (IM), were compared with natural herbaceous cover and conventional tillage. Soil and plant community root samples collected in 2023 and 2024 were analysed using targeted metagenomics to study bacterial and fungal communities and evaluate CCs' influence on soil health and beneficial symbionts.

Results showed that seasonal variations had a stronger effect on soil microbial communities than CC treatment, with shifts in dominant bacterial and fungal groups between years. However, CC treatments influenced microbial composition of plant community roots, as 16S sequencing showed differences between CC treatments and natural cover, while ITS sequencing indicated no significant alpha-diversity differences. Multivariate analyses confirmed treatment-dependent microbial variations, suggesting that CCs may shape belowground biodiversity.

The ability of CCs to foster beneficial microbial associations suggests the need for further analyses, aimed at integrating molecular and physiological data, allowing the selection of CCs able to enhance soil fertility with limited water/nutrient competition with trees, to optimize olive grove management in Mediterranean agroecosystems, under varying climatic conditions.

Keywords: Soil microbial communities, Mycorrhizal symbiosis, Soil fertility, Cover crop plants

Session: Biodiversity in cropping systems

Innovative management for organic ornamental crops in nurseries

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The ornamental sector of organic outdoor ornamental plants needs innovative technical means that can improve the sustainability and competitiveness of production companies.

There are numerous difficulties that stand in the way of adopting organic production protocols and the most limiting are the poor availability of certified organic technical means. Pot cultivation is very difficult to manage in an organic regime. Another problematic phase in ornamental nursery is propagation, usually carried out by cutting with the aid of synthetic products with hormone-like action. Last, but not least, is the challenge of controlling biotic and abiotic stress with remedies permitted in organic farming, which implies a holistic approach to the problem with respect to the use of synthetic chemical products, possible above all thanks to a conscious use of bioactive substances. Of particular importance are the control of weed flora and the defense against soil-borne pathogens. In this context, the BIOVIVO project aims to analyze with a multidisciplinary approach, also thanks to the direct support of horticultural companies, the critical points of the production process - propagation, growing substrates, fertilization, defense - with the aim of outlining a cognitive framework that can support the organic cultivation process for ornamental shrub plants in pots to be used in green spaces. The project will use technical means of natural origin such as biostimulants to improve the production process of ornamental plants in nurseries.

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Keywords: Substrates, Propagation, Weeds, Nutrition, Bioactive substances

Session: Biodiversity in cropping systems

Investigation of local Italian varieties of *Ficus carica* L.: morphological, physicochemical and aromatic characterization

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Mediterranean fig (*Ficus carica*), is one of the earliest cultivated fruit trees, it is a classical fruit tree of Mediterranean region, where is concentrated more than 80 % of production. The fruit is an important resource of the MD and it is consumed fresh, dried, and processed. In recent years, fig has been gaining increasing attention, for the fruit's nutritional properties and exceptional resilience to climate change. Among fruit trees, the fig is recognised as an extremely polymorphous species with a rich varietal platform. The Italian fig germplasm consists of a large number of cultivars, more than 300. This number is approximate; there are many genotypes that are still poorly known and studied that may possess interesting agronomic traits, especially in terms of response to climate change. Therefore, it is necessary to characterize the local germplasm to identify genotypes with desirable traits. Aim of this study was to describe Italian fig cultivars through fruit morphological parameters, physicochemical characteristics, and aromatic profiles.

11 pomological parameters, physicochemical characteristics (TSS, pH, and TA), and flavor profiles (HS-SPME/GC-MS analysis) of the pulp and peel were evaluated on fruits of 15 varieties of *Ficus*

Significant differences were observed in fruit pomological traits, as well as in the physicochemical parameters and the aroma profiles. Volatile compounds detected in flesh and skin being distributed by distinct chemical classes: aldehydes, alcohols, ketones, esters, monoterpenes and sesquiterpenes. All varieties presented a similar volatile profile, although with different intensities between the varieties. Peels exhibited a greater number of volatile compounds, especially for sesquiterpenes, compared to the pulps.

The results obtained highlight that among the fig varieties studied, some exhibit superior morpho-chemical characteristics; these could be used in breeding programs and in the establishment of commercial orchards.

Keywords: Fig tree, Local varieties, Morphological parameters, Qualitative analysis, Volatile profile

Session: Biodiversity in cropping systems

Metabolites and nutritional value of different local varieties of tomato (*Lycopersicon esculentum* Mill.) cultivated in Salento

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Tomato (*Lycopersicon esculentum* L.) is one of the world's most consumed vegetables, known as nutritional and healthy food. Thus, various cultivars have been developed to attract more consumers. The beneficial effects of tomatoes such as antioxidant, anti-inflammatory, anti-cancer and anti-atherogenic properties, reducing risk of cardiovascular diseases, have been widely studied. Therefore, it is interesting analyze metabolic differences and nutrient content of different cultivars. We carried out a metabolomic analysis and an evaluation of nutritional properties of four different tomato local accessions from Salento area, named pomodoro di Morciano, pomodoro leccese, pomodoro racalino, pomodoro di Salve.

Results show that cultivars have a lower level of lycopene, but the same content of α -carotene and β -carotene than commercial tomato varieties. Moreover, the four cultivars were characterized by their high content of all-trans lutein, a carotenoid used for protecting retinal damages.

In addition, total phenolic and flavonoid contents were evaluated.

The data obtained provide valuable information of different characteristic in various tomatoes, which can be considered for improving tomato cultivars.

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Keywords: *Lycopersicon esculentum*, Local varieties, Phenolic compounds, Carotenoid

Session: Biodiversity in cropping systems

New insights into the diversity of Apulian wheat landraces known as Saragolla

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Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) has been cultivated in Southern Italy since ancient times. Historically, farmers cultivated landraces that evolved in response to environmental conditions in which they were traditionally grown. Among the Italian durum landraces, the "Saragolla group" played a significant role in Southern Italy until the mid-20th century. This group included landraces such as "Saragolla o duro di Puglia" "Saragolla Lucanica", and "Saragolla Siciliana", all characterized by long grains and semolina used for pasta production.

The release of improved durum cultivars led to the progressive decline of the landraces, including the "Saragolla group" which now survives in small marginal areas of Southern Italy. Recently, through the Rural Development Program, seven durum landraces were recovered in Apulia region, one of which named "Saragolletta" while the others were collectively referred to as "Saragolla".

In this study, the genetic variation within and among these landraces was assessed using biochemical markers. The gliadin profiles revealed considerable genetic diversity within the "Saragolletta" which appeared to be a mixture of three genotypes. The main distinguishing feature in the gliadin profiles of the six "Saragolla" landraces was the presence/absence of components in the slow-moving ω -gliadin region. This result allowed us to split the Apulian "Saragolla" into two subgroups. Two of the "Saragolla" landraces, polymorphic for ω -gliadin, were further genotyped using Single Nucleotide Polymorphism (SNP) markers, alongside a panel of durum (landraces and cultivars) and Khorasan (*Triticum turgidum* L. subsp. *turanicum* (Jakubz.) Á. Löve & D. Löve) accessions. Phylogenetic analysis confirmed the genetic diversity among the Apulian "Saragolla", suggesting that the term "Saragolla" includes landraces consisting of a mixture of genotypes from different *T. turgidum* subspecies.

Keywords: Durum wheat, Genetic diversity, Landraces

Session: Biodiversity in cropping systems

Old and new paths for seeds of European vegetable landraces

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In Europe, the commercialization of vegetable seeds is only permitted for varieties registered in the Common Catalogue of Varieties of Vegetable Species, after satisfying the distinctness, uniformity and stability (DUS) requirements. This regulation therefore totally excludes the possibility of marketing landraces or participatory varieties which, due to their high variability, do not meet the DUS requirements. To overcome this limitation, Directive 98/95/EC introduced two derogation regimes: Conservation Varieties (CV) and varieties with no intrinsic value (or amateur varieties, AV). This paper analyses: (i) the effectiveness of these regimes with respect to the European seed market and (ii) the dissemination channels - formal and informal - used for European vegetable seeds. In 2024, in the Common Catalogue, 1,133 AV and 235 CV were registered out of a total of 22,821 varieties, equal to 5% and 1.1% respectively of marketable vegetable varieties. The diffusion of landraces and, more generally, of farmer's varieties, takes place mainly through informal channels, such as custodian farmers, seed savers, associations or Community Seed Banks. The European seed system therefore needs to be renewed in order to offer more formal distribution channels for landraces, especially in light of their increasingly common use in non-conventional agricultural systems, and to protect agrobiodiversity. In this sense, with Regulation (EU) 818/2018 and with the Implementing Directive (EU) 2022/1648, the European seed system has adopted two additional regimes: the Organic Heterogeneous Material (OHM) and the Organic Varieties of vegetable species suitable for organic production (OV). These, together with new regulations that will be discussed in the European Union in the coming months, could open up new and unexpected scenarios for the seeds of European vegetable landraces.

Keywords: Conservation varieties, Amateur varieties, Landraces, Seed regulation, in situ conservation

Session: Biodiversity in cropping systems

The garlic landrace of Resia, Eastern Italian Alps. The plant genetic resource managing in a Slavic enclave, twenty years later

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At the crossroad of Latin, Slavic and Germanic culture, Friuli - Venezia Giulia region (FVG) is a true land of differences. Despite being reasonably small, it includes 3 out of the 5 European biogeographical regions. A few languages, dialects and idioms are spoken here. The Alpine valley of Resia stretches 21 km W-E and is closed towards the Slovenian border by the Canin massif (2580 m). A group of alpine Slavic shepherds settled in the valley, between the 7th and 9th centuries AD. A high level of genetic insulation for Resia people has been recently assessed. The landrace (strok in Resian idiom) is a hardneck (bolting) garlic, putatively assigned to the *Ophioscorodon* group. Through reproductive stages, a long and curving scape is developed, then the scape uncoils and the lumping shape of the umbel capsule become evident, with a white spathe that includes sterile flowers and topset.

Since 2003, the garlic was included in the FVG Repertoire of Landraces and by 2005 it entered among the Slow Food Presidia of FVG. This casted light at national level, so the garlic prices rose dramatically. Phenotype characterization was performed by standard IPGRI descriptors as well as agronomic evaluation in comparison to other bolting materials.

Intrinsic biological and agronomic difficulties interacted to limit the crop area enlargement and some frictions among local horticulturists arose, so in recent years the number of farmers involved with the Presidium dropped significantly. After 20 years, the Municipality of Resia, the Prealpi Giulie Natural Park and the University of Udine are being striving to foster the garlic landrace: agronomic evaluation, on farm management practices and communication measures have been put in place, in order to safeguard the *Allium* genetic resource: an identity flag for the Resia community.

Keywords: *Allium sativum* ophioscorodon, Landrace, Resia, SlowFood Presidium

Session: Biodiversity in cropping systems

The potential use of Sicilian chili pepper accessions for ornamental purposes

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Chili pepper (*Capsicum* spp.) in recent decades has been rediscovered as an ornamental plant mainly due to its efficient sexual propagation, short crop cycle, high temperature tolerance, and variability in fruit color and plant height. In the genera *Capsicum*, *Capsicum annum* is the most widely cultivated species for ornamental purposes. Although ornamental chili pepper plants are self-pollinators, entomophilous pollination may occur with cross-pollination which can varies from 2% to 90%. Nurseries do not always provide adequate isolation among plants to promote self-pollination, so hybridization among cultivars may occur. Our preliminary observations suggest that there is considerable variation among Sicilian genotypes, perhaps due to cross-pollination. Consequently, a specific study to characterize Sicilian chili pepper accessions for ornamental purposes is needed. In our research, the germplasm was established in an experimental field of the Department of Agricultural, Food and Forestry Sciences of the University of Palermo for 2 consecutive years. Nineteen chili pepper accessions were grown. The characterization of the genotypes was accomplished using the IPGR (International Plant Genetic Resources Institute) descriptors. Results pointed out that chili pepper accessions A33, A27, G1 and A1 attained good attitudes for ornamental purposes since they revealed high visual quality traits. Our findings also showed different yield traits among the accessions evaluated. This suggests a greater degree of variability among genotypes. Remarkably, the study indicated that the visual quality scores of the plants increased as the number of fruits per plant decreased. Thus, the variability detected could suggest an overtime selection pressure for yield or ornamental purposes.

Keywords: *Capsicum*, Sicilian biodiversity, IPGR descriptors, Visual quality

Session: Biodiversity in cropping systems

Valorizing Apulian chickpea (*Cicer arietinum* L.) biodiversity: a multidisciplinary approach

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Legumes have been cultivated in Italy since ancient times. Each Italian region has its own group of landraces, belonging to different legume species. They are deeply embedded in the traditional agricultural practices, as well as in the local landscape, dishes and cultural heritage. In the Apulia region, chickpea exhibits considerable unexplored variability, particularly in seed traits. In the PSR project SavegrainPugliaLeg, nine landraces were characterized using a multidisciplinary approach to uncover their potential for more sustainable farming systems. A sample of seeds representative of each landrace was acquired from farmers who traditionally cultivated it. The genetic variation within and among the landraces was assessed through biochemical and genetic analyses, including the evaluation of some genes involved in drought stress tolerance.

An unequivocal genetic fingerprint was obtained for each landrace. The genetic diversity indices revealed a moderate level of polymorphism. As expected, due to the strictly self-pollinating nature of chickpea, the observed heterozygosity (H_o) was lower than the expected one (H_e), resulting in positive inbreeding coefficients. The analysis of the mRNA accumulation of two genes encoding for Kunitz-type trypsin inhibitor, which are responsive to water deficit, revealed a higher expression of CaTPI-1 transcript in roots of the “black chickpea from Murgia dei Trulli” in the presence of the stress. In addition, this black chickpea showed also the highest CaKPI transcript expression. In terms of seed nutritional quality, all the landraces showed protein contents within the typical range for chickpea. Higher levels of some nutraceutical compounds, such as carotenoids and total phenolic compounds, were recorded for the “black chickpea from Murgia dei Trulli”. Collectively, these findings suggest that this landrace is a promising genetic resource for chickpea crop improvement.

Keywords: Drought, Chickpea, Landraces, Apulia

Session: Biodiversity in cropping systems

Virtual fencing as a tool for biodiversity-oriented pasture management in alpine grasslands

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While fulfilling a valuable productive role, pastoral systems based on extensive grazing provide and maintain numerous ecosystem services, such as high levels of plant and habitat diversity. These functions become even more relevant in the alpine environment, where the complexity of the landscape is often associated with high levels of biodiversity. However, inappropriate stocking rates and deficient pasture management can lead to environmental degradation and a loss of ecosystem functionality due to overgrazing or pastures' abandonment. A rational and adaptive management of livestock distribution is therefore essential to preserve the ecological integrity of these grassland systems, though difficult to apply in extensive mountain grazing environments. In this context, the use of Virtual Fencing (VF) GPS collars, a precision livestock technique that consists of defining the grazing areas from remote and confining the animals through an acoustic and electric stimulus, allows for an almost real-time and spatially explicit regulation of livestock movement without the need to set physical fences. This technology was tested during the 2023 grazing season in an alpine dairy farm in the Eastern Italian Alps (Mount Zoncolan, UD, 1700 m a.s.l.). The animals (35 Bruna dairy cows) reacted well to the coupled stimuli and grazed inside the imposed virtual boundaries for most of the time. Such a fine-scale control enabled the implementation of adaptive and rational grazing patterns. Under a biodiversity conservation approach, a rotational grazing scheme can be used to enhance a differentiated use of the pasture and maintain habitat heterogeneity or, on the other hand, to protect sensitive ecological areas by excluding them from animal grazing during specific periods. As such, VF may represent a powerful tool to align productive livestock farming with biodiversity conservation through an intelligent and adaptive pasture management.

Keywords: Grasslands, Pastures, Adaptive Grazing, Virtual Fencing

Session: Biodiversity in cropping systems

Crop Wild Relatives (CWRs): a promise for the future of the European farming systems

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Crop Wild Relatives (CWRs) are the COUSINs of crops, which can have an important role for broadening the genetic basis of European farming systems. They can contribute to enhancing crop resilience to biotic and abiotic stresses, promoting the agroecological transition, in alignment with the EU Green Deal and the United Nations Sustainable Development Goals.

Currently, 75% of the world's food is generated from only twelve plant species and since the 1900s, plant diversity has been lost as farmers worldwide have left their diverse set of local varieties for genetically uniform, high-yielding varieties. In this frame, 26 partners from 12 European countries joined their effort to set up the European Union-funded project "Crop Wild Relatives Utilization and Conservation for Sustainable Agriculture" (COUSIN - Project: 101135314 - HORIZON-CL6-2023-BIODIV-01). Working on five flagship crops (barley, brassica, lettuce, pea, wheat), the COUSIN main objectives are to: i) identify pathways to use CWRs to strengthen sustainable agriculture; ii) recognize in situ genetic reserves; iii) determine stakeholder-demanded characteristics of CWRs; iv) implement CWRs into breeding and farming activities; v) provide information about CWRs in an accessible format to stakeholders and potential users; vi) train and raise awareness about the value of CWRs in the society. COUSIN will provide new organic heterogeneous materials (OHMs) to use in organic farming to increase the resistance to biotic and abiotic stress and the organoleptic and nutraceutical traits of the produce. CWRs need to be included into national strategies on Plant Genetic Resources for Food and Agriculture, but they are hardly considered. In Italy, for example, the National Biodiversity Strategy to 2030, approved in 2023, doesn't not include any action to protect and conserve CWRs. It is important to raise awareness on the importance of CWRs starting from the scientific community.

Keywords: Crop wild relatives, Organic breeding, Organic heterogeneous materials, National biodiversity strategy

Session: Biodiversity in cropping systems

The European funded project pro-wild: protect and promote crop wild relatives

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Crop wild relatives (CWR) are wild plant species genetically related to cultivated crops. Their untapped diversity can enhance resilience to biotic and abiotic stress and the nutritional quality of modern crops. Wheat, sugar beet, and oilseed rape were selected by Pro-wild because of their importance to food security and EU farmers and because some of their wild relatives are endemic to Europe. Moreover, these CWRs constitute a rich and under-exploited resource needed to face challenges linked to climate change and the transition to lowinput agriculture. The genetic diversity and vulnerability of these CWRs must be better characterized to optimize their conservation and utilization. The objectives of Pro-Wild are to identify priorities for in-situ conservation of the selected CWR gene pools, to survey and complement CWR genebanks collections, and to increase the use of CWRs in crop improvement.

Coordinated by the National Research Institute for Agriculture, Food and Environment (INRAE, France), Pro-Wild associates 18 partners from 11 EU and associated countries with expertise in ecology, conservation, genomics, pathology, microbiology, plant breeding, agriculture, and sociology. Pro-Wild will compile and analyse CWR occurrence maps and perform new in-situ collections. It will predict the vulnerability of several CWR species and populations to ongoing climate changes. Ex-situ collections will be complemented with endangered CWRs accessions. Pro-Wild will investigate the resilience of CWR collections for relevant biotic and abiotic stresses. The identification of CWRderived desired traits and their transfer into elite backgrounds will be done to promote CWR use. Overall, Pro-Wild specific goals will be coordinated with input from breeders, farmers, and consumers. Pro-Wild outcomes will contribute to European Green Deal initiatives through research, education, and training. It will serve the EU biodiversity and the Farm to Fork strategies by preserving, characterizing, and utilising wild species that have unique importance for the resilience of our food systems.

Keywords: Agrobiodiversity, Plant breeding, Plant genetic resources, Biodiversity, Conservation biology, Conservation genetics, Beet, Brassica, Wheat, Genebank, Microbiota, Tolerance to biotic and abiotic stress

Session: Insect biodiversity in the modern era

Exploring biodiversity with molecular and morphological analyses to define species boundaries of the ectoparasitoid *Sclerodermus cereicollis* Kieffer

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The genus *Sclerodermus* comprises over 80 cosmopolitan species of idiobiont ectoparasitoids of many insect taxa, among which many xylophagous or stored product insect pest associated to anthropized environments are present. This genus exhibits high biodiversity, including various morphotypes and pronounced sexual dimorphism. Its role as a biological control agent of pest species and a cause of sting dermatitis in humans makes it a key genus in Bethylinidae family. Understanding *Sclerodermus* biodiversity is challenging due to simplified morphology, limited diagnostic traits, outdated descriptions, difficult access to type specimens, and a lack of identification keys. *Sclerodermus cereicollis* Kieffer, includes syntypes from Annobón Island (Afrotropical) and Giglio Island (European). According to the concept of allopatric speciation, these sites - separated by the Atlantic Ocean and the Sahara Desert - suggest that the two syntype groups likely represent different species. The syntypes of *S. cereicollis* are deposited in the Nat. Hist. Museum of Genoa and were redescribed following Lanes et al. (2020). Two Italian field specimen groups were compared with the type series and used for molecular analysis. We analyzed the mitochondrial Cytochrome Oxidase I gene and reconstructed a Maximum Likelihood phylogeny with seven *S. cereicollis* specimens and 11 outgroups, including other *Sclerodermus* species and bethylids. Molecular analyses showed intraspecific genetic distances of 0-3%, and, together with morphological analyses, confirmed that all analyzed specimens belong to the species *S. cereicollis*. To stabilize taxonomy, we designated a lectotype. Although the syntypes belong to populations found in such distant geographical areas, their morphological differences do not justify classifying the Afrotropical syntypes as a new species. These bethylids often spread through infested furniture, which could explain the presence of two distinct populations in distant geographical areas.

Keywords: Bethylinidae, *Sclerodermus*, Xylophagous insect pest, Stored product insect pest

Session: Microbial biodiversity in foods: evolving insights and uncharted horizons

A 50-year evolutionary history of *Lactobacillus helveticus* associated with the natural whey starters (NWS) of the traditional dairy environment

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This research investigates the genotypic and phenotypic variability between and within *Lactobacillus helveticus* strains isolated in two different periods to explore potential changes that have been undergone over the past 50 years. This approach can reveal species' evolutionary adaptation, aiding the identification of strains with technological and functional traits.

The study leverages a unique collection from the Department of Agricultural and Food Sciences (University of Bologna) of *L. helveticus* strains isolated in 1970 from natural whey starters used in traditional cheese-making. These 20 never-before-revived strains were compared to recent isolates (2023) from the same area, representing the current biodiversity of the species in this environment. Molecular genotyping has been performed by Amplified Fragment Length Polymorphism (AFLP). Safety features were assessed, including antibiotic resistance to EFSA-recommended antibiotics (2012). Phenotypic growth performances and metabolic features were studied by monitoring bacterial growth in culture media and using GENIII microplates on an OMNILOG instrument (Biolog, Inc.).

Preliminary AFLP results of the molecular genotyping suggested significant genetic diversity between the strains from the 1970s and modern strains. Historical strains exhibit lower antibiotic resistance, potentially offering a sustainable approach to mitigate the spread of resistant genes within the dairy sector. Data revealed different growth performances among the tested strains, not only depending on the time of isolation but also highlighting notable biodiversity among the present biotypes.

Understanding the genotypic and phenotypic traits that *L. helveticus* strains have adapted over time is crucial for valorising a unique 1970 microbial collection, harnessing phenotypic reservoirs for innovative applications and fostering the sustainable utilization of microbial diversity in dairy production.

Keywords: *Lactobacillus helveticus*, Microbial biodiversity, Dairy application, Natural whey starters

Session: Microbial biodiversity in foods: evolving insights and uncharted horizons

Citizen Salad: investigating plant leaf microbiota with a citizen science approach

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Lettuce is a widely consumed raw vegetable, making it essential to understand and predict its leaf-associated microbial communities for both plant and human health. While environmental factors strongly influence plant microbiomes, the role of plant-specific traits and the origins of microbial diversity remain unclear.

To bridge these knowledge gaps, we launched a citizen science experiment partially funded through crowdfunding. The campaign attracted 144 donors and recruited 100 participants from across Italy to grow two lettuce varieties and collect soil, environmental, and leaf samples. The project involves diverse contributors, including primary and secondary schools, eco-museums, urban gardens, and botanical gardens.

We will analyze bacterial communities using 16S rRNA amplicon sequencing to disentangle the contributions of plant morphology, soil, and environmental microbial communities.

By integrating public engagement with rigorous, hypothesis-driven research, Citizen Salad aims to advance our understanding of plant microbiomes while fostering scientific literacy and community involvement.

Keywords: Leaf microbiota, Environment, Human health, Citizen science

Session: Microbial biodiversity in foods: evolving insights and uncharted horizons

EPS-producing microbial communities from water kefir in gluten-free sourdough production

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Kefir water is valued for its stable microbial communities associated with kefir grains, which produce exopolysaccharides (EPS) with significant technological potential. These compounds, along with the microorganisms responsible for their synthesis, can enhance food texture and stability. Their application in gluten-free bakery production may introduce variations in microbial communities, leading to new functional attributes. This study aimed to screen and select microbial communities based on their EPS production. Commercial kefir grains were used to ferment kefir water, which was then applied as a starter for rice-flour-based sourdough, routinely back-slopped. The different microbial communities, including lactic acid bacteria (LAB) and yeasts, were characterized using MRS agar with 0.0005% aniline blue and 5% sucrose for LAB and PDA agar with 0.008% ruthenium red and 5% sucrose for yeasts. A total of 70 isolates were analysed, with the vast majority from kefir water demonstrating EPS production. Moreover, 80% of the isolates from sourdough retained EPS production. These findings confirm that kefir-related microbial communities can adapt to the sourdough habitat, unveiling bio-functional properties in terms of EPS production and fermentation. Gluten-free sourdough produced from water kefir could improve the structural and sensory properties of gluten-free leavened products improving their clean label.

Keywords: EPS, LABs, Yeasts, Sourdough

Session: Microbial biodiversity in foods: evolving insights and uncharted horizons

Food-derived *Levilactobacillus brevis* M3R3 as an emerging probiotic: genomic and functional study of Bile Salt Hydrolase activity

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Bile salt hydrolase (BSH) deconjugates bile acids (BA), regulating lipid and glucose metabolism, and microbial homeostasis. BSH, a probiotic trait, is widespread among Lactobacillaceae gut bacteria. *Levilactobacillus brevis*, a probiotic species with QPS status, is found in fermented foods, with many strains exhibiting cholesterol-lowering effect linked to BSH. However, the characterization of bsh genes in *L. brevis* remain unclear. Here we studied the BSH activity in *L. brevis* strain M3R3.

M3R3 genome was sequenced with Illumina platform. bsh genes were identified and kinetic growth response to BA was analyzed. bsh gene expression was determined by RT-qPCR in presence of BA compared to MRS medium. BA profiling was carried out by UHPLC/HR-MS.

78 available *L. brevis* bsh proteins were phylogenetically clustered into three groups (bsh1, bsh2, and bsh3), with several strains with more than one protein. Strain M3R3 had a 2.30 Mbp long genome, with 2375 CDS. Neither AMR genes nor virulence factors were found. M3R3 possessed bsh2 and bsh3 genes, expressed both in control and in presence of BA. BAs exposure decreased kinetic parameters A and λ . M3R3 completely deconjugated glycine-conjugated BAs and app. half of the tauro-conjugated BA, respectively.

By combining genomics and metabolomics, we identified *L. brevis* M3R3 as promising BAs-modulating probiotic with two bsh proteins.

The project was founded by FAR2024 (University of Modena and Reggio Emilia) and by the NRRP, Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n. 3175 of 18 December 2021 of the Italian Ministry of University and Research funded by the European Union - NextGenerationEU. Project Code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP E93C22001090001, "National Biodiversity Future Center – NBFC".

Keywords: *Levilactobacillus brevis*, Probiotic candidate, Sourdoughs, Bile salt hydrolase activity

Session: Microbial biodiversity in foods: evolving insights and uncharted horizons

Galactose utilization profiles in *Streptococcus thermophilus* strains

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Streptococcus thermophilus is widely in the dairy industry. While glucose is rapidly consumed, many *S. thermophilus* strains lack efficient galactose (GAL) uptake or catabolism, leading to its accumulation in the final product. High residual galactose is undesirable due to potential implications for product quality; in fact, its occurrence is correlated with browning of Mozzarella cheese upon heating. In this study, the galactose phenotype of 100 presumptive *S. thermophilus* strains from dairy origin has been studied. The strains were identified through a species-specific PCR. Moreover, strains were typed by RAPD-PCR using GTG₅ primer. The content of lactose, glucose, and galactose was determined by UPLC. All strains were identified as *S. thermophilus*. The analysis of galactose residues among *S. thermophilus* strains revealed substantial variability in galactose utilization efficiency, suggesting the presence of distinct fermentation phenotypes. Residual galactose concentrations ranged from 0.65 to 4.16 g/L, with the majority of strains (92 out of 100) falling between 1 and 2 g/L. In 4 samples, the content of residual galactose was <1 g/L. Based on these values, strains were categorized into three phenotypic groups: low-GAL (≤ 1.3 g/L), intermediate-GAL (1.31–2 g/L), and high-GAL (>2 g/L) users. Approximately one-third of the strains exhibited low residual galactose levels, indicating efficient uptake and metabolism of galactose. Conversely, several strains—most notably those with GAL values exceeding 3 g/L—demonstrated limited galactose utilization, potentially reflecting a gal⁻ phenotype. The glucose was depleted by all strains, suggesting a preferential glucose consumption consistent with carbon catabolite repression. These results showed a galactose fermentation diversity among *S. thermophilus* strains, which could be exploited to develop tailored starter cultures.

Keywords: *Streptococcus thermophilus*, Galactose metabolism, Mozzarella cheese

Session: Microbial biodiversity in foods: evolving insights and uncharted horizons

Gempipe: a computational tool for exploring strain-level bacterial metabolic diversity and screening strains for desired metabolic traits

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Strains of the same species may have phenotypic differences due to mutations, gene losses and horizontal gene transfers, sometimes related to niche adaptation. This diversity is relevant in biotechnology industry: the ability to synthesize specific metabolites varies between strains and can be harnessed for applications in health, food, and bioprocessing.

Genome-scale metabolic models (GSMMs) are in silico tools describing a cell's metabolic potential. They are built on a genome by annotating enzyme-encoding genes, which are translated into a network of metabolic reactions. Assuming a steady state and providing a biomass composition, the flux-balance analysis of a GSMM allows growth simulations on a specified nutritive input and predicts specific abilities for each sequenced strain.

Given the increased availability of genomes, we present Gempipe, a tool that enables the simultaneous reconstruction of hundreds of GSMMs, providing a comprehensive view of strain-specific characteristics and offering valuable insights for comparative and applied studies in biotechnology. Building upon large genome datasets and an optional reference GSMM, it rapidly constructs a pan-GSMM, which is then leveraged to derive and analyze strain-specific GSMMs, enabling the identification of auxotrophies, preferred growth substrates, and potential for target metabolite production.

Gempipe was validated on public datasets and outperformed other state-of-the-art tools in strain-specificity studies, thanks to its hybrid reconstruction mode. A case study on 1000+ *Limosilactobacillus reuteri* genomes demonstrated its ability to cluster strains by metabolic potential, highlighting phenotypic variability beyond the six subspecies proposed in the literature.

Gempipe predicts strains' metabolic potential, helping microbial collections unlock bioresources. Per-strain GSMM availability enables rapid, cost-effective analysis, improving selection beyond trial-and-error screening.

Keywords: Genome-scale metabolic models, Strain-level metabolic diversity, Rational screening, Phenotype predictions

Session: Microbial biodiversity in foods: evolving insights and uncharted horizons

Polyphasic characterisation of lactic acid bacteria diversity associated with sheep milk from “Gentile di Puglia”: starter culture design for Pecorino cheese and for cereal-based fermented beverage

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Preserving and valorising Apulian agrobiodiversity is a key strategy to promote the innovation of agro-food systems associated with regional Marginal Areas (MAs), including a diverse range of animals, plants, and micro-organisms. Fermented foods, in particular, are defined as “those foods or beverages made through controlled microbial growth and enzymatic conversions of major and minor food components”. Fermentation and fermented products represent interesting elements to ensure the transition of food systems toward greater environmental, economic, and social sustainability. “Gentile di Puglia”, literally “Gentle Apulian” in reason of the fine wool, is an autochthonous sheep breed of millenary origins typical of the South of Italy, particularly associated with Foggia province (i.e. Subappennino Dauno and Tavoliere areas). “Gentile di Puglia”, genetically adapted and resilient to the environmental conditions of marginal areas, is also recognised for cheese production. Cross-over fermentation has been defined by Dank et al. as “processes in which a microorganism from one traditional fermentation process is introduced onto a new substrate and/or to a new partner”. Here, we report a case study related to the marginal area of the Subappennino Dauno/Monti Dauni, in the north of Apulian Region, i) isolating lactic acid bacteria (LAB) from sheep milk (breed “Gentile di Puglia”) and ii) performing a polyphasic characterisation of LAB for the design of multi-strains starter culture for Pecorino cheese and the design of a cross-over cereal-based fermented beverage (“Senatore Cappelli” durum wheat variety).

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Keywords: Microbial diversity, Innovation, Plant-based, Fermentative cross-over, Lactic Acid Bacteria

Session: Microbial biodiversity in foods: evolving insights and uncharted horizons

Production of a “fermented lactic beverage” with health and probiotic properties from “Almond milk” of almond Apulian varieties

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The almond represents one of the most common fruit crops in Puglia and is a food rich in unsaturated fats, protein, vitamins and flavonoids. Some of the Apulian most deserving varieties, processed into almond milk, can be used to obtain a highly digestible novel food product that is a vehicle for useful and potentially probiotic microorganisms.

This study involved the production and comparison of two fermented almond milk products one to a mixture of three microorganisms (one yeast and two bacteria) isolated from kefir granules, and a second product obtained using the combination of three strains of lactic acid bacteria (WCFS1, probiotic; B2, riboflavin producer; Lp90, exopolysaccharide producer).

The microbial consortium and the three strains of lactic acid bacteria were separately used for the production of different fermented lactic beverages using three almond milk preparations of the Apulian almond varieties “Montrone”, “Ferrara”, and “Calia”, prepared using a homogenizer and ultrasonic treatment.

The fermented products showed a difference in the final structure of the obtained product. In fact, the one obtained by the microbial consortium from the Eastern European kefir granules exhibits greater homogeneity, the product obtained with the three strains of lactic acid bacteria has two phases, a whey and a clot, both of which are organoleptically sound.

Results from the analysis of fresh almonds and fermented products, obtained by HPLC, gas chromatography coupled with mass spectrometers, GC-FID E-Nose and sensory analysis evaluation will be discussed.

Keywords: Almond, Fermented, almond milk, Probiotic, Microorganisms

Session: Microbial biodiversity in foods: evolving insights and uncharted horizons

Unraveling *Lactobacillus helveticus* species inhabiting cultivable fraction of natural whey starter: a genotype and phenotype-based study

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The lactic acid bacterium (LAB) *Lactobacillus helveticus* is a key player in thermophilic dairy fermentation. LAB also inhabit the human gut, which has often been exploited as a reservoir of potential novel probiotics. We analyzed the cultivable fraction of 12 natural whey starters (NWS) sampled in 2 different sampling times through a DNA barcoding e genotypic approach. Twenty-five candidates were evaluated for tolerance to bile acids (BA), low pH, and lysozyme as probiotic traits.

Pentaplex-PCR and 16S-ARDRA analyses were used as DNA barcoding techniques. (GTG)₅ REP-PCR was carried out to estimate the intra-species diversity (Simpson's index) and dereplicate the clones library. BA tolerance and pH tolerance were assessed by cultivating strains in the presence bile salt mixture (0.05%, 0.3%, 0.6%, and 1%), and at pH of 3.5, 4.5, and 5.4, respectively. Lysozyme (0.1 mg/mL) was used to test lysozyme tolerance.

Inter-species diversity was >0.9 Simpson's index for NWS enriched in *L. helveticus* (type-H), while was slightly lower in NWS enriched in *L. delbrueckii* (type-H). Huge phenotypic diversity was found within a pool of 25 genotypically unique strains. Two candidates showed the best growth response to all three stresses tested.

Two *L. helveticus* strains were found as good probiotic candidates.

The project was founded by Consorzio del Formaggio Parmigiano Reggiano and by the NRRP, Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n. 3175 of 18 December 2021 of the Italian Ministry of University and Research funded by the European Union - NextGenerationEU. Project Code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP E93C22001090001, "National Biodiversity Future Center – NBFC".

Keywords: Bile salts tolerance, pH tolerance, Lactic acid bacteria, *Lactobacillus helveticus*, Natural whey starter

Session: Plant biodiversity: environmental preservation to face global change

Biotechnological approaches of OMIBREED project for sustaining olive genetic resources: micropropagation and in vitro conservation

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The conservation of plant biodiversity has gained worldwide attention, not only for endangered species but also for those with important cultural and economic values, such as *Olea europaea* L. In this context, the Italian Ministry of Agriculture, Food Sovereignty and Forests (MASAF) has funded the project “OMIBREED - Characterization and Valorization of Agrobiodiversity through Multiomic and Next-Generation Breeding Approaches for Resistance to *Xylella fastidiosa*”. The main objective of this research is to identify genetic traits that confer resistance to *Xylella fastidiosa* (Xf) and to adopt advanced techniques such as micropropagation, slow-growth storage, and cryopreservation for the sustainable management of olive tree genetic resources. To achieve this goal, micropropagation in vitro was initiated with explants collected from various varieties. These explants were disinfected and placed on Rugini Olive medium (OM) supplemented with zeatin, mannitol, and Gelrite. For conservation at low temperatures, healthy and high-quality micropropagated shoots were cultured on OM medium containing different concentrations of zeatin, both in the presence and absence of light. Preliminary results have shown the effectiveness of micropropagation protocols and in vitro storage at low temperatures for short periods only for some varieties of olive. This finding will enable rapid clonal propagation, conservation, and enhancement of valuable olive biodiversity, including the genotypes selected by the project and centuries-old plants threatened by Xf in olive groves in the Apulia region. Since cryopreservation protocols are not yet optimised, studies are being conducted to verify how olive genotypes respond to the cryogenic process. Finally, the outcomes of OMIBREED could suggest a strategy to protect and propagate this vital genetic material through clonal methods, ensuring maximum diversity and phytosanitary safety.

Keywords: *Olea europaea*, in vitro culture, Slow-growth storage, Cryopreservation, *Xylella fastidiosa*

Session: Plant biodiversity: environmental preservation to face global change

Characterisation of ancient citrus cultivars from Florentine Medici Villas by the use of Machine Learning techniques

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Originated in the Eastern world, citrus plants now include countless varieties and have been used for centuries for pharmaceutical, cosmetic, food, and ornamental aims, which have made them very desirable among collectors.

In the 16th century, the Medici family gathered and hybridised many citrus varieties, which are now hosted in the Florentine Medici Villas and taken care of following strict techniques, being placed outdoors from April to October while protected in citrus greenhouses during winter. These ancient varieties represent a great potential for the future of agriculture, holding unique morphological characteristics which reflect the historic agronomic practices and the adaptation to specific agricultural techniques.

The aim of this study was to perform a morphological characterisation of the citrus belonging to Medici's ancient collections hosted in Boboli Gardens and Villa Castello - both located in Florence -, using a mapping which dates back to the 1980s.

The dataset was digitised and the variability among varieties was evaluated considering morphological characters of fruit, seed, flower, and leaf, including both quantitative and qualitative variables. Analysis of variance was accompanied with: clustering, to identify homogeneous groups; feature importance, using models based on decision trees (random forest) to examine the contribution of every characteristic to the definition of the groups; PCA, to explore the overall variability.

The preliminary results reveal that some of the ancient varieties show strong morphological similarities, suggesting possible genetic correlations or convergences due to cultivation techniques. In addition, the feature analysis states that fruit weight, seed shape and leaf dimension are among the most discerning variables.

In conclusion, this study represents a substantial step towards the valorisation of the historic agronomic heritage of the Medici Villas, providing useful, objective data for conservation programs.

Keywords: *Citrus* cultivars, Medici Villas, Machine Learning, Morphology, Digitisation

Session: Plant biodiversity: environmental preservation to face global change

Evaluating biostimulant effects on aromatic and medicinal plants cultivation within sustainable Mediterranean agroecosystem

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Mediterranean agro-ecosystems are increasingly vulnerable to climate change with challenges such as soil degradation and biodiversity loss impacting the farming practices. However, the region's dry climatic conditions, limited water resources and rich biodiversity offer unique opportunities for cultivating crops, resilient to both abiotic and biotic stresses. Aromatic and Medicinal plants (MAPs) are well-suited to organic farming, due to low pest susceptibility and reduced input requirements. Greek oregano is a high-value crop with growing global demand and holds a prominent position among MAPs in the Mediterranean. Sustainable fertilization practices can improve oregano's yield and overall product quality. Biostimulants emerged as a promising solution, promoting plant development and improving nutrient use efficiency. This study investigates the effect of various biostimulant treatments on Greek oregano, focusing on agronomic performance and essential oil yield and composition.

Four fertilization treatments were applied to experimental oregano fields using a randomized block design:

1. Control (no treatment)
2. Growers standard fertilization scheme
3. Basic fertilization + Biostimulant 1 + nitrogen fertilization
4. Basic fertilization + Biostimulant 1 + Biostimulant 2.

The essential oils were obtained by using hydrodistillation and the chemical composition was analyzed by GC/MS.

The results indicated that Treatment 4 was the most effective in enhancing essential oil yield, reaching 6.4%. Carvacrol was identified as the main essential oil compound, with the highest content observed in Treatment 3 (72.3%), followed by Treatment 4 (70.8%). Treatments 3 and 4 demonstrated the potential of biostimulants to enhance both essential oil yield and quality as indicated by high carvacrol content. In conclusion, the use of biostimulant-based fertilization schemes can reduce chemical inputs in oregano cultivation and improve the quality.

Keywords: MAPs, Greek oregano, Biostimulants, Sustainable agriculture, Mediterranean agroecosystems

Session: Plant biodiversity: environmental preservation to face global change

From genetic diversity to phenotypic diversity: characterization and potential of a Historical Lemnoideae Collection

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The Lemnoideae, an aquatic subfamily of the Araceae, diverged from their terrestrial ancestors ~40-50 Mya. Fossil evidence suggests a common pre-clade in North America, followed by rapid global dispersal. This enabled morpho-physiological adaptation to a floating lifestyle in eutrophic freshwater bodies across different climate zones.

The renewed interest in Lemnoideae, long known for water remediation, is currently driven by their remarkably high growth rate and valuable biomass, rich in proteins and starch, making them suitable for both animal and human consumption. Four species have been recently approved by EFSA as novel food, making them an emerging crop.

To study their genetic diversity and evolution, we started the molecular characterization of about 600 accessions from the historical Landolt collection, now at IBBA. The principal method employed was the Tubulin-Based Polymorphism (TBP), based on length polymorphisms in intron regions of β -tubulin genes, which provided a reliable clustering of accessions at species level. Plastid marker sequencing, genome size measurement and chromosome counting integrated this nuclear marker. Despite similar morphologies, results revealed high genetic diversity and phylogenetic complexity, including the existence of cryptic interspecific hybrids and variable ploidy in *Lemna*. Morphology-based classifications often proved inaccurate.

To assess ecophysiological diversity, we compared clones of representative species for their growth performance on dairy effluents, converting nutrients into valuable biomass. Results showed both inter- and intra-specific variation, with a triploid clone of *L. × mediterranea* exhibiting superior biomass increase and a specific *L. minor* accession displaying higher stress tolerance.

Such diversity supports duckweed exploration in agrifood systems, their role as bioindicators in ecotoxicological assays, and their use in nutrient recovery within a circular economy framework.

Keywords: Lemnaceae, Molecular Phylogeny, Emerging crop, Phytoremediation, Ecotoxicological assays

Session: Plant biodiversity: environmental preservation to face global change

Genetic analysis of Sicilian forest trees as a tool to improve the management and conservation of local resources in face of global changes

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Several surveys have highlighted the enormous value of the genetic heritage of Sicilian forests, which, despite covering less than 10% of the island, host the richest tree floras in Italy. Many species live here at the southernmost edge of their distribution range; hence may have developed particular adaptations to extreme environmental conditions, which make them of high interest for reforestation in an increasing alarming scenario of global warming. In this context, the study of the genetic diversity of local trees is of fundamental importance to implement management and conservation measures for the island's forest. Our research (financed by the Sicilian Regional dept. of Rural and Territorial Development) focused on studying the intra- and interpopulation diversity of several forest trees as *Acer* (*platanoides*, *pseudoplatanus*, *obtusatum*, *monspessulanum*), *Alnus glutinosa*, *Quercus petraea*, *Fraxinus excelsior*, *Ostrya carpinifolia*, *Malus sylvestris* and *Prunus mahaleb*. Indices of genetic diversity within population as expected and observed heterozygosity (H_e , H_o), inbreeding coefficient (F_{is}) and differentiation among populations (F_{st}) were calculated. A Bayesian approach was performed, using the software STRUCTURE v.2.3.4, to identify the different gene pools. The results highlight a low genetic variability of *A. platanoides* stands and the presence of complex introgression processes between *A. pseudoplatanus* and the other two maple species. As for *O. carpinifolia* and *M. sylvestris*, the presence of the same gene pool was recorded in all stands on the island, while a clear genetic divergence emerged between the stands of *P. mahaleb*. We provide indications on the biogeographical history, the natural dispersal capacity and the possible impact of human activities on the current genetic pattern of the species. Moreover, we underline the importance of studying genetic diversity in order to implement conservation and management programs.

Keywords: Genetic diversity, Conservation, Global change, Forest Trees

Session: Plant biodiversity: environmental preservation to face global change

Genetic and functional traits variability of Sardinian populations of strawberry tree (*Arbutus unedo* L.)

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Strawberry tree (*Arbutus unedo* L.) is an evergreen shrub belonging to the Ericaceae family and a common element of the flora and vegetation of the Mediterranean basin. It is a highly vigorous resprouting species that can form forests, particularly in wildfire prone regions. At the genetic level, the species is diploid ($2n = 26$) with an estimated genome size of 450 Mb approximately. Only few papers dealing with the genetic characterisation and the determination of the population structure of strawberry tree (ST) are available, usually based on medium throughput approaches or on a defined number of markers, and no in-depth research have been carried out so far in Italy. In this study, 116 single plants belonging to 8 wild populations of ST from different sites in Sardinia (Italy) were sampled and analysed by using the Genotyping by Sequencing approach. Sampling sites were selected in areas, where STs grow spontaneously, characterised by different altitude, climate and geography in terms of sun exposition, temperature, water availability, soil types and composition. One of the sites was subject to a major fire a few years ago. Further, plant materials from all the individuals, fully expanded leaves, shoots of the year and leaves of different size, were sampled to assess functional traits: Huber Value, Leaf Mass per Area, Leaf Dry Matter Content, Leaf Size. A high level of genetic variability was observed, with each plant being unique and different from the others. Concerning the 8 populations, two of them were clearly different, while all the others present a very high level of intra-population variability, higher than the inter-population variability, preventing them to be clearly separated. Removing the two most different populations from the analyses, plants from other populations appeared to be more dispersed and other intermediate clusters could be defined. At the phenotypic level it is possible to see that all four traits vary between sites, but only moderately.

Keywords: Strawberry tree, Genotyping by sequencing, SNPs, Functional traits, Genetic variability

Session: Plant biodiversity: environmental preservation to face global change

Micropropagation and in vitro conservation of "Patata dei Cortigli" germplasm

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Patata dei Cortigli, also known as patata del bosco, is a local Apulian variety of *Solanum tuberosum* L., found in clearings and forest edges on the Gargano promontory, between San Giovanni Rotondo and San Marco in Lamis. It represents a significant example of native agrobiodiversity, the result of both natural and human selection processes, which have enabled the variety to adapt to the specific soil and climate conditions of the area. As part of the project "Biodiversity of non-fruit vegetable species of Puglia - BiodiverSO VEG" (Rural Development Programme Puglia 2014-2020, action 10.2.1), a study was initiated on the potential application of micropropagation protocols to this genotype. Tubers provided by the Department of Agricultural Sciences, Food, Natural Resources and Engineering of the University of Foggia were conserved at 4 °C in the laboratory of micropropagation and microscopy at the Department of Soil, Plant and Food Sciences (UniBa). Subsequently, they were placed in a mixture of blond peat, brown peat, and perlite to induce sprouting. Axillary buds of approximately 0.2 cm were collected from the resulting sprouts and sterilized for in vitro introduction. The buds were then placed on a nutrient substrate (base medium - MB), solidified with agar and enriched with different concentrations of 6-Benzylaminopurine (BAP). After about three weeks, the shoots that had developed at least 6 nodes were multiplied using binodal cuttings and transferred to a renewed hormone-free nutrient medium. From that point on, the culture was subcultured every 25 days, showing a tendency toward tillering, resulting in an average multiplication index higher than 8. Rooting was observed without the need to add auxinic plant growth regulators. The plantlets obtained will soon be transferred to appropriate conditions for slow-growth conservation.

Keywords: *Solanum tuberosum* L., Local variety, Tuber, Shoots, 6-benzilaminopurine

Session: Plant biodiversity: environmental preservation to face global change

Morphological traits of *Olea europaea* leaves in Italian Germplasm Collections

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The olive tree (*Olea europaea* L.) is a crop of global importance, with worldwide olive oil production exceeding 3 million tonnes in the 2024/2025 season (ISMEA, 2024), marking a 23% increase compared to 2023/2024. In contrast, Italy's production declined to 224 thousand tonnes, reflecting a 32% reduction from the previous season. Unfavorable conditions linked to the pathogen *Xylella fastidiosa*, responsible for the death of thousands of plants, and climate change have severely impacted southern Italy's production. Thus, the recovery, study, and conservation of olive agrobiodiversity are essential to maintain a genetic pool for breeding programs. This study aimed to morphologically characterize twenty-four olive cultivars from the Italian germplasm.

Twenty-four olive cultivars grown in CNR (Florence, Italy) collection field under uniform agro-environmental conditions were analyzed. Mature leaves were subjected to:

- Color analysis (Konica Minolta CR-400),
- Macro- and micro-morphological measurements (Leica DM LB2 microscope; TESCAN GAIA3),
- Chlorophyll a fluorescence transient and JIP-test (Handy-PEA, Hansatech Instruments).
- Statistical analyses included ANOVA and multivariate methods (Cluster Analysis, PCA).

All examined traits exhibited significant variability among cultivars, highlighting distinct leaf characteristics. Two leaf color groups were identified: Light green ($L^* > 40$, e.g., "Sant'Agostino", "Picholine") and Dark green ($L^* \approx 34$, e.g., "Leccio Maremmano"). Anatomical analysis revealed statistically significant differences in total leaf thickness, ranging from 330.6 μm ("Maurino") to 488.6 μm ("Nocellara del Belice"), indicating structural diversity. The Fv/Fm index (about 0.8) suggested good photosynthetic efficiency.

The parameters evaluated in this study could facilitate the screening of cultivars resilient to adverse climatic conditions (e.g., drought, high temperatures).

Keywords: Olive cultivar, Trichomes, Leaf anatomy, Color, Fv/Fm

Session: Plant biodiversity: environmental preservation to face global change

Nutritional profile of edible wild species from “Laguna del Re” Oasis

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This study aims to determine the key nutritional and anti-nutritional compounds and the antioxidant capacity of four spontaneous edible species (sea beet, bristly-ox-tongue, soft thistle, golden thistle). These obligate/facultative halophytes were collected during the spring period from “Laguna del Re” Oasis (Siponto, Manfredonia, Foggia province), a coastal wetland along the Adriatic Sea with high ecological and agricultural significance for the conservation and valorisation of edible wild species. Sea beet (along bristly-ox-tongue) exhibited the highest concentration of cations as well as carotenoids, phenolic compounds, and vitamin C. Sea beet also proved the highest antioxidant capacity. Sea beet showed the highest nitrate and oxalate levels, though both remained within safe limits for human consumption. Bristly-ox-tongue stood out for its high anthocyanin and flavonoid content. In contrast, soft thistle exhibited the lowest concentration of cations, antioxidant compounds and antioxidant capacity. Both soft-thistle and golden thistle were distinguished by their highest iodine content (129 µg 100 g f.w., on average), indicating that a 120 g serving portion could meet the Recommended Dietary Allowance for an adult. The examined species had an average Na content of 3.6 g kg⁻¹ f.w. and a relatively high Na/K ratio (1.5), attributed to the saline conditions of their habitat. To avoid exceeding the recommended daily Na intake (2 g day⁻¹), consumption should be limited to 500 g per day.

This study highlights the nutritional potential of spontaneous species from bio-saline environments; in particular, sea beet and bristly-ox-tongue stand out for their antioxidant and mineral content, while soft thistle and golden thistle stand out for their iodine content.

Keywords: Antioxidants, Iodine, Sea-beet, Bristly-ox-tongue, Soft- and golden-thistle

Session: Plant biodiversity: environmental preservation to face global change

Propagation of *Achillea maritima* for its re-introduction in the Marine Protected Area of Torre Cerrano (TE), Italy

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The Marine Protected Area (AMP) "Torre del Cerrano" (Abruzzo, Italy), recognized as a Special Area of Conservation (SAC IT7120215) by the EU, hosts dune ecosystems with highly valuable species of psammophilous vascular flora. The SEED (Spread and Store Coastal Biodiversity) project, funded by Spoke 8 NBFC, involved the creation of a Germplasm Bank for ex-situ plant conservation and propagation used for rehabilitation of degraded dune sites. Particular attention was paid to an Asteraceae, which has disappeared from much of the Abruzzo coastline, with the aim of reintroducing it into the AMP: *Achillea maritima* (L.) Ehrend. & Y.P. Guo ssp. *maritima*. Antibacterial, antifungal, anti-inflammatory, and anticancer effects have also been ascribed to this plant.

Collection, cleaning and preservation of germplasm of native psammophilous plant species, including this species. On the latter: assays of in vivo and in vitro propagation protocols in controlled growth chambers; morphological study at M.O. Axio Zoom V16; analysis of chlorophyll and carotenoid content and antioxidant activity.

In vivo seed germination at 9 weeks reached about 20% while in vitro propagation required several trials to achieve sterility and a longer germination period. Physiological analyses performed on the aerial part of the plants showed a fair content of carotenoids (44.06 µg/g FW) and chlorophylls (average content 406.48 µg/g FW). Differences emerged between early germination stages and adult plants, particularly in chlorophyll content, 620.93 µg/g FW and 458.40 µg/g FW respectively. Antioxidant activity showed values of 30-31 IC50, indicative of strong antioxidant capacity.

The preliminary results obtained on *A. maritima* are being used for its propagation and reintroduction into the wild. An ecological characterization of the optimal germination conditions (salinity level and seed dormancy period) will be the next step in the research.

Keywords: Psammophilous flora, Germoplasm bank, Antioxidant activity, Seed germination, Plant conservation

Session: Plant biodiversity: environmental preservation to face global change

The “ancient apple” varieties of “Central Italy” an inexhaustible source of biodiversity and health

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The varieties of ancient apples from Central Italy are very numerous, highlighting how “fortunately” the biodiversity of these fruits is still wide. Furthermore, in many cases they also have high contents of phenolic compounds and anthocyanins with antioxidant and anti-inflammatory properties, which increase their “health” value. In this work, the content of these bioactive molecules of some varieties of apples were studied: Sanguinella and Limoncella from Molise, Roggia and Rossa di Todi from Umbria.

The apples were cooled, homogenized and then extracted with a 70-30 water/ethanol solution. The extracts were stained with Folin-Ciocalteu reagent and read spectrophotometrically against a standard calibration curve of gallic acid at 765 nm to determine the total phenolic compounds. For total anthocyanins, the extracts were read directly spectrophotometrically at 518 nm. The antioxidant capacity was also determined with the ORAC degree by measuring the ability to protect fluorescein from degradation induced by radical activity, then expressing the results as TE (Trolox Equivalents), according to the method (Cao et al., Free Radic. Biol. Med., 1993, 14, 303-311).

Sanguinella and Limoncella apples showed a total phenolic compound content of 2248±67 and 2346±72 mg/Kg respectively referred to the dry substance, while Roggia and Rossa di Todi apples showed a content of 2985±87 and 2588±82 mg/Kg respectively. For the Roggia apple, the ORAC degree was then determined which gave a result of 4570±275 mmol TE/100g.

These preliminary results are very interesting and deserve a molecular investigation by evaluating the LC-MS profile of both phenolic compounds and anthocyanins to obtain an antioxidant and anti-inflammatory “Finger Print” of these very interesting ancient apples from Central Italy.

Keywords: Ancient apple, Central Italy, Phenolic compounds, Orac, anthocyanins

Session: Plant biodiversity: environmental preservation to face global change

The "Traditional" varieties of grains, an inexhaustible repository of biodiversity: the "Saragolletta Rossa Molisana"

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The "Saragolletta Rossa Molisana" has been almost abandoned and it is very difficult to find data, there are only studies on the characterization of the Saragolle (*Triticum turgidum* subsp. *durum* of Abruzzo) that have shown how these varieties are a precious repository of biodiversity. Previous studies have identified genetic differences between *Triticum aestivum* and Saragolletta del Sannio. In this study we want to verify whether the population of seeds currently found in Molise, is a distinct variety or overlapping with the Saragolle that insist in the same area.

Three different plots were sown in the municipality of Castel del Giudice (IS) and the sowing will then be repeated for two consecutive years in the same conditions, with a basic technique. We expect significant differences in production due to different slopes and shading and for this purpose, we have geolocalized the plots by creating a grid with five square meter meshes. In each sector, surveys were carried out and then cross-referenced the data with those of the nearest meteorological station, to verify the existence of correlations with temperature and precipitation.

At the moment, data are available for the primary phenological phases developed so far, as the three plots were sown at an altitude of over 800 meters. In fact, the cultivation is still in the tillering phase, so in addition to the soil horizons, only the germination times, dry matter and the formation of culms for the tillering potential were evaluated. The protein content (14.1 ± 0.4 g/100g of dry matter) and total phenolic compounds (65 mg/Kg as gallic acid) were also determined on a small portion of unsown grain.

Obviously, it will be necessary to wait for the first definitive results in June to evaluate the effect of the particular area and the sowing altitude on the characteristics of the grain which, especially in terms of proteins, appear to be already good.

Keywords: Saragoletta Rossa Molisana, Repository of biodiversity, Saragolle, Protein, Phenolic compounds

Session: Plant biodiversity: environmental preservation to face global change

Uncovering the genetic diversity of Tunisian monumental olive trees to promote the olive sector

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Centenary olive trees in Tunisia have a great symbolic cultural value of resilience. Neither the genetic diversity of these historical trees nor their commercial potential have been studied so far. Our study represents the first molecular analysis of historical olive trees in Tunisia.

The genomes of twenty-eight ancient olive trees from archaeological sites of Tunisia were analysed using nine highly polymorphic microsatellite markers. The genetic profiles were then compared with those of local varieties and other regions of the Mediterranean basin.

The study revealed the remarkable polymorphism of these ancient trees, which showed a high degree of observed heterozygosity and a remarkable allelic richness. Relatedness and structural analyses have shown that the centenary trees share a common genetic background with some Tunisian cultivars but also with varieties from Italy, Spain and Greece. These results indicate that the Tunisian germplasm has a common ancestry and that there has been a significant genetic exchange between olive plants in the Mediterranean region. The conservation of these unique trees is crucial and requires the implementation of effective conservation measures and strategies to ensure ecological benefits for future generations.

Keywords: Olive, Microsatellites, Genetic exchange, in situ conservation

Session: Agro-environmental microbiology: emerging perspectives and new frontiers

Agroecological innovation through green mulch transplanting: enhancing soil health and biodiversity in mediterranean organic horticulture

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SORBioTraP (Development of an Organic Horticultural System Based on Transplanting into Green Mulch) is a multi-stakeholder project financed by the Italian Ministry of Agriculture, Food Sovereignty, and Forests (MASAF), supporting agroecological transition and biodiversity conservation in Mediterranean horticultural systems. It addresses the critical challenge of weed management in organic farming by applying an innovative transplanting technique into green mulch (living or terminated cover crops). This strategy aims to improve soil fertility, reduce mechanical inputs and irrigation needs, and enhance crop resilience.

The project operates across different environments (open field and greenhouse), seasons, and mulch compositions (e.g., rye-vetch, Sudan grass), employing a sod transplanting machine adapted from Northern Europe. The project is focused on a microbiology-based evaluation of soil health. A dual analytical strategy integrates microscopic methods for assessing viable and metabolically active microorganisms, with metabarcoding (NGS) to characterize the taxonomic diversity and functional traits of bacterial and fungal communities. This approach will provide insights into microbiome composition, diversity, and ecological responses under the different soil management treatments highlighting their impact on microbial composition and activity. Complementary assessments will include soil mesofauna, weed flora, crop productivity, water use efficiency, and socio-economic impacts. The involvement of farmers in shaping project strategies ensures both contextual applicability and scalability of the proposed practices.

SORBioTraP will contribute to enhance Mediterranean biodiversity through soil conservation, reduced external inputs, and agrobiodiversity valorization. These outcomes align with EU Farm to Fork and Soil Health and Food strategies, offering a replicable model for sustainable organic horticulture.

Keywords: Agroecology, Green mulch, Soil microbiome, Organic horticulture, Sustainable farming

Session: Agro-environmental microbiology: emerging perspectives and new frontiers

Exploiting yeast isolates from pear cv. Abbé Fétel carposphere as biocontrol candidates against *Stemphylium vesicarium*, the causal agent of the brown spot disease

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Microbial biodiversity is essential for plant health. The EU Farm to Fork Strategy restricted pesticide use in agriculture. This study explored the potential of yeasts inhabiting pear cv. Abbé Fétel carposphere as candidate biocontrol agents (BCA) against *Stemphylium vesicarium*, causal agent of brown spot of pear (BSP).

In 2024, pear fruits were collected from thirteen Emilia-Romagna orchards conducted with organic and integrated pest management, respectively. Yeasts were isolated on YPDA medium, morphologically characterized, and in vitro screened for their antimicrobial activity against *S. vesicarium*. Then, a selection of 25 yeast isolates were molecularly identified by sequencing the rDNA ITS region.

A biobank of 260 isolates was established, displaying a mycelia growth inhibition of *S. vesicarium* ranking from -15.71% to 42.48%. The yeasts were attributed to *Aureobasidium pullulans*, *Metschnikowia* spp., *Rhodotorula babjevae*, *Pichia kluyveri*, and *Hanseniaspora uvarum*. One isolate belonged to *Papiliotrema horticola*, an endophytic yeast previously associated with apple fruits. Sequencing of ITS amplicons failed for *Metschnikowia* spp. isolates, according to the well-documented rDNA heterogeneity within this clade. When yeast BCA candidates were tested against different *S. vesicarium* strains, their antagonistic activities were slightly variable, depending on the *S. vesicarium* strain used.

The presence of diverse yeast species with antagonistic activity against *S. vesicarium* highlights the pear carposphere as a reservoir of potential novel BCAs for effective and sustainable pathogen management. In planta experiments will investigate their ecological role and potential in controlling BSP.

Funded by the Emilia Romagna region within Reg. Law 17 del 27/10/2022; Operation: "Urgent interventions to support the agricultural, agri-food, fishing and reclamation sectors" Art. 1 - Project "A.MA.PERO".

Keywords: Epiphytic yeasts, *Pyrus communis*, Biocontrol, Agro-environmental microbiology, Sustainable agriculture.

Session: Agro-environmental microbiology: emerging perspectives and new frontiers

Fermented milk fortified with polyphenols extracted from olive leaves and functional strains: preliminary data

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Consuming foods enriched with functional ingredients could reduce the risk of chronic diseases and improve physical and mental well-being. The project FUNDAIRIES (granted by PNRR, NextGenerationEU - PE_00000003) aims to develop new functional dairy products by adding probiotic strains and bioactive polyphenols recovered from olive leaves (PE). This study was carried out to assess the effect of PE on the growth of two probiotic strains, *Bifidobacterium animalis* subsp. *lactis* BB12 and *Lactiplantibacillus plantarum* UNICA B26, in fermented sheep's milk (FM).

A preliminary screening was conducted using the broth microdilution method to analyze the antibacterial activity of PE and establish the MIC towards the two probiotic strains. The ability of the strains to tolerate PE concentrations of 20 and 10 mg GAE 100 g⁻¹ was then assessed. The FM was produced using either the BB12 or the UNICA B26 strain with and without PE at 20 mg GAE 100 g⁻¹. FM samples underwent microbiological analyses to evaluate the impact of PE on probiotic growth and viability, as well as sensory analyses to assess the influence of probiotics on the sensory profile of the final product.

Both strains were not affected by PE concentrations tested under the experimental conditions applied. The acidification curves of FM showed a similar pH decrease for samples added with probiotics only and with PE, suggesting that polyphenols do not affect the starter activity. FM added with BB12 and UNICA B26 plus PE showed similar microbial counts during the whole shelf-life. Differences in sensory characteristics were only observed for the samples fortified with PE.

Overall, data suggests that the addition of PE to FM did not negatively affect the acidification process or the viability of both probiotic strains during refrigerated storage. This supports the potential use of polyphenols to enhance the functionality of probiotic fermented dairy products.

Keywords: Functional food, Probiotic, Polyphenols, Dairy products

Session: Agro-environmental microbiology: emerging perspectives and new frontiers

Isolation and characterization of yeast diversity in nectar of *Onosma* spp.

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Over the last ten years, significant research has been conducted on the microorganisms present in floral nectar and how they affect nectar composition. These microorganisms transform sugars, amino acids, and various compounds, leading to alterations in the chemical profile of nectar. Due to its high sugar concentration, along with proteins and secondary metabolites (NSCs), nectar acts as a selective barrier against microbial colonization. NSCs not only possess antimicrobial properties but also potentially affect interactions with other organisms. Some yeast species are specifically adapted to flourish under the high osmotic pressures found in nectar, yet their diversity and ecological significance remain largely neglected. Investigating the connection between yeast diversity in nectar and other plant-related microhabitats could provide valuable insights into their ecological functions. It is essential to establish a standardized method for collecting and characterizing yeasts from nectar to facilitate further research. The genus *Onosma* (Boraginaceae: Lithospermeae) includes plants that are particularly suitable for such investigations because of their abundant nectar production, as well as their floral morphology and orientation, which minimize dilution from rainfall. These characteristics may reduce random variability and mitigate potential errors associated with limited nectar availability volumes.

This study aims to investigate yeast diversity in floral nectar, their ecological roles, and their interactions with secondary metabolites, using the genus *Onosma* as a model system.

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Keywords: Microhabitat, Antimicrobial properties, Ecological functions

Session: Agro-environmental microbiology: emerging perspectives and new frontiers

Multispecies bioinoculant enhances sugarloaf chicory (*Cichorium intybus* L.) performance while modulating rhizosphere microbial diversity and functionality

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The use of bioinoculants in organic farming is increasing due to their ability to enhance crop yields, boost immunity, and suppress soil-borne pathogens. They offer a sustainable alternative to chemical fertilizers, which harm soil health and the environment. However, the large-scale introduction of microorganisms raises concerns about potential disruptions to native microbial communities, which are crucial for soil resilience. Understanding these effects is key to sustainable bioinoculant use.

This study evaluated Micosat F® UNO-a bioinoculant based on a mixture of mycorrhizae (*Rhizophagus* spp.), beneficial fungi (*Trichoderma harzianum*), and other microbes-on sugarloaf chicory (*Cichorium intybus* L. var. *foliosum*) cultivated in an organic farming system. Bioinoculated plants had higher post-transplant survival (99.8% vs. 97.2%), reduced disease incidence (88.7% vs. 72.7%), and greater net yield (397 vs. 320 q/ha) than controls. Illumina shotgun metagenomic sequencing was performed to evaluate shifts in rhizosphere microbiota. Alpha diversity measurements showed significantly higher evenness in bioinoculated soils ($t(17) = 2.35$, $p = 0.0312$). Moreover, bioinoculation altered the beta diversity of microbial assemblages, as supported by PERMANOVA of Bray-Curtis dissimilarity ($p = 0.0091$, $R^2 = 0.10687$). Functional gene characterization showed no significant difference in total gene abundances, though treated samples had lower overall functional gene abundances. These findings suggest that bioinoculant-induced microbiota variations enhance plant growth and may sustain long-term fertility, paving the way for further research on plant-microbiome interactions.

Keywords: Bioinoculant, Soil biodiversity, Metagenomics, rhizosphere, Sugarloaf-chichory

Session: Agro-environmental microbiology: emerging perspectives and new frontiers

Surviving the cold: unraveling molecular strategies of *Mrakia gelida*'s resilience in freezing conditions

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Cold-adapted yeasts, such as *Mrakia gelida*, not only play a critical role in organic matter turnover in cold habitats but are also considered valuable biotechnological resources, thanks to their ability to thrive in very low-temperature environments. However, further genetic investigation is required to comprehensively elucidate the mechanisms underlying their cold-stress responses. In this study, three strains of *M. gelida* were grown in YPD liquid media at their optimal temperature (15°C), then exposed to cold stress (4°C), and subsequently returned to 15°C. Biomass from each phase was processed for RNA extraction and RNA-seq analysis to characterize transcriptomic changes associated with cold adaptation and recovery. Preliminary findings indicate that, when comparing cold stress to the control, there is an overexpression of CDSs related to membrane transporters, detoxification-related proteins, and enzymes involved in expanding the range of utilizable carbon substrates under cold stress conditions. Additionally, in the comparison between stress and restored conditions, these CDSs show expression levels similar to the control, suggesting a return to baseline transcription after cold stress. These findings suggest that *M. gelida* utilizes diverse strategies to preserve cellular homeostasis and metabolic flexibility during cold stress. The results enhance our knowledge of psychrophilic yeast biology and may contribute to future biotechnological advancements, including the development of enzymes and other products tailored for cold environments.

Keywords: *Mrakia gelida*, Psychrophilic yeasts, Cold stress response, Transcriptomic analysis, RNA-seq

Session: Agro-environmental microbiology: emerging perspectives and new frontiers

Synthetic microbial communities assembly for bacterial cellulose synthesis optimization

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Bacterial cellulose (BC) is a biopolymer with many industrial uses. Within PRIN 2022-SynBioCell project (code: 20228Z34PF), to enhance BC production, Synthetic Microbial Communities (SMCs) consisting of acetic acid bacteria (AAB) as core microbial components, lactic acid bacteria (LAB), and yeasts (Y) were developed and studied.

Komagataeibacter xylinus K1G4, K2G30, K2G30 Δ gdh, *K. rhaeticus* K2G14 (AAB), *Lacticaseibacillus casei* N87, *Lactiplantibacillus pentosus* O17, *Levilactobacillus brevis* LB12 (LAB), *Brettanomyces bruxellensis* KCO149, *Kluyveromyces lactis* UMCC701, *Saccharomyces cerevisiae* UMCC165 (Y) strains were used for SMC assembly. A Plackett-Burman design (PBD) was used to screen strain significance in BC production (static growth system, HS medium, 28°C). Top SMCs were reassembled to study AAB, LAB, and Y interactions during BC production, and the best SMC was tested for LAB's stimulatory effect on BC production. Single AAB strains served as control. Finally, a second PBD was used to select top SMCs by modelling key factors affecting BC yield.

SMC2 (K2G30, K1G4, O17, N87, LB12), SMC9 (K2G30, K2G30 Δ gdh, O17, KCO149, UMCC701) and SMC11 (K2G30 Δ gdh, K1G4, K2G14, N87, UMCC701) were top BC-producing while SMC7 (K2G30 Δ gdh, N87, O17, UMCC165, KCO149) was the worst. Unlike Y, LAB actively moved to BC layer. O17 and LB12 were the most stimulant LAB, while K2G30 and K1G4 were the most performing AAB strains. AAB and LAB settled in BC layers. PBD revealed incubation temperature and carbon source amount as the key factors affecting BC yield, identifying SMC9 as the highest BC-producing SMC during the process.

A selected SMC optimized BC production, unravelling interactions among AAB, LAB and Y. Lactic acid and ethanol exchange likely boosted BC production by AAB, highlighting LAB and Y as effective helpers. Further metabolic and transcriptomic studies will be performed to clarify SMC members interactions and roles.

Keywords: Synthetic microbial communities, Acetic Acid Bacteria, Bacterial cellulose, Microbial interactions, Microbial communities

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ABSTRACT BOOK

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1- CHALLENGES FOR RUMINANT TICKS AND TICK-BORNE DISEASES CONTROL IN THE MEDITERRANEAN REGION

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Vector-borne diseases (VBDs) are widely distributed across the globe. The majority of these diseases, classified as metazooses, can be transmitted to both humans and domestic or wild animals through invertebrate arthropod hosts, particularly ticks. According to the WHO, VBDs account for more than 17% of all infectious diseases worldwide, resulting in over 700,000 human fatalities annually. The global “One Health” concept brings together three constantly relevant and inherently interconnected components: human health, animal health, and the environment. It emphasizes the identification of risk factors, modes of transmission, as well as the development of prevention and control measures for both zoonotic and non-zoonotic diseases. This triad can be disrupted by factors such as population growth, globalization, urbanization, climate change, travel, and changes in land use, all of which contribute to the emergence of VBDs. From an economic perspective, the impact of tick-borne diseases extends to four major populations: humans, economically significant livestock, companion animals, and wildlife. The annual economic losses associated with these diseases amount to billions of dollars. Despite the wide range of climatic conditions that support tick abundance and the development of all life cycle stages, significant efforts have been directed toward reducing parasitism with these arthropods. The occurrence and spread of tick-borne diseases are directly dependent on the presence of competent vector species. Understanding the biology of these bacterial, parasitic and viral pathogens requires the identification of one or more host species, and their geographic distribution may be narrower than that of their tick vectors. Among the tick species involved in pathogen transmission, the most frequently reported—ranked in descending order—are *Ixodes ricinus*, *Hyalomma marginatum*, and *Dermacentor marginatus*. In recent decades, multiple chemical control strategies have been implemented, particularly in developing countries, to mitigate the effects of tick infestations. However, the methods for controlling both ticks and the diseases they transmit remain unclear. Our contribution highlights a rising occurrence and prevalence of tick-borne Ruminants diseases in Mediterranean region, underscoring the need for thorough monitoring of farms and vector hotspots—ideally within a “One Health” framework.

Keywords: Mediterranean region, ruminants, ticks, One Health.

2- CLINICAL EXPERIENCE WITH INFECTIOUS DISEASES IN CATTLE IN THE MEDITERRANEAN REGION

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Introduction

Since the Mediterranean Region is located in the subtropical region, many infectious diseases are observed. The close border of the Mediterranean Region with the tropical region in Africa increases the risk of infectious diseases. Turkey is a country located in the Eastern Mediterranean Region. However, it also borders and is close to Middle Eastern countries and West Asian countries such as Iran. Therefore, while infectious diseases are seen in Turkey like other Mediterranean countries, diseases from Asian and Middle Eastern countries are also seen from time to time.

Ephemeral fever has been observed in Turkey since 1985 in the south-southeast of Türkiye with outbreaks every 3-4 years. Ephemeral fever, also known as 3-day disease, may have high morbidity, but mortality is low, because it usually recovers in 3 days. However, in the outbreaks after 2008, the outbreaks did not progress as recovered in 3 days as before, and the mortality rate was higher with serious lung problems. Because ephemeral fever caused by Taiwan serotype was observed in these years. In recent years, live vaccine has been used in the region and thus there is no significant problem.

Lumpy skin disease (LSD) was first detected in Kahramanmaraş in the Southeast Region in 2013. In 2014, it caused a widespread epidemic in Turkey. It causes serious systemic problems in cattle and may cause deaths. The disease has been taken under control with annual vaccination against this disease in spring and has not been seen in recent years.

Epizootic Hemorrhagic Disease (EHD) was first clinically detected in Turkey by us in Muğla in 2007. This disease was laboratory confirmed to be EHD-6. Akabane caused a widespread outbreak in 2010. In recent years, akabane is not seen as a significant problem.

Foot-and-mouth disease (FMD) has been seen in Turkey for many years. There are 7 main serotypes of this infection, namely A, O, C, SAT1-SAT2, SAT3 and Asia1. A, O and Asia 1 serotypes have caused outbreaks in Turkey for many years. For many years, trivalent vaccine was used against A, O and Asia1. In 2023, SAT2 serotype was observed in Africa and the Middle East and tetravalent vaccine was administered against A, O, Asia1 and SAT2 in Turkey. At the beginning of 2025, SAT1 Bahrain strain was detected in Turkey. Today, pentavalent vaccine is used.

Conclusion

Since Mediterranean countries are located in a risky climate zone, they should pay much more attention to biosecurity, vaccination and quarantine practices to prevent epidemic diseases. Veterinary medicine services should be implemented and developed together with all countries in the region.

3- GLOBAL THREAT, LOCAL RESPONSE: MONITORING AND MANAGING FOOT AND MOUTH DISEASE

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Foot-and-Mouth Disease (FMD) is a highly contagious viral disease that poses a serious threat to livestock health, rural economies, and food security across many regions. While FMD does not affect human health, its impact on animal welfare, productivity, and profitability can be profound, especially for communities reliant on livestock. The disease's complex and evolving epidemiology—marked by multiple serotypes, rapid mutations, and unpredictable cross-regional spread—continues to challenge both FMD-free and endemic countries.

FMD outbreaks are particularly severe in intensive livestock systems and can be devastating when introduced into previously disease-free zones. For these areas, the consequences extend beyond animal health, affecting trade and market access due to the loss of official FMD-free status. Common obstacles to effective global control of the disease include weak surveillance, inconsistent vaccination coverage, lack of risk information sharing, limited biosecurity and regulation of animal movements, insufficient private sector involvement and resource mobilization, low awareness of the disease's socioeconomic impact, and inadequate contingency planning.

Since 2012, a global FMD control strategy is in place and regional roadmap meetings have been conducted within different viral pools to review countries' progress in FMD control and evaluate FMDv circulation within the regional ecosystems to better understand risks and inform vaccination strategies and other control measures. Training and job aids can significantly contribute to the capacity development of the veterinary and animal health workforce to address some of the critical gaps identified.

Despite differing priorities and resources, both endemic and FMD-free countries can benefit from a comprehensive, context-sensitive approach to disease control. Success depends on realistic goals tailored to the needs of endemic regions and on understanding the behaviors, motivations, and incentives of livestock keepers within those settings.

4- STRENGTHENING PREPAREDNESS: TRAINING STRATEGIES FOR FOOT-AND-MOUTH DISEASE OUTBREAK RESPONSE

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Introduction

Foot-and-mouth disease (FMD) represents a highly impactful and widely distributed transboundary animal disease with substantial implications for livestock health and trade. Its recent incursion into the European Union has elicited significant concern, prompting heightened awareness and reinforcing the imperative for rigorous surveillance systems. Given the acute infectivity and rapid transmission dynamics of FMD, legislative frameworks mandate specialized training for veterinarians engaged in animal health management to ensure effective preparedness and response.

Objective

This study aimed to underscore the pivotal role of training strategies in the effective management of FMD, focusing on courses for veterinarians that were strategically implemented between 2019 and 2025 to optimize disease control outcomes and enhance the overall quality of professional development in animal health management. The primary objective was to review the diverse training methodologies employed and to compare their strengths and benefits.

Materials and methods

Training methodologies were categorized into three distinct formats:

1. Fully online courses delivered entirely through remote platforms, emphasizing theoretical instruction, without any in-person engagement;
2. Field-based real-time training organized by the European Commission for the control of FMD (EuFMD). This format involves immersive field training conducted on-site within areas affected by ongoing outbreaks. Participants engage directly in outbreak investigations, allowing for hands-on skill development under authentic epidemiological conditions;
3. Hybrid courses integrating online theoretical instruction with face-to-face practical sessions. In-person activities are conducted in a disease-free environment.

Anonymous surveys were conducted to assess the extent to which participants applied the knowledge acquired during the course and their level of satisfaction.

Results

The three educational formats have distinct advantages and limitations. While fully online courses offer broader accessibility, real-time training provides immersive experiential learning, so that participants will be able to recognize FMD in a real-life situation and cascade their knowledge to colleagues.

Considering EuFMD training, in 2022-2023, surveys were sent to participants of 18 training programmes: 43% of participants reported applying what they learned to a great extent and over 51% said that the courses helped improve their country's control programme. In addition, in-person training participants tend to provide more detailed feedback, especially concerning the practical application of content and the cascading of knowledge within their organizations.

Regarding the hybrid model, it facilitates dynamic, interactive learning environments while maintaining adherence to essential biosecurity standards. In addition, it also represents a good compromise from an economic standpoint. Multiple training sessions have been conducted in 2025 across the regions of Piedmont, Lombardy, and Emilia-Romagna (Italy). The target audience for the training programs consisted of local and

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regional level veterinarians, resulting in the training of approximately 120 veterinary professionals. The questionnaire results indicated a high level of participant satisfaction, with the course being perceived as highly relevant to their professional needs. In response to the evolving needs of veterinary public health, especially in the context of emergency preparedness and response, the hybrid training approach offers a balanced compromise between effective knowledge dissemination and cost-efficiency.

Conclusion

Positive feedback was consistently reported across the various training editions, reinforcing the critical importance of preparedness in the effective management of transboundary animal diseases.

5- EPIDEMIOLOGICAL INVESTIGATION OF THE PREVALENCE AND RISK FACTORS OF FOOT AND MOUTH DISEASE (FMD) IN SMALL RUMINANTS IN BAHAWALPUR DIVISION, SOUTH PUNJAB, PAKISTAN

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Introduction

Foot-and-Mouth Disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals and remains a persistent threat to livestock productivity and rural livelihoods in endemic regions. Despite huge efforts to control the disease, FMD is endemic in most regions of Pakistan and FMD outbreaks have been reported throughout the years and mainly in cattle and buffaloes. With regards to Punjab province, several outbreaks of FMD affecting almost all the districts of the province are being reported every year. This could be associated with various factors such as lack of biosecurity measures, poor husbandry practices, vaccines availability, vaccination coverage and practices and excessive cross-border trading. Pakistan is part of the west Eurasian FMD pool, where three FMD serotypes (A, O, and Asia-1) are commonly identified.

Objective

Sheep and goats' population in Pakistan are typically not targeted by regular surveillance and vaccination efforts and their epidemiological role in the dissemination of the disease in this region is not clear. The fact that FMD most often assumes a subclinical condition in sheep and goats makes these host species a potential hidden source of infection for cattle and buffaloes in mixed livestock systems and further increasing the risk of virus dissemination through animal movement and international trade.

This study investigates the seroprevalence and risk factors associated with FMD in small ruminants (goats and sheep) population within the Bahawalpur Division of Pakistan. Bahawalpur Division has a sheep and goat population of about 4 million animals.

Materials and methods

A cross-sectional study design was used to collect blood samples and epidemiological data from 1,440 animals across 360 households. This study employed a multistage cluster sampling strategy, based on proportional allocation to ensure representative coverage across Bahawalpur Division. In each selected household, structured interviews were conducted with animal owners by means of an hoc developed epidemiological questionnaire, and blood samples were collected from small ruminants to assess FMD virus circulation. Serum samples were transported to the National Veterinary Laboratory in Islamabad for laboratory analysis by means of a ELISA test targeting non-structural protein antibodies. This test enables the differentiation between vaccinated and naturally infected animals. Univariable analysis and two multivariable logistic regression models were used to investigate the influence of putative risk factors toward the FMD positive households and animals respectively.

Results

The overall true seroprevalence of FMD in small ruminants was 34%, with the highest prevalence observed in Cholistan (43% among goats) and the lowest in Rahim Yar Khan (25% among goats). Univariable and multivariable logistic regression analyses revealed significant associations between FMD seropositivity and factors such as grazing practices, education level of household heads, and implementation of seasonal disease control measures. Notably, animals that grazed outside had nearly three times higher odds of being seropositive, while secondary education and seasonal FMD measures were protective at the household level. Biosecurity assessments highlighted critical gaps in practices such as quarantine, vaccination, and sanitation, despite high awareness levels.

Conclusion

These findings underscore the need for integrated control strategies that combine farmer education, targeted vaccination, practical biosecurity interventions, and enhanced disease surveillance. The study contributes to the understanding of FMD dynamic in small ruminants and provides a foundation for informed policy and resource allocation to improve animal health outcomes in southern Punjab.

6- VALIDATION OF TWO MULTIPLEX LATERAL FLOW DEVICES FOR THE RAPID DETECTION AND TYPING OF FOOT-AND-MOUTH DISEASE VIRUSES

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Introduction

In foot-and-mouth disease (FMD) endemic regions, which often lack adequate transport systems and equipped laboratories, Lateral Flow Devices (LFDs) represent the simplest tool for rapid on-site diagnosis. Other experimental LFD prototypes offer a user-friendly disease confirmation tool, but lack serotyping capacity, whilst serotype identification is crucial to identify regional transmission patterns and to ensure appropriate vaccine selection in countries where multiple serotypes co-circulate.

Objective

This study aims to depict the validation of the multiplex prototypes LFD1 (Pan-FMDV, serotypes O, A and Asia1) and LFD2 (Pan-FMDV, serotypes SAT1 and SAT2), developed starting from well-characterized monoclonal antibodies (MAbs). The long-term outcome is to finalise the setting up of the devices for on-field FMD diagnosis (Pan-FMDV) and simultaneous serotyping.

Materials and methods

The ability of LFDs to serotype the widest range of FMDV lineages was evaluated testing cultured viruses of 98 different lineages; 49 type O, 27 type A and 7 type Asia1 with LFD1 and 6 type SAT1 and 9 type SAT2 with LFD2.

The diagnostic sensitivity of the virus detection was evaluated on 214 FMDV epithelium homogenates previously tested-positive with real-time RT-PCR; namely 160 for LFD1 (74 serotype O, 43 serotype A, 38 serotype Asia1 and 5 untyped samples) and 64 for LFD2 (31 serotype SAT1 and 33 serotype SAT2).

The diagnostic sensitivity of the serotyping abilities was assessed using as comparison the IZSLER Antigen-ELISA (Ag-ELISA) kit, instead. The test involved 178 serotyped FMDV positive samples for LFD1 (95 serotype O, 54 serotype A and 29 serotype Asia) and 92 for LFD2 (52 serotype SAT1 and 40 serotype SAT2).

Diagnostic specificity was evaluated testing 60 samples, negative for FMDV, collected in Italy by various species of cloven-hoofed animals.

A score from 0 to 4 was assigned to the results, reflecting the colour-intensity of the line in the test window.

Results

LFD1

- In 87.5 % of FMDV positive samples, the presence of virus was correctly detected with LFD1.
- The evaluation of serotyping abilities revealed that LFD1 correctly serotyped 96% of type O, 94% of type A and 83% of type Asia1.

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LFD2

- In 70% of type SATs positive samples FMDV was correctly detected with LFD2.
- LFD2 correctly serotyped 77% of SAT1 and 82% of SAT2 samples.

Both the devices correctly detected and typed all the tested tissue-cultured strains.

The evaluation of diagnostic specificity reached 100% for both the devices, with only few inter-serotypes cross-reactions. LFD1 and LFD2 demonstrated a solid agreement with real-time RT-PCR and Ag-ELISA (considering respectively the detection and the serotyping of FMDV).

Conclusion

The two devices demonstrated to be able to detect FMDV and to type 5 serotypes with high diagnostic sensitivity and specificity, showing a high concordance with the other diagnostic methods (real-time RT-PCR and Ag-ELISA). This validation process confirmed that using the two LFDs together leads to a reliable on-field detection and serotyping of various FMDV strains belonging to all the 7 pools.

7- A PROSPECTIVE, BLINDED, OPEN-LABEL CLINICAL TRIAL TO ASSESS THE ABILITY OF FLUORESCENT LIGHT ENERGY TO ENHANCE WOUND HEALING AFTER CASTRATION IN GOAT KIDS

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Introduction

Castration of male goat kids is a common procedure, with the purposes to obtain control of reproduction in the flock (preventing unwanted pregnancies and indiscriminate reproduction); to have more docile animals, to modify the organoleptic characteristics of a particular product category or, when castrated at an age of over 10 weeks, to improve the economic management of animals of different age groups and to increase the slow growth of those animals that have not reached slaughter weight before the onset of puberty. When performed surgically, castration is not free from complications, such as wound infection, incision dehiscence, seroma formation, scrotal self-mutilation, hernias and hemorrhage. Since these are food-producing animals, special attention must be paid to the use of drugs, especially antimicrobial drugs. Perioperative antimicrobial therapy is commonly used to prevent surgical complications including surgical site infections (SSI) during surgery. Fluorescent light energy (FLE), could represent a valid alternative to reduce the use of drugs promoting wound healing thanks to its antibacterial, anti-inflammatory, immunomodulatory and tissue regeneration-promoting effects.

Objective

Objective of this research was to evaluate the ameliorating effect of FLE on wound healing after castration surgery in small ruminants. The endpoint of the study was the assessment of the healing status of the surgical wound at day 5 after surgery.

Materials and methods

Twelve male goats were recruited after castration, performed according to standard surgical procedures. During anesthesia recovery, the animals received the application of FLE, only in one of the two surgical incisions. FLE consisted of the application of a 2 mm layer of biophotonic gel illuminated with the blue LED device that delivers non-coherent blue light with a peak wavelength between 440 and 460 nm and a power density between 55 and 129 mW/cm², for 2 min, at approximately 5 cm distance. After illumination, the gel was gently removed using sterile gauze immersed in sterile saline solution. The FLE illumination was repeated twice, one minute apart. Wound photographs (day 0 immediately after surgery, 1 and 5 post-surgery) were uploaded to a shared folder to allow the principal investigator, who remained blinded to the type of treatment received, to assess the wound using the Modified Hollander Cosmesis Score System and the Modified Draize Wound Healing Score.

Results

All goat kids tolerated very well FLE application and no adverse reaction was recorded. Statistical analysis revealed a significant ameliorative effect of FLE in erythema, edema, and serous discharge that were found to be statistically lower for those wound parts managed with FLE. In addition, the presence of step-off borders, contour irregularities, and excessive distortion were significantly lower in illuminated wound area, in comparison with control area that not received FLE.

Conclusion

These results showed a better healing process after FLE application on post-castration wounds in goat kids. Inappropriate wound management negatively affects wound healing time, affects the quality of life of animals, increases drug use in veterinary medicine (with possible repercussions on human health regarding drug residues) and reduces the profitability of small ruminant farming. FLE represents a cost-effective therapeutic alternative that reduces wound healing time and care for breeders. FLE could reduce the costs related to the use of drugs (antibiotics, antiinflammatory) and the problem of residues in food of animal origin that is highly desirable from a one-health point of view.

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8- A CLINICAL EVALUATION OF TWO DIFFERENT VOLUMES OF EPIDURAL ANAESTHESIA IN CALVES UNDERGOING CASTRATION

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Introduction

Castration is one of the most common and routine surgeries performed on calves in field conditions. The procedure aims to reduce aggressive behaviour, increase sexual activity and improve carcass quality. Several orchiectomy techniques have been described in the livestock context over the years. All procedures are associated with intra-operative and post-operative algic stimuli, i.e. nociceptive stimuli and pain. The European Community places great importance on the management of pain in livestock animals when it comes to animal welfare. This growing interest has prompted many research groups to address the issue of preserving the sustainability of the production chain. Over the years, some sedative and analgesic molecules administered systemically have been investigated in calves, but with varying degrees of effectiveness and often unsatisfactorily. Loco-regional anaesthesia in companion animals has increased exponentially over the last 20 years. In contrast, loco-regional anaesthesia, particularly epidural anaesthesia, has always been an effective solution for managing nociception during surgery in livestock. In fact, an epidural is a loco-regional neuroaxial anaesthesia technique involving the administration of a local anaesthetic in the epidural space. Despite the widespread use of short-acting local anaesthetics in cattle, the literature is lacking studies that describe different peridural lidocaine volumes, especially in calves where dosing is still performed on an empirical basis. The aim is to desensitise the somatic and visceral nerve afferents of the affected segment to prevent pain without causing motor desensitisation. Our hypothesis is that the proposed volumes can provide adequate analgesia for an orchidectomy without affecting motor blockade.

Objective

The aim of the study is to evaluate the clinical effect of two different volumes of lidocaine at the same concentrations (high volume 0.1 ml/kg vs low volume 0.05 ml/kg) administered in the epidural space in calves undergoing elective orchiectomy.

Materials and methods

A group of 25 Friesian crossbred calves, aged between 25 and 30 days from the Zooprophyllactic Institute (IZS) in Teramo, were brought to the University of Teramo to be castrated. The project was approved by the University Ethics Committee, University of Teramo with protocol no. 35/2025. After obtaining written informed consent from the owner (IZS Teramo), the calves were randomly and blindly divided into 3 groups: Control group (CG) which received a sterile physiological saline solution, epidural-lidocaine group 0.05 ml/kg live weight at low volumes (LLVG), epidural-lidocaine group 0.1 ml/kg live weight at high volumes (LHVG). All calves were premedicated with xylazine 0.1 mg/kg (Rompun® 20 mg/ml, Elanco Italia S.p.A.) and midazolam 0.4 mg/kg (Dormazolam® 5 mg/ml, Le Vet. Beheer B.V. Netherland) intramuscular via (IM). After 20 minutes, the calves were taken to the preparation room, a jugular catheter was placed in a sterile manner and surgically anchored. Meanwhile, an operator who was not involved in the surgical procedure or anaesthesiological assessments prepared the sterile material for the epidural with the drug mixture, ensuring blind work. Once the sedation plan had been approved, the epidural site was shaved at the sacro-coccygeal level (C5–S1). If the sedation plan was deemed inadequate, a top-up of midazolam 0.3 mg/kg (Dormazolam® 5 mg/ml, Le Vet. Beheer B.V., Netherland) and ketamine 0.5 mg/kg Ketavet®; MSD Animal Health, Kenilworth, NJ, USA) was administered slowly to deepen the sedative effect. The epidural was always performed by the same operator unrelated to the surgical procedure and anesthesiologic assessments. During the epidural procedure, the anaesthetist in charge of the clinical case was absent, meaning he could not determine which group the case belonged to. A 21G hypodermic needle (40 mm x 0.8, Sterican®, B. Braun, USA) was used for all calves. Once the needle was in the correct position, the drop test and/or loss of resistance to anaesthetic administration were performed. Once we were sure that we were in the epidural space, the anaesthetic was administered slowly to prevent problems with ataxia. After allowing five minutes for the drugs to be distributed and absorbed, all calves were gently positioned in right lateral recumbency with the

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contralateral limb facing forward, allowing the surgeon to visualise the testes clearly. Approximately 20 minutes prior to surgical incision, amoxicillin and colistin (Potencil®, 100 mg + 2500.00 IU/ml, Virbac, Italy) were administered IM. Flow-by oxygen was administered at 53 L/min. The following vital parameters were monitored and noted: heart rate (HR), respiratory rate (fR), systolic, mean and diastolic blood pressure (SAP, MAP and DAP) in a non-invasive manner (NIBP), and oxygen saturation (SpO₂). If there was a change of at least 20% in two out of three vital parameters (HR, RF and mean blood pressure) from the value recorded in the preceding five minutes, the subject was considered positive for the algic stimulus and was therefore treated. A bolus of 100 mg/kg ketamine (Ketavet®, MSD Animal Health, Kenilworth, NJ, USA) was administered. Intraoperative algic stimuli were divided into three time points: skin incision; first testis incision; and second testis incision. At the end of the procedure, all calves were moved to the wake-up box and received 2.2 mg/kg of Flunixin Meglumine (Fluvex®, SP Veterinaria S.A., Milan) subcutaneously (SC) as an anti-inflammatory. An external operator recorded the timing of recovery of the sternal and quadrupedal positions. Over the next eight hours, the timing of rumination, formation of tympanism, resumption of drinking, and any signs of discomfort in the calf, including scratching, redness of the surgical site, vocalisations, and other signs of discomfort, were assessed and recorded. Any clinical signs of ataxia were considered and recorded. Any signs of complications attributable to the epidural procedure were observed and noted. After the six-hour observation period, if no complications were present, the calves were discharged.

Results

A total of 25 mixed-breed Friesian calves, aged 25–30 days and weighing 40–50 kg, were divided into three groups: two experimental groups and one control group. Homogeneity tests for demographic data were conducted without showing statistically significant differences. The significance was set at $p < 0.05$. The other variables taken into account were the response to the nociceptive stimulus, rescue analgesia, pain reaction to skin, first and second testicles for intraoperative period. For these variables, the following was applied Fisher's Exact Test and relative post hoc. Rescue analgesia and response to nociceptive stimuli were significant for the LHV group vs C group with a p value of 0.03 for each variable. Skin incision, first and second testis were not significant with p 0.37, 0.37 and 0.18 respectively. For the post-operative period, awakening time was evaluated with Kruskal Wallis and results significant in LLV vs C with $p=0.01$ and LHV vs C with $p < 0.0001$. Standing time was analyzed with ANOVA test and was significant in both groups with $p < 0.0001$ and $p = 0.0002$, LLV and LHV respectively. Tympanism and ataxia were analyzed with Fisher's Exact Test. In the first variable significance is present for LLV vs C with a $p=0.04$. Ataxia is not significant for groups ($p=0.26$).

Conclusion

In conclusion, epidural anaesthesia performed at the C5-S1 level is an effective technique for reducing systemic analgesia at both high (0.1 ml/kg) and low (0.05 ml/kg) doses. The proposed dosages also facilitate faster recovery times, enabling calves to ruminate more quickly and decreasing the risk of rumen distension. The LHV group appears to provide more intense analgesia during the operation than the LLV group. No significant differences were found in terms of ataxia or post-operative pain between the groups. No subjects in the epidural group developed complications attributable to the technique, such as infection, walking problems, ataxia or longer hospitalization time.

9- EFFECTIVE DISINFECTION PRACTICES IN ANIMAL HUSBANDRY THROUGH PROPER PROTOCOL ADHERENCE

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Maintaining robust health and productivity in ruminant livestock (cattle, sheep) is paramount for advanced animal husbandry. This necessitates a multifaceted approach to disease prevention. This work will delve into various types of disinfectants (biocides) and their correct application, leading to the formulation of targeted and specific biosecurity plans for individual farms. Reducing the bacterial load in livestock operations drastically decreases antimicrobial use and consequently increases profitability, always in line with a market where consumers demand healthy and certified products.

10- MANAGEMENT OF HEAT STRESS IN DAIRY COWS

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In today's world, the impact of climatic changes on livestock production is becoming increasingly pronounced, with heat stress in dairy cattle emerging not only as an environmental factor but also as a significant threat to animal welfare and economic sustainability. Temperatures exceeding the cows' optimal thermal comfort zone—typically between 5 °C and 25 °C—disrupt physiological homeostasis, resulting in reduced feed intake, decreased milk yield, and impaired reproductive performance; the multifaceted consequences of heat stress are clearly illustrated by embryonic losses, an increased incidence of lameness, and a heightened susceptibility to mastitis. Addressing this complex issue requires a management approach that is not only reactive but also proactive, beginning with improvements to the environment through active cooling strategies such as shade structures, misting systems, and ventilation fans, which enhance thermal comfort by facilitating heat dissipation and lowering body temperature. However, nutritional interventions are equally critical: to compensate for decreased dry matter intake and meet elevated energy demands in heat-stressed cows, rations should prioritize highly digestible energy sources like bypass fats and starch-rich grains, while incorporating digestible neutral detergent fiber (NDF) sources remains essential to support rumen function and reduce the risk of subacute ruminal acidosis. Ultimately, alongside these strategies, providing unrestricted access to clean, fresh water at all times is paramount, as the comprehensive management of heat stress in dairy cattle must be addressed through a holistic framework that combines environmental modifications with nutritional strategies aimed at enhancing physiological resilience, thereby laying the groundwork for more ethical and resilient livestock production systems through improved management efficiency, maximized productivity, and enhanced welfare.

11- EVALUATING THE IMPACT OF HEAT STRESS ON OXIDATIVE STATUS IN HEALTHY LACTATING COWS IN SERUM

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Introduction

Heat stress is defined as the sum of several external environmental factors, including ambient temperature (T), relative humidity (RH), solar radiation, and wind speed, which increase body temperature and trigger physiological and behavioral adjustments.

To know the degree of heat stress to which these animals are exposed, the temperature-humidity index (THI) parameter is used, which estimates the magnitude of heat stress. The optimal range in temperature for dairy cattle is between 5°C and 25°C, and in relative humidity from 40% to 70%. Lactating cows start to suffer from heat stress from 66 THI and for each unit of THI above 72, milk production decreases by 0.2 kg/cow/day. When the upper critical THI (THI > 87) is exceeded, the risk of death in dairy cows is maximum.

One of the alterations in homeostasis necessary to understand the physiological changes that occur in animals when heat stress increases is oxidative stress.

Objective

This study aims to investigate the impact of heat stress in healthy dairy cattle by measuring oxidative stress in serum, specifically oxidative stress index (OSI), total oxidant status (TOS), and total antioxidant capacity (TAC).

Materials and methods

32 healthy lactating Holstein dairy cows with heat stress were evaluated against 32 without heat stress. These animals were from south-eastern Spain, and blood samples were collected, centrifugated at 3000g for 10 min to obtain serum. To determine the heat stress condition, the THI was calculated as follows: $THI = (1,8 \times T + 32) - (0,55 - 0,00555 \times RH) \times (1,8 \times T - 26)$, where T = ambient temperature (°C) and RH = relative humidity (%).

Serum TOS and TAC levels were quantified using enzymatic assays previously validated. The OSI was calculated using means TOS and TAC coefficient. For TOS a commercial peroxide assay (Pierce™ Quantitative Peroxide assay, Thermo Scientific, United States) was used, and the total TAC was measured using the ferric-reducing antioxidant power assay. OSI was then calculated as the ratio TOS/TAC.

Statistical analysis was developed with GraphPad Prism 10.3.1. software. Data distribution was evaluated with the Shapiro-Wilk test, homoscedasticity with the Fligner-Killeen test, and the parameters were analyzed with the unpaired t-tests with Welch's correction to search for differences between cow under heat stress and cows without heat stress. The Spearman association between parameters was also evaluated.

Results & Discussion

In cows under heat stress, the THI median was 78.75 while in cows without heat stress the THI was 52.54 ($p < 0.0001$). The mean temperature in heat stress condition was 27.76°C, compared with 15.23°C without heat stress, while the mean humidity was 77.69% and 61.91%, respectively. So high T with high RH impact in the heat stress, as it was expected.

Serum TAC concentration levels were similar in both conditions while TOS levels were statistically higher in cows under heat stress compared to no heat stress condition (13.52 vs 10.18 $\mu\text{M/L}$ peroxidase equivalents, $p = 0.0163$), which could indicate a direct relationship with THI. OSI was higher when the heat stress was present, with statistical significance ($p = 0.0071$). TAC and TOS showed a positive association (0.76), and TAC had a low negative association with THI (-0.11), while almost null (0.09) TOS and THI correlation was observed.

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Conclusion

Heat-stressed dairy cows showed oxidative stress according to serum analysis. The lack of antioxidants could be the cause of the oxidative stress observed under heat stress conditions, failing to balance the large increase in oxidants released.

12- ASSESSMENT OF HEAT STRESS EFFECTS ON PHYSIOLOGICAL RESPONSES AND REPRODUCTIVE EFFICIENCY IN DAIRY CATTLE UNDER OCEANIC CLIMATE CONDITIONS IN NORTHWESTERN SPAIN

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Introduction

Climatic conditions in northwestern Spain have undergone notable changes over recent years, despite the region's characteristic oceanic climate. In 2023, record high temperatures were recorded, which had detrimental effects on dairy farming. Heat stress has become an increasingly frequent concern, particularly regarding its impact on reproductive performance in dairy cattle. The Temperature-Humidity Index (THI) is commonly used to quantify heat stress and evaluate its effects on animal physiology and fertility.

Objective

The objective of the present study was to evaluate the presence or absence of heat stress based on the Temperature-Humidity Index (THI). Additionally, the study aimed to assess the impact of heat stress on uterine and ovarian physiological parameters, as well as on the reproductive efficiency of dairy cattle under these recurrent environmental conditions.

Materials and Methods

A total of 227 Holstein cows were randomly assigned to two experimental groups.

-Experiment 1: 63 cows were synchronized using a modified Double-Ovsynch protocol and inseminated to improve fertility under heat stress conditions. Physiological variables—including rectal temperature, vulvar viscous fluid secretion, uterine tone, uterine and endometrial thickness, and preovulatory follicle length and width—were measured on the day of insemination.

-Experiment 2: 96 cows were inseminated without prior synchronization. This group was used to evaluate the impact of THI on pregnancy rate at first insemination.

Logistic regression analysis was performed to identify physiological variables associated with reproductive success under heat stress conditions.

Results

High THI levels negatively affected reproductive physiology. Specifically: Altered estrus expression, reduced uterine blood flow and altered progesterone (P4) levels in synchronized cows (P4 = 0.41 ng/mL in cool months vs. P4 = 0.49 ng/mL in warm season; $p < 0.05$).

In Experiment 2, non-synchronized cows exposed to $\text{THI} \geq 72$ showed a lower pregnancy rate (29%) compared to cows exposed to $\text{THI} < 72$ (33%).

Conclusion

Even mild heat stress during the summer negatively impacted the reproductive physiology of lactating dairy cows, leading to decreased pregnancy rates at first artificial insemination when THI was ≥ 72 . The use of a modified Double-Ovsynch synchronization protocol was effective in improving conception rates under heat stress conditions.

13- EARLY DIAGNOSIS OF HEAT STRESS IN DAIRY GOATS THROUGH INFRARED THERMOGRAPHY AND BEHAVIOURAL INDICATORS: TOOLS TO VALIDATE THEIR POTENTIAL AS MODEL SPECIES IN THE CLIMATE CHANGE ERA

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Introduction

In the context of global climate change, heat stress is a shared challenge affecting various animal species in dairy farming. Veterinarians should be able to recognize early clinical signs of thermal distress in ruminants by adopting non-invasive methods that respect animal welfare, such as Infrared Thermography (IRT) and behavioural observation.

Objective

The research evaluates the effectiveness of Infrared Thermography (IRT) in assessing thermal stress and anticipating behavioural responses in dairy goats exposed to heat stress conditions. IRT was employed to measure the Superficial Skin Temperature (SST) across different body regions, with the aim of identifying the best thermal window for detecting variations related to environmental parameters. Behavioural observation determined the most indicative behaviour of thermal distress experienced by the animals. The association between these two results defined the earliest clinical sign for identifying the level of heat stress based on the Temperature-Humidity Index (THI).

Materials and methods

The study was conducted on a dairy goat farm, located at the Department of Veterinary Medical Science, University of Bologna, in Ozzano Emilia. 17 Alpine dairy goats (11 lactating and 6 dry) were examined over six days between July and August 2023, at 7 AM and 7 PM in their three pens. Goats were video recorded for 10 minutes per session over the six days, and behavioural changes related to increased heat dissipation, water conservation, and reduced thermogenesis were analysed using an *ad libitum* sampling method. Following each recording, IRT images were captured using a portable thermal camera at the same distance of 80 cm from body regions of interest, including the left eye lacrimal caruncle, left flank, loins, carpal area, and distal part of forelimbs. Additionally, in lactating goats, SST of the udder was recorded before and after milking. Finally, a clinical examination of each animal was performed every morning, recording the rectal temperature measurements to monitor for hyperthermia. Environmental temperature and humidity were monitored using a weather station and three levels of heat stress were defined: mild ($THI < 75$), moderate ($75 \leq THI \leq 80$) and severe ($THI > 80$). Statistical analysis was performed in the R environment to examine the association between environmental parameters (independent variables) and SST values using linear regression models. THI classes and behavioural changes were associated using generalised linear models: behavioural states, evaluated by their duration (seconds), were analysed with a Poisson distribution, while behavioural events, evaluated by their frequency (number of manifestations), with a negative binomial distribution.

Results

Based on the statistical analysis, the best thermal window for performing IRT under heat stress was the distal part of forelimbs, while the reduction of time spent ruminating while standing represented the most indicative behaviour of thermal discomfort in dairy goats. The association between these two results, compared with a logarithmic function at three different levels of heat stress, showed that thermal fluctuations in the distal part of forelimbs can anticipate variations in animal behaviour.

Conclusion

This study highlights the crucial role of IRT as an early, non-invasive, and real-time diagnostic tool, enabling the timely implementation of appropriate strategies to preserve the health, welfare, and productivity of valuable

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livestock species, such as goats. Furthermore, IRT also represents a useful method for identifying animals with greater resistance, tolerance, and resilience, traits that are significant to continuously changing climate. Among livestock, the goat emerges as an ideal model in the era of global warming: its dairy products contribute not only to the economic stability of industrialized countries but also play a vital role in ensuring food security and livelihoods in developing countries.

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14- EFFECT OF HEAT STRESS ON ENDOCANNABINOID LEVELS AND OXIDATIVE STATUS IN DAIRY COWS

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Introduction

Heat stress (HS) elevates body temperature, triggering the activation of the hypothalamic-pituitary-adrenal (HPA) axis and leading to increased cortisol levels, which in turn suppress immune function. The endocannabinoid system (ECS), comprising N-arachidonyl ethanolamide (anandamide; AEA) and 2-arachidonoylglycerol (2-AG), plays a key role in modulating stress responses by regulating corticosterone release and inflammation. HS has been shown to downregulate ECS gene expression in adipose tissue, suggesting that thermal stress can impair ECS activity. Additionally, HS negatively affects fertility by enhancing cellular metabolism, which leads to oxidative stress. This condition compromises the redox balance of oocytes due to increased production of reactive oxygen species (ROS), along with decreased glutathione levels and antioxidant enzyme activity.

Objective

The aim of the study was to evaluate ECS components and oxidative status in dairy cows at heat under two different conditions of environmental temperature (winter vs summer).

Material and methods

The study involved 10 Italian Fresian cows recruited from farm located in the rural area of Bari, Apulia. The study was conducted in January 2023 (winter) and in July 2023 (summer). Ambient temperature and humidity were recorded using a data logger placed in the feeding area. The temperature humidity index (THI) was calculated.

Before any experimental procedures, all cows underwent a thorough clinical examination. During oestrus, all enrolled cows underwent blood sampling from the coccygeal vein. eCBs (AEA, 2-AG, palmitoylethanolamide-PEA, oleoylethanolamide-OEA) were analyzed using high-performance liquid chromatography (HPLC). ROS were assayed by colorimetric reaction capable of assaying reactive oxygen metabolites (dROMs) while BAP (Blood Antioxidant Potential) was determined with the BAP test using a photometric system. Statistical analysis was performed using SPSS 19, considering $p < 0.05$ as significant.

Results

Average THI values recorded in winter and summer were 58.14 ± 0.96 and 75.63 ± 1.56 , respectively, showing a statistically significant difference between the two seasons ($p < 0.001$). Considering all enrolled animals, no significant differences were observed for AEA, PEA, and OEA concentrations between the two seasons analyzed. In contrast, a higher concentration of 2-AG was found in winter ($23,05 \pm 9,82$) than in summer ($16,77 \pm 8,59$) ($p < 0.05$).

Regarding oxidative status, statistically significant differences were found between the two seasons considered for both dROMs ($p < 0.05$) and BAP ($p < 0.001$) values.

Conclusion

The findings of this study indicate that the two primary eCBs are present at higher concentrations in winter than in summer, although a statistically significant difference is observed only for 2-AG. It is known that heat stress induces a systemic inflammatory state in dairy cows, leading to increased cyclooxygenase-2 (COX-2) activity, which promotes the production of pro-inflammatory prostaglandins. COX-2 enzymes are known to metabolize both AEA and 2-AG, and this may explain the observed reduction in eCB levels during the summer. Additionally, heat stress is known to trigger an increase in fatty acid amide hydrolase (FAAH), the enzyme

that metabolizes AEA. This could help to explain the lower AEA concentrations observed during the summer compared to winter. Chronic exposure to heat stress further contributes to the depletion of the endocannabinoid system. As oxidative status, it also influenced by heat stress that induces an increase in ROS production and antioxidant reduction. Overall, this study highlights that heat-stressed cows experience a marked reduction in endocannabinoid levels ed un aumento dello stress ossidativo, suggesting the potential for future research on dietary supplementation to enhance and support eCB system function and improve the oxidative status.

15- PILOT SURVEY OF *HAEMONCHUS CONTORTUS* INFECTION IN SHEEP FROM CROATIA USING PNA DETECTION METHOD

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Introduction

The nematode *Haemonchus contortus*, commonly known as the barber's pole worm, is a widespread and highly pathogenic parasite affecting ruminants. It inhabits the abomasum, where it feeds on the host's blood, often causing severe clinical symptoms and, in some cases, death, particularly in young or immunocompromised animals. The prevalence of *H. contortus* varies depending on climatic conditions, the age of animals and their living environment.

In various regions, the parasite shows notably high prevalence rates: 88.95% in Serbian goats, 76.9% in the Basilicata region of Italy, and it has been documented in the alpine chamois population in Slovenia. Even in colder climates, such as Sweden, the prevalence of *H. contortus* at 72% has been recorded. Diagnostic methods include coprological analysis, PCR and larval cultures. A promising method, but not yet routinely used is lectin binding to specific nematode eggs, especially peanut agglutinin (PNA) for diagnostics of *H. contortus*.

Despite the increasing number of small ruminant farms in Croatia over the past decade, no comprehensive study on the prevalence of *H. contortus* and its associated risk factors has been conducted.

Objective

This pilot study aimed to assess the presence and potential risk factors of *H. contortus* infection in two distinct Croatian regions: the Lika-Senj region (highlands, mountain climate) and the Vukovar-Srijem region (lowlands, continental climate). Additionally, this study represents the first application of the PNA detection method in Croatia.

Materials and methods

Twenty-seven sheep farms of different flock size, breed and housing conditions were included in this study during the spring of 2022. Twenty farms were located in Vukovar-Srijem region and seven in Lika-Senj region. Approximately, 20% of each flock was sampled. Initial calculation of sample size was based on cluster sampling method, proportionally to sheep populations in different clusters. Fecal samples were collected by the licensed veterinarians during regular procedures. Samples were gathered from the ground and pooled, with each pooled sample consisting of 10 individual samples.

Fecal egg counts (FEC) were determined using the FLOTAC method as previously described. The pooled sample with the highest strongyle egg count from each farm was selected for PNA testing, following the previously described protocol. If all pooled samples from a farm tested negative for strongyle-type eggs, all samples were pooled and included in the PNA procedure as one single sample.

Farm owners completed questionnaires covering general flock information (breed, size), housing, quarantine options, pasture access, presence of other animals, deworming protocols, and observed clinical signs. Statistical analysis using Fisher's exact test and Pearson's test was used to determine statistically relevant correlations between presence of *H. contortus* and possible risk factors on the farms.

Results

Out of 27 farms, 17 (62.96%) proved positive on PNA testing. FEC values in PNA-positive farms ranged from 15 to 360 eggs per gram (epg). Only 7.40 % (3/27) of farmers use quarantine for newly acquired animals, but none did deworming before introduction to the flock. On 25.92% of farms, sheep are kept in stables, without pasture access. Deworming protocols varied from 44.44 % of farms that deworm sheep once a year to 3.7% of farms that deworm three times a year. The most used anthelmintic was ivermectin (62.96 %).

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Statistical analysis using Fisher's exact test and Pearson's test revealed a significant correlation between housing type and infection status: farms with stabled animals without pasture access were more likely to test negative for *H. contortus*. No significant associations were found between *H. contortus* infection and other factors. The limited sample size may have influenced these findings.

Conclusion

This pilot study provides the first data on *H. contortus* prevalence in sheep from Croatia using the PNA detection method. Given the ongoing effects of climate change, haemonchosis is expected to become an increasing challenge for ruminant production, even in regions where it has not been historically prevalent. Further large-scale studies are needed for better understanding of the epidemiology and risk factors of *H. contortus* infection in Croatia.

16- FASCIOLOSIS CONTROL IN THE BORU KUTABER ALASHA VALLEY PERHAPS A PROJECT, PERHAPS DREAM

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Since many years the Vet for Africa Onlus is working in Ethiopia visiting different Veterinary Faculties with the aim to share experiences and improve and integrate mutual knowledge. Unfortunately, isolation, economic hardship and ultimately spotted wars make everything extremely complex. A great satisfaction for us was the request from our colleagues at the University of Dessie to help them in the control of liver fluke in a very particular area. This is the origin of the present project to be realized in a valley that remain flooded for two months every year.

Objective - Realize epidemiological studies to reduce the impact of fasciolosis on human and animal health from a one health perspective.

Material and methods – animals

1. Collect all the paper on trematodes published in Ethiopia (also grey literature)
2. Bring together all the experiences (present but not organized) made on animal flukes, intermediate hosts and environment.
3. A special form will be prepared
4. To obtain the coprological prevalence in the main hosts bovine (100) and sheep (100). For each head we need to know: owner, breed, sex, age, treatments and geographic origin.
5. The feces should be analyzed in Dessie University by Mini Flotac Technique using flotation solution at 1350 S G.
6. This tool allows to work quickly and to obtain qualitative and quantitative results at the same time.
7. The results should be statistically analysed.
8. The suspicion of inefficacy of triclabendazole utilized should be clarified with controlled scientific criteria. The drug is imported in Ethiopia from three countries: Europe, China and India.
9. With the collaboration of bovine and ovine slaughterhouse we can collect adult parasite, feces from positive animals and 5 ml of bile from 100 randomized heads. All the tested animals should be noted using the form of the point 3. If possible, this point allows to include also the point 4 more difficult to be realized in alive animals.
10. The bile collected will be analysed by a stereomicroscope and the flukes eggs identified (*Fasciola*, *Paranfistomum*, *Dicrocoelium*) and counted. Considering the presence of eggs in the bile as a gold standard this data permit the evaluation of the sensitivity and specificity of the Flotac technique.
11. The adult flukes (10 from bovine and 10 from ovine) of *Fasciola* spp collected will be sent to the parasitology unit of Bologna (Italy) to molecularly confirm the identification.

Material and methods – environment

1. Collect snails (100), define the species and the level of positivity for trematode cercariae with a special techniques developed in laboratories of Dessie.
2. This experience should be repeated for the different seasons and in three levels of altitude of the valley.
3. The data should be obtained also in pastures used during the flood months of the valley.
4. The Ethiopian colleagues should get geographic data of the valley also with remote (GIS) cartography
5. With this last data the Bologna colleagues will try to ask for the collaboration of the University hydraulic engineers with the aim of understanding the possibility of regulating flood waters without incurring excessive cost and without affecting the ecology of the area.

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Material and methods – humans

1. Data should be obtained from hospital to understand the positivity levels in different categories of people: type of diagnosis, age, sex, working activity, origin, eating habits.

In compliance with international rules, all biological material introduced in Italy will have to transit, subjected to specific agreements through the IZS of Teramo (Italy)

Expected results

All this data should aid in understanding the unique epidemiology of flukes in the valley and support decision-making regarding animal treatment, with the goal of reducing the economic impact by controlling fasciolosis and improving the health of both humans and animals, in alignment with the One Health approach within a specific environmental context.

17- TICK DIVERSITY AND RELATED TRANSMISSIBLE PATHOGENS IN FIVE DIFFERENT LIVESTOCK FACILITIES FROM SOUTH TANZANIA

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Introduction

Vector-borne diseases are increasingly influenced by climate change, which affects the biology of arthropod vectors and drives their geographic expansion. In African countries such as Tanzania, where livestock plays a critical role in food security and the economy, these diseases contribute to reduced animal productivity and increased mortality, leading to substantial economic losses. However, in certain regions, data on tick-borne diseases remain scarce.

Objective

This study aimed to investigate the distribution of tick species and the presence of tick-borne pathogens in livestock and slaughtered cattle in the Ruvuma Region of Tanzania, a region with limited prior research on these ectoparasites and their associated pathogens.

Material and methods

Samples were collected from four livestock farms and the main regional slaughterhouse in Songea municipality. Blood samples from 49 cattle were used to prepare May-Grünwald Giemsa-stained smears for microscopic examination of hemoparasites. Ticks were systematically collected and general clinical examinations were performed on 113 animals (97 cattle, 7 dogs, 6 goats, and 3 sheep). A total of 301 ticks were preserved in 70% ethanol and identified morphologically; species identification was confirmed by sequencing the cytochrome c oxidase subunit I (COI) gene. Molecular screening for pathogens was conducted on 62 ticks, selecting at least one representative per species per host—to detect DNA from apicomplexan hemoparasites, *Anaplasmataceae*, *Rickettsia* spp., and *Borrelia* spp. Positive PCR results were confirmed through sequencing.

Results

All animals appeared clinically healthy, and all 49 blood smears tested negative for hemoparasites. Tick specimens belonged to three genera (*Rhipicephalus*, *Amblyomma*, and *Haemaphysalis*), with seven species identified: *Rhipicephalus microplus*, *R. muhsamae*, *R. decoloratus*, *Rhipicephalus* sp., *Amblyomma variegatum*, *A. eburneum*, and *Haemaphysalis elliptica*. PCR analyses revealed the presence of DNA belonging to *Theileria mutans*, *T. velifera*, *Theileria* sp., *Babesia* sp., *Anaplasma* sp., *Ehrlichia* sp., *Coxiella burnetii*, *Rickettsia africae*, *Rickettsia* sp., and a *Borrelia* sp.

Conclusion

This study expands the known distribution of tick species in Tanzania, reporting *Haemaphysalis elliptica* and *Rhipicephalus muhsamae* for the first time in the country. Although no pathogens were detected in blood smears, molecular analysis of ticks revealed circulation of multiple pathogens in the Ruvuma Region, many of which are reported here for the first time. Notably, the detection of zoonotic agents such as *Rickettsia africae*, *Coxiella burnetii*, and *Borrelia*, for which molecular analyses are ongoing to identify the exact species, highlights the importance of ongoing surveillance for tick-borne pathogens, both for animal health and public health implications

18- CRYPTOSPORIDIOSIS IN LAMBS: DIAGNOSTIC FEATURES ON COPROMICROSCOPY AND INTESTINAL EXAMINATION

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Introduction

Unlike cattle, detailed investigations into *Cryptosporidium* infections in sheep across Europe remain limited, with most studies focusing on prevalence surveys in lambs presenting with diarrhoea. In Italy, available data indicate a 17.4% seroprevalence in lambs from the Abruzzo region ([Paoletti et al., 2009](#)), and a 10.1% prevalence of *Cryptosporidium* spp. oocysts in sheep from Sardinia (92/915), with 34.4% of the farms raising lambs carrying zoonotic subtypes of *Cryptosporidium parvum* and *C. ubiquitum* ([Dessi et al., 2020](#)).

Objective

The aim of the present study is to make a practical contribution about the clinical and laboratory diagnosis of cryptosporidiosis outbreaks in Sarda breed lambs, through the copromicroscopical monitoring and direct diagnosis in a sheep farm with the evaluation of lambs mortality.

Materials and methods

The study was carried out between December 2024 and January 2025, during an outbreak of cryptosporidiosis involving 272 sarda breed pre-weaning lambs, aged 7-30 days, raised in Sardinia (Campeda highland - 40° 19' 09" N - 08° 43' 51" E). Fifty-six fecal samples were randomly collected soon after defecation and macroscopically observed to determine the consistency as normal, pasty and diarrheic.

Faecal samples were then examined for the presence of *Cryptosporidium* spp. oocysts by microscopy of faecal smears stained using the modified Ziehl-Neelsen technique according to Angus (1987). For positive samples, the intensity of oocyst shedding was evaluated semi quantitatively according to the average number of oocysts in 10 fields at × 400 magnification, expressed as oocysts per field (OPF).

In the meanwhile, the whole intestines of two deceased lambs were collected and dissected in their segments (duodenum, jejunum, ileum, cecum, colon and rectum). After removing the intestinal content, 10 smears were obtained from each intestine segment, stained and examined as described above.

Results

Cryptosporidium oocysts were detected in 96.4% (54/56) of examined lambs, particularly in diarrheic and pasty samples 100% (26/26 and 14/14 respectively) but also in 87.5% (14/16) of normal fecal samples ($\chi^2=1.048$; $P=0.592$). The mean intensity of oocyst was evaluated based on fecal consistency, resulting significantly higher in pasty samples (3.62 ± 2.08) and diarrheic (3.52 ± 3.82) than in normal feces (0.075 ± 0.06) (Anova test: $F=4.29$; $P=0.000$).

All intestinal tracts of the two naturally deceased lambs had at least one mucosal smear positive for *Cryptosporidium* oocysts. More in details, significantly higher mean OPF were observed in the ileum (26.4 ± 11.7), cecum (9.7 ± 6.9) and jejunum (6.6 ± 3.2), compared to other intestinal segments, as the duodenum (0.6 ± 0.8), colon (4 ± 2.4) and rectum (1.8 ± 1.9) (Anova Test: $F=52.91$; $P=0.000$).

Conclusion

The results here obtained showed how in severe cryptosporidiosis outbreaks in Sarda breed lambs with mortality cases, the oocyst shedding are seen in almost all examined animals (96.4%), particularly in those excreting pasty and diarrheic feces. Furthermore, all intestinal tracts are involved and can harbor evolutive forms of the protozoa, being the ileum the most affected, that should be firstly examined during necropsy.

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19- COCCIDIOSIS CONTROL IN RECENTLY WEANED LAMBS VIA HURA CREPITANS (L) SEEDS: A SUSTAINABLE PHYTOTHERAPEUTIC APPROACH

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Introduction

Coccidiosis, a common parasitic disease in young lambs caused by protozoa of the genus *Eimeria*, poses a significant challenge to sheep production due to its negative impact on growth and animal health. In light of increasing resistance to conventional anticoccidial drugs and the global demand for residue-free products, natural alternatives have gained relevance. In this context, *Hura crepitans* seeds (a tropical tree) stands out as a promising species due to its bioactive compounds and antiparasitic efficacy, offering a sustainable approach to coccidiosis control in livestock systems, particularly in resource-limited regions.

Objective: Coccidiosis in small ruminants, particularly lambs, results in considerable economic losses due to mortality and reduced growth. This study aimed to evaluate the efficacy of *Hura crepitans* seeds as a natural antiparasitic treatment against intestinal coccidia in lambs, compared to conventional drugs.

Materials and Methods

Twenty-four recently weaned lambs were randomly assigned to three treatment groups (n = 8/ group): toltrazuril (20 mg/kg BW, oral), sulfonamide + trimethoprim (340 mg/kg + 68 mg/kg BW, oral), and *Hura crepitans* seeds (6.0 g/kg BW, single oral dose). Fecal samples were collected at multiple times over a 29-day period to determine oocyst counts. Productive performance was assessed via total weight gain (TWG), daily weight gain (DWG), and feed conversion (FC).

Results

Lambs treated with *Hura crepitans* showed a significant reduction in oocyst counts, comparable to or exceeding that of toltrazuril and sulfonamide + trimethoprim. By day 29, both the *Hura crepitans* and toltrazuril groups exhibited the lowest oocyst counts. Productive performance variables (TWG, DWG, FC) showed no significant differences among the groups.

Conclusion

The results suggest that *Hura crepitans* seeds exhibit significant anticoccidial effects in lambs without compromising growth performance. This plant-based approach represents a sustainable and environmentally friendly alternative to conventional anticoccidial drugs, and these findings support the potential integration of these seeds into phytomedicine-based strategies for parasite control in livestock, in line with sustainable animal health practices.

20- CROSS- REACTIONS OF THE MONOSPECIFIC HYDATID CYST SERA WITH SOME HELMINTHES ANTIGENS IN SHEEP

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Echinococcus Granulosus is the causative agent of *Hydatidosis*, which is an important zoonosis with worldwide distribution. This study aimed to identify the cross- reaction of the hydatid cyst fluid antisera with antigens of *Fasciola hepatica*, *T.hydatigeana* (*CysticercusTenuicollis*), *T.thysaniezia*, and *Moniezia expansa* by application of *Immunoblot* test and dot ELISA techniques. Mono- specific serum was obtained by immunizing rabbits against the 54, 38 and 27 KDa antigens individually; and Sera were collected from rabbits before injecting the antigens and used as negative control.

The results showed that serum induced by bands which have molecular weight 54 KDa antigen reacted with antigens of *CysticercusTenuicollis* and *Moniezia expansa*, while, serum induced by band 38 KDa antigen strongly reacted with antigens of *T.thysaniezia* and *Fasciola Hepatica*. On the other hand, serum induced by band 27 KDa antigen reacted with antigens of *T. thysaniezia* and *Moniezia Expansa*. However, there was no cross - reaction between antigens of *Fasciola hepatica* and *Cysticercus tenuicoillis* with antiserum band 27 KDa.

It is concluded that there is an antigenic relation between *Hydatid* cyst fluid and different studied parasites. It is proposed that subunit with molecular weight 38 KDa is satisfactory antigen would be used in serodiagnosis for detection of *hydatidosis* antibodies in Syrian sheep.

Keywords: Hydatid cyst antigens, cross- reaction, sheep.

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21- FERTILITY AS A MIRROR OF MANAGEMENT: A CRITICAL ANALYSIS OF WEAK POINTS IN DAIRY CATTLE FARMS

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Fertility in dairy cattle is not only a technical indicator, but also a genuine reflection of the overall quality of farm management. Although many reproductive protocols and tools are now well established, field experience shows that several practical weaknesses still persist. When neglected, these critical points can significantly compromise reproductive performance and farm profitability. This presentation will examine the ideal reproductive parameters required for efficient economic management (calving-to-conception interval, first service conception rate, average number of services per pregnancy) and compare them with real-world data from dairy farms in the Mediterranean region. Key underestimated management factors will be highlighted, including:

- inadequate monitoring of animal welfare,
- nutritional and sanitary quality of feedstuffs (e.g., mycotoxins, fat oxidation, nitrate excess),
- technical errors in artificial insemination procedures, often considered trivial but frequently performed inaccurately,
- poor operational compliance with synchronization protocols,
- improper use or underutilization of herd management software and estrus detection systems.

Through practical examples, scientific data, and economic considerations, the presentation will propose a critical approach to reproductive management, where the prevention of organizational errors and attention to technical details are key to improving farm efficiency.

22- THE SEMENTUSA PROTOCOL: REPRODUCTIVE CONTROL IN SHEEP AND GOAT FARMING

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The Sementusa Protocol refers to a comprehensive series of technical procedures implemented in both dairy and meat sheep and goat farms. This protocol mandates the systematic application of standardized veterinary medical activities across both extensive and intensive livestock operations.

The primary objective of this protocol is to elevate the fertility rates of production systems to above 97%, with many farms achieving rates exceeding 99% through its application.

Management of the protocol is facilitated by the Sementusa Mobile application, which enables the collection of all farm-specific data. This data collection streamlines managerial decisions for veterinarians and nutritionists alike, and the application generates a detailed farm efficiency graph.

Ultimately, the implementation of the Sementusa Protocol leads to a significant enhancement in the overall efficiency of sheep and goat livestock systems.

23- COLOUR DOPPLER EVALUATION OF THE CORPUS LUTEUM IN CATTLE: APPLICATIONS AND PERSPECTIVES

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Introduction

Since 1980, B-mode ultrasound has been adopted in bovine reproduction research and veterinary medicine as a complementary examination in bovine reproductive management [1]. This technique was initially used for pregnancy diagnosis [2,3] but was soon utilised to study follicular dynamics, corpus luteum [4,5]. Miyazaki et al. [6] describe the Doppler method for human medicine to quantify luteal blood flow (LBF) in cows. Conventional Doppler ultrasound, like continuous-wave Doppler and pulsed-wave Doppler, is applicable to measure high [blood flow](#) velocities. Since blood vessels of the [corpus](#) luteum (CL) have a very low blood flow velocity, Power mode [ultrasonography](#), an advanced method of colour Doppler ultrasonography that detects the number of red [blood cells](#) moving through the vessel per time unit and shows them as coloured pixels, is advantageous for LBF measurement [7].

Discussion

One of the most interesting applications of colour Doppler is CL evaluation. Analysis of circulating concentrations of progesterone is the most reliable way to assess CL function, since the primary function of a CL is to secrete progesterone during dioestrus and pregnancy. The CL is a highly vascularized structure during dioestrus and pregnancy, yet undergoes an extensive decrease in blood perfusion and luteal tissue volume during luteolysis [8]. Several studies have utilised colour Doppler to characterise morphological and functional changes in the CL during this period [9-11]. Interestingly, luteal blood perfusion decreases before structural regression [10,12]. The correlation between CL blood perfusion and circulating concentrations of progesterone is greater during luteolysis than the correlation between CL area and progesterone [11]. Due to the possibility of evaluating LBF, some Authors suggested this technique for non-pregnancy diagnosis (NPD) around 20 days after timed artificial insemination (TAI). Several authors demonstrated that the process of CL regression can be identified in bovines. This is of significant interest, given that most non-pregnant females undergo luteolysis before performing a conventional ultrasound scan for diagnosis [13-15]. Routinary pregnancy diagnosis (PD) is performed around 28 days after TAI. This is based on studies that show a decrease in accuracy when conventional ultrasound examination is performed earlier than 28 days after TAI [16]. NPD had two different approaches: absence of colour in the central region of CL [13]; the CL area with blood perfusion signals was $\leq 25\%$ or when their CL area was $< 2 \text{ cm}^2$ [14]. When colour Doppler was used for pregnancy diagnosis at 20 weeks post-AI, the false negative (FN) rate was 0.6% for Siqueira et al. [13], while other authors reported an FN rate of 0% [14; 17-19]. However, the authors reported a false positive rate of $7 < 25\%$ [13-19]. A potential contributing factor to the variation in the number of false-positive results in these studies is the percentage of cows that experience early embryonic mortality between days 20 and 30 of gestation, as a greater proportion of dairy cows' experience early embryonic mortality compared with beef cows [20; 21].

Conclusion

In conclusion, although the potential of colour Doppler ultrasound to improve reproductive monitoring in cattle has been demonstrated, its current limitations, related to cost, operator dependence, physiological variability and lack of standardisation, must be addressed. Achieving wider dissemination in both research and commercial practice necessitates targeted research, technological innovation and a coordinated training effort. Now, with the production of relatively affordable ultra-portable ultrasound machines, it will be possible to apply Doppler technology in daily veterinary practice. This could be a breakthrough towards greater standardisation and refinement of early PD, contributing to improved cattle breeding.

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24- EVALUATING THE RELATIONSHIP BETWEEN ULTRASONOGRAPHIC PARAMETERS AND SEMEN QUALITY IN ANGORA BUCKS

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Introduction

Since male fertility is essential for successful breeding in livestock, the usage of a non-invasive tool as ultrasonographic (US) imaging capable to evaluate reproductive capacity in Angora bucks could be a useful and reliable method.

Objective

This study aims to investigate the association between ultrasonographic parameters and semen quality in Angora bucks.

Materials and methods

Thirty-six bucks were categorized into three groups based on the Computer-Aided Ultrasonography (CAU) appearance of the testicular parenchyma, defined by the Testicular echotexture Index (TEI) which reflects the extent of the tissue changes observed in the testicles through ultrasound. In the first phase of the experiment scrotal circumference, testicular volume, and pixel grey intensity (PGI) of the testis and vesicular glands were evaluated. Semen was collected using an artificial vagina, evaluated for both fresh and post-thaw characteristics. In the second phase, semen was collected via transrectal massage and compared with transrectal ultrasonographic findings.

Results

Group 3 exhibited the highest scrotal circumference and testicular volume, while group 2 showed best fresh and post-thaw semen parameters including the concentration and motility. Semen collected via transrectal massage showed lower motility and viability comparing to artificial vagina, emphasizing the impact of collection method. While scrotal circumference and testicular volume did not differ significantly between groups in rectal collections, testicular PGI was highest in Group 3.

Conclusion

Although the testicular echotexture index (TEI) is not a precise diagnostic tool, higher echogenicity negatively affects sperm quality, particularly post-thaw motility and viability. Accessory gland ultrasonography, especially of vesicular gland and bulbourethral glands, may support fertility assessment. Ultrasonography provides insights into semen quality and freezeability, with PGI and Computerized-aid ultrasonography as potential diagnostic markers for breeding soundness.

25- MULTIPLE FACTORS CAUSING INFERTILITY IN A HERD OF LIMOUSIN BREEDING BULLS UNDER EXTENSIVE FARMING CONDITIONS

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Introduction and Objective

Fertility assessment is especially important in herds operated under extensive systems, such as those in Alto Porma, in the Province of León (Spain), mostly located above 1,000 m above sea level. In this region, cattle are farmed under extensive systems except during the winter, playing a fundamental role in land management, environmental preservation, the use of natural resources, and the generation of quality products, as well as population stabilization in areas with harsh climates.

Since some bulls have shown signs of infertility, an attempt has been made to identify the possible causes, conducting both semen and blood studies.

Materials and methods

Several Limousine bulls with signs of infertility were assessed on two herds in the area. Six bulls underwent semen analysis, hematological analysis, macromineral (Ca, P, Mg) and trace element (Cu and Se) assessment, and ELISA to determine the presence of antibodies against epizootic hemorrhagic disease (EHD).

Results

After seminal analysis of the six bulls, four were classified as unfit for reproduction due to low sperm concentration, poor motility, and abnormal shapes. The other two bulls had less dramatic semen characteristics, although they did not reach acceptable levels. When ELISA was performed for EHD, the four bulls with the poorest semen quality were highly positive for this disease, having passed between six and eight months since contact with the virus and being asymptomatic at the time of examination. The hematological and serum values for Ca, P, Mg, and Cu were considered consistent with those reported in the literature; however, particularly deficient values for selenium were found.

In these geographical areas, fertilizers are not used, given the large areas where the animals graze, and the mineral supplements provided are usually limited to blocks of common salt. Therefore, the shortage of this trace element may be a consequence of the low intake of pasture and the lack of supplementation.

Conclusion

The association of severe selenium deficiency with epizootic hemorrhagic disease may be a cause of infertility in these breeding bulls from the León Mountains.

26- SERUM MINERALS AS PREDICTORS OF REPRODUCTIVE SUCCESS IN PRIMIPAROUS DAIRY COWS DURING THE PERIPARTUM TRANSITION

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Introduction

Since the middle ages, ruminants in the Mediterranean region have been essential for agricultural sustainability, particularly dairy cattle. During the transition period, primiparous cows undergo significant physiological and metabolic changes that increase their nutrient demands. The role of minerals—macro- and microelements—has been well-established in health and milk production, yet their relationship with reproductive performance in first-calving cows remains poorly explored.

Objective

The objective of this research was to assess whether serum mineral levels are associated with reproductive outcomes in primiparous dairy cows during the peripartum period, focusing on indicators such as days open, calving interval, and the number of inseminations.

Materials and Methods

Twenty-five healthy Holstein primiparous cows from a commercial herd in Galicia (Spain) were enrolled. Serum mineral levels were measured at four time points: one month before calving, one week before calving, one week postpartum, and one month postpartum. Blood samples were analyzed for electrolytes, macrominerals, and trace elements using standardized biochemical and spectrometric methods. Reproductive performance was tracked for up to four years, and data were correlated using principal component analysis (PCA) and Spearman's rank correlation.

Results

Electrolyte levels (Na, K, Cl) significantly declined after calving but recovered by one month postpartum. Optimal prepartum K and Cl concentrations were associated with shorter calving intervals and fewer days open. Macrominerals such as calcium and magnesium followed a similar trend, with the lowest concentrations observed one week postpartum. Among trace elements, selenium levels at one month postpartum showed a significant association with reduced calving intervals. Principal component analysis identified three mineral clusters: PC1 (Na, K, Cl), PC2 (Ca, Mg, Fe), and PC3 (Mn, Co, Mo), explaining over 50% of the total variance. PC2 and PC3 showed negative correlations with days open and calving interval, suggesting a beneficial effect of higher mineral availability on reproductive efficiency. Additionally, a body condition score (Δ BCS) loss of ≥ 1 point during the transition period was significantly associated with longer days open and a delayed return to fertility.

Conclusion

This study demonstrates for the first time that serum mineral concentrations during the peripartum period are closely linked to reproductive efficiency in primiparous dairy cows. Optimal levels of key electrolytes and trace elements, particularly potassium, chloride, selenium, and magnesium, appear to promote earlier conception and improved calving intervals. Furthermore, minimizing excessive loss in body condition may support better fertility outcomes. These findings underscore the importance of mineral monitoring and tailored dietary strategies during the transition period to enhance reproductive performance in dairy herds.

27- EPIDEMIOLOGICAL INSIGHTS INTO DAIRY CATTLE BOTULISM: ANALYSING THE OUTBREAKS IN PARMA AND PIACENZA PROVINCES (NORTHERN ITALY)

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Introduction

Botulism is a sporadic disease characterized by a neuromuscular syndrome, resulting from botulinum neurotoxins (BoNTs) exposure. BoNTs are thermolabile toxins produced by a heterogeneous group of bacteria, the most representative of which is *Clostridium botulinum*. In cattle farms, botulism causes substantial economic losses and is most frequently associated to ingestion of feed contaminated with BoNT-producing bacteria spores and BoNTs (toxoinfection and/or intoxication).

Objective

During the years 2014-2022, an increase of cattle botulism outbreaks was observed in a well-defined area bordering the Ongina and Arda rivers, within the Provinces of Parma and Piacenza, accounting for 27 out of 28 outbreaks reported in the Emilia-Romagna region (Northern Italy). This situation led to the need for funding a research project to define a risk area and implement indirect prophylactic measures.

Materials and methods

The epidemiological investigations conducted in 2023-2024 included:

- (i) Risk factor assessment, carried out through both retrospective and prospective administration of a checklist (147 items and 3 open-ended data entry forms). A total of 19 outbreaks in 17 farms were investigated using the checklist (9 outbreaks reported in 2020-2022; 10 newly emerged ones).
- (ii) Detection and genotyping of BoNTs-producing *Clostridia* using a multiplex real-time PCR and MLVA. Samples were collected from newly emerged outbreaks, a subset of farms affected in 2020-2022 (retrospective analysis), and environmental sources within the risk area. Molecular screening was performed on 270 samples from 19 outbreaks.
- (iii) Evaluation of vaccines efficacy (commercial vs autogenous), using an in-house indirect ELISA and a mouse protection assay against DC toxin. 65 sera from 4 different farms vaccinated with the commercial vaccine and 79 sera from 3 farms vaccinated with the autogenous vaccine were screened using the ELISA test (samples were collected before vaccination-T0 and 30 days after the booster-T2). The mouse test was performed on sera collected in T2 (25 sera from 6 farms vaccinated with the commercial vaccine and 19 sera from 2 farms vaccinated with the autogenous one).

Results

- (i) Significant identified risk factors included: the use of farm manure as fertilizer; pests depopulation procedures lacking adequate carcass management; inconsistent application of vaccination protocol; wild animals' abundance; and cultivation of forage within the delineated risk area.
- (ii) The chimeric DC toxinotype was confirmed in the outbreaks. 95 positive samples were genotyped using MLVA, revealing the circulation of genetically related but distinct strains within the risk area. Samples from the same outbreak and farm confirmed the persistence of identical strains.
- (iii) ELISA tests demonstrated seroconversion in vaccinated animals, with similar antibody titers elicited by both vaccines. The mouse test showed a significantly higher mean neutralizing antibody titer induced by the autogenous vaccine than the commercial one.

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Conclusion

The majority of the outbreaks (77%) in 2023–2024, occurred within or were epidemiologically linked to the Parma and Piacenza risk area. These findings confirmed the persistence of a known health issue in the region. They allowed for a more detailed understanding of shared risk factors, genetic diversity of circulating strains, and pathogenetic mechanisms.

Vaccination remains a valuable tool for the prevention and control of the disease; however, the autogenous vaccine proved significantly more effective at eliciting neutralizing antibodies than the commercial-one.

Epidemiological and molecular analyses identified key risk factors, notably using farm manure as fertilizer, which consistently tested positive during outbreaks.

These results contribute to a clearer understanding of an ongoing and concerning epidemiological situation in the region, and provide essential knowledge for managing and controlling this emerging animal health issue.

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28- *NERIUM OLEANDER* POISONING IN A DAIRY HERD: CLINICAL FINDINGS AND TREATMENT OUTCOMES

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Introduction

Toxic plant ingestion remains an important but often overlooked cause of decreased productivity and health impairment in ruminants worldwide. *Nerium oleander*, an ornamental shrub containing potent cardiac glycosides, poses a significant risk when accidentally ingested by cattle, leading to severe cardiotoxicity and systemic effects that compromise animal welfare, health and productivity. Recently, a suspected case of oleander poisoning in dairy cattle was reported to the Clinic for Ruminants of the University of Bologna, Italy.

Objective

To report the details of an outbreak of *Nerium oleander* poisoning in a dairy herd in Italy, describing the clinical presentation, treatment challenges, and long-term impacts on cattle health and reproductive performance.

Materials and Methods

The study was conducted on a dairy farm in Italy with 86 Holstein cows. The animals were housed in two different barns: Barn 1 consisted of dry and low-yielding cows and Barn 2 of lactating cows. Hay fed to Barn 1 was inspected by the farmer that has identified the presence of leaves of *Nerium oleander*. The hay offered to Barn 2 was different from those in Barn 1 and no signs of oleander were observed. A herd health visit was performed eighteen hours after ingestion of contaminated hay. All the animals housed in Barn 1 (n=26) showing general decrease of mental status and with anorexia were clinically examined. Serum biochemistry profiles were performed on 6 affected cows. Animals were monitored at 3, 6, 15, 24, 35, and 42 hours (T₁, T₂, T₃, T₄, T₅, T₆ respectively) post-treatment, followed by reassessments of 15 days and 5 months to evaluate recovery and productivity.

Results

On arrival to the farm (T₀), the cows housed in Barn 2 appeared to be healthy. On Barn 1, three cows were already dead and clinical examination of the remaining animals revealed lethargy, absent pupillary reflexes, moderate to severe dehydration (8–12%), ruminal atony, agalactia, and anorexia in all animals, bradycardia in 58% of the animals (average of 40 bpm) and cardiac arrhythmia in 69%. Serum biochemistry showed moderate hemolysis with hemoglobin concentrations estimated at 100 mg/dL, panhyperbilirubinemia (mean total bilirubin: 0.25 mg/dL; reference: 0.1–0.2 mg/dL; mean direct bilirubin: 0.05mg/dL; reference: 0 mg/dL; mean indirect bilirubin: 0.21 mg/dL; reference: 0–0.01 mg/dL), hypoglycemia: 41.5 mg/dL; reference: 57–79 mg/dL, azotemia: 71 mg/dL; reference: 7–19 mg/dL, hypercreatinemia: 2.34 mg/dL; reference: 0.4–0.9 mg/dL, and hypermagnesemia: 3.4 mEq/L; reference: 1.6–2.5 mEq/L. At T₀, each animal received symptomatic treatment consisting of 500 g activated charcoal diluted in 20 L of water, administered orally twice; 10 mg atropine sulfate intravenously; 250 ml of 33% glucose infusion; 250 g brewer's yeast orally; drenching with water; and parenteral supplementation with vitamins A, B and E. Despite intervention, one cow died. The proportion of animals presenting with bradycardia requiring therapy decreased from 58% at T₀ to 16% at T₁ (3 hours post-treatment) and eventually reached 0% at T₆ (42 hours after treatment initiation).

Cardiovascular symptoms improved significantly with the implemented therapy within 42 hours after oleander ingestion, with resolution of bradycardia and arrhythmia. Post-therapy, mortality was 3.85% and morbidity 96.15%, indicating high treatment efficacy compared to previous studies. Fifteen days post-exposure, ruminal hypomotility, anorexia, and reproductive abnormalities were noted, including persistent anoestrus and poor body condition score. Follow-up at five months revealed two cows delivered stillborn calves, while three cows were culled due to infertility and deteriorated condition.

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Conclusion

Nerium oleander intoxication severely impairs dairy cattle health, leading to acute cardiotoxicity and long-lasting systemic signs including impaired ruminal activity. While symptomatic treatment can improve survival and cardiac recovery, ruminal dysfunction and reproductive failure compromise long-term productivity. This case highlights the critical importance of rapid therapeutic intervention. Our study suggests that oleander poisoning might have long term effects in fertility, however more studies are needed to decipher the physiopathogenic mechanisms. Veterinary practitioners and farmers should remain vigilant for plant poisoning as a cause of unexplained herd health issues.

29- APPLICATION OF THORACIC ULTRASOUND IN THE DETECTION AND MANAGEMENT OF OVINE PULMONARY ADENOCARCINOMA IN A SHEEP FLOCK

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Introduction

Ovine pulmonary adenocarcinoma (OPA), also known as ovine pulmonary adenomatosis or jaagsiekte, is a contagious tumor primarily affecting sheep and, occasionally, goats. This progressive respiratory disease predominantly occurs in adult animals and has a worldwide distribution. OPA is caused by a betaretrovirus, the jaagsiekte sheep retrovirus (JSRV), which is distinct from non-oncogenic ovine lentiviruses. The virus induces the formation of lung tumors, leading to respiratory compromise, wasting, and ultimately death. As no effective treatment exists, early detection of affected animals is essential. Recently, thoracic ultrasonography (TUS) has emerged as a valuable non-invasive antemortem diagnostic tool for identifying OPA in live sheep.

Objective

The aim of this study was to evaluate the usefulness of thoracic US in detecting OPA-affected sheep and to assess its value as a flock management tool for selecting animals for culling.

Materials and methods

A flock investigation was performed in February 2025 in Switzerland after unexplained mortality in a flock of 250 with five different meat-focused breed sheep. Anamnesis revealed 20% lamb mortality within the first month of life during the previous year and recurrent ewe deaths with wasting. A slaughterhouse inspection revealed the presence of lesions compatible with OPA in an ewe. Consequently, flock health problem solving visits were performed and diagnostic procedures included clinical examination, coprology, TUS, and post-mortem investigations (gross- and histopathology). TUS was performed on adult ewes using a 6.5 MHz sector transducer over the 5th to 7th intercostal spaces from dorsal to ventral, targeting the ventral lung lobes where OPA lesions are common. Ultrasound gel was applied to a 5 cm vertical strip of skin extending from the point of the elbow. The following possible scores were considered: score 0, no abnormalities observed; score 1, any abnormal echogenicity, typically a small hyperechoic effect indicating a small region of damage to the integrity of the pleural surface but dissimilar to the training set of OPA images; score 2, multiple abnormalities at the pleural surface and/or small areas (>1 cm) of echogenicity consistent with consolidation associated with disruptions of the pleural hyperechoic reflection in the ultrasound field of the cranio-ventral and/or ventral diaphragmatic lobes; score 3, larger areas (>2 cm) of echogenicity consistent with consolidation associated with disruptions of the pleural hyperechoic reflection, extensive areas of increased echogenicity indicating pleural damage in the cranio-ventral and/or ventral diaphragmatic lobes or larger areas of consolidation with or without abscessation. TUS findings were recorded and compared with clinical signs to identify animals showing both ultrasonographic and clinical evidence of disease.

Results

Clinical examination of the flock revealed that 35% of adult ewes had poor body condition (BCS 1–2), and approximately 30% exhibited respiratory signs, including coughing, nasal discharge, and tachypnea. Necropsy confirmed OPA in two lambs and one adult ewe, demonstrating that the disease affected multiple age groups. TUS identified pulmonary lesions consistent with OPA TUS score 3 in approximately 15% of adult ewes. Lesions were characterized by consolidations with >2 cm, often associated with pleural disruption. Analysis of BCS in relation to lung lesion scores suggested a trend: ewes with lower BCS tended to have higher TUS scores, while animals in better condition generally showed lower or no lung abnormalities (p-value<0.1). TUS proved effective as an antemortem diagnostic tool, enabling early detection of affected animals. Ewes testing positive with a TUS score of 3 were selectively culled, which aimed to reduce infectious pressure within the flock. The combination of clinical assessment and TUS provided a practical approach to identify subclinical and clinically affected animals, supporting both immediate disease control and long-term flock management.

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Conclusion

OPA was identified as a significant contributor to both mortality and chronic morbidity within the flock, particularly affecting adult ewes. Thoracic ultrasonography proved to be a reliable, non-invasive antemortem diagnostic tool, capable of detecting pulmonary lesions before severe clinical signs appeared. The application of TUS enabled the identification of high-risk animals, and selective culling of ewes with a TUS score of 3 effectively reduced the infectious pressure within the flock. Beyond immediate disease control, this approach supports long-term flock health management by limiting viral transmission, improving overall productivity, and providing a practical strategy for farms lacking other definitive diagnostic options. These findings highlight the value of integrating TUS into routine flock health monitoring programs, emphasizing its role not only as a diagnostic tool but also as a proactive measure for sustainable OPA management.

30- FIRST REPORT OF SPORADIC BOVINE LEUKOSIS IN A MARCHIGIANA CALF: CLINICAL AND PATHOLOGICAL CHARACTERIZATION OF A RARE CONDITION

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Introduction

Bovine leukosis is a neoplastic disease with global distribution and occurs in two main forms: enzootic bovine leukosis (EBL), which has a viral etiology and typically affects adult cattle, and sporadic bovine leukosis (SBL), a rare condition of unknown etiology that is not considered contagious. SBL occurs in three forms: juvenile, thymic, and cutaneous. The juvenile form typically affects calves between 3 and 6 months of age and is characterized by multicentric lymphoma, often accompanied by clinical signs such as weight loss, anorexia, and weakness. Fever, hepatomegaly, ataxia, and diarrhea may also be present. The clinical course of SBL is rapid and usually fatal. While several cases of leukosis have been described in the literature, most refer to the enzootic form. To date, the juvenile form has not been documented in the Marchigiana breed.

Objective

The aim of this study is to describe the clinical and anatomo-pathological findings in a juvenile Marchigiana calf affected by SBL, from a herd located in the province of Ancona, Marche region, Italy.

Materials and methods

A three-month-old male Marchigiana calf from a beef herd in Ancona province underwent clinical examination after the breeder reported abnormal enlargement of peripheral lymph nodes. The calf presented with hyperthermia, jaundice, and generalized lymphadenomegaly. Two days later, it developed acute enteritis with diarrhea and died within three days. A necropsy was performed at the Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati". Samples of mediastinal, inguinal, mesenteric, and prescapular lymph nodes, as well as liver, kidney, and spleen, were collected for histopathological evaluation. Additionally, spleen/lymph nodes and kidney samples were submitted for molecular testing for EBL and leptospirosis, respectively.

Results

The calf weighed 85 kg but was in poor body condition. External examination revealed jaundice and marked generalized lymphadenomegaly, with lymph nodes enlarged approximately 8 to 10 times their normal size. Perineal staining from diarrhea was also noted. On opening the abdominal cavity, hepatosplenomegaly and fibrinous ascites were observed. The liver and kidneys appeared pale and were extensively infiltrated with disseminated nodules. The thoracic cavity contained haemothorax and hemopericardium, and petechial haemorrhages were present on the epicardium and thymus. Systemic lymphadenomegaly involved the haemolymph nodes. All lymph nodes had a soft, greyish-yellow "cerebroid" appearance, with spontaneous extrusion of the cut surface, areas of necrosis, and haemorrhagic petechiae. Histopathological examination revealed that all sampled tissues were diffusely infiltrated by a monomorphic population of round neoplastic cells, morphologically consistent with intermediate to large cell lymphoma. Molecular analyses were negative for EBL and leptospirosis.

Conclusion

Although rare, juvenile sporadic bovine leukosis should be included in the differential diagnosis for calves presenting with generalized lymphadenopathy. Further investigation is warranted to better understand the pathogenesis and breed susceptibility of this fatal condition in young cattle.

31- BOVINE MODELS FOR GENETIC NEUROMUSCULAR DISEASES

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Introduction

Bovine models offer unique advantages for studying genetic neuromuscular diseases due to their physiological and anatomical similarities to humans. Although less commonly used than rodent models, cattle provide a more human-like muscle and nerve architecture, a larger body size suitable for clinical procedures, and a longer lifespan that enables longitudinal studies. Moreover, the natural occurrence of certain neuromuscular disorders in cattle enhances their value as spontaneous disease models, making them highly relevant for translational research into human neuromuscular conditions.

Objective

The aim of this project is to investigate inherited neuromuscular diseases in cattle as a privileged model to better understand comparable human conditions. Suspected or affected animals were expected to be reported for inclusion in the study. These animals were examined either on-farm or, when feasible, hospitalized at the Teaching Hospital of the Department of Veterinary Medical Sciences, University of Bologna. When hospitalization was not possible, animals were included in an on-farm protocol involving clinical and genealogical data collection and blood sampling for genomic analysis. Primary focus was given to Congenital Pseudomyotonia (PMT), Bovine Spastic Syndrome (BSS), and Bovine Spastic Paresis (BSP).

Materials and methods

All animals examined, either at the clinic or on the farm, underwent clinical and morphological evaluations, including a full phenotypic description and in-depth neurological examination. Photographs and videos were collected to allow for the objective interpretation of clinical signs.

Genealogical studies were conducted to investigate potential modes of inheritance. Biological samples (EDTA blood or semen for bulls) were collected from affected animals and, when possible, their dams and sires. Genetic investigations included genome-wide association studies (GWAS), short-read whole genome sequencing (WGS), and targeted nanopore sequencing using a trio approach (case, dam, sire).

Post-mortem examinations were conducted on animals that died or were euthanized due to poor prognosis. For histopathology, central nervous system and peripheral nerve samples were fixed in 10% buffered formalin, while muscle biopsies were frozen in isopentane precooled in liquid nitrogen and stored at -80°C.

Results

Over an 18-month period, 2 adult cows and 19 calves were reported for suspected neuromuscular disease. Thirteen animals were hospitalized, six were examined on the farm, and two were evaluated remotely via WhatsApp video recordings.

Final diagnoses in hospitalized animals included: BSS (1 adult cow), arthrogryposis (1 calf), intracranial abnormalities (4), osteoarthritis/arthrosis (3), fractures (2), atresia coli (1), and unknown etiology (1).

Among animals examined on the farm, one adult cow was diagnosed with PMT and three related calves were clinically healthy; two calves exhibited postural abnormalities; and video recordings of two additional calves showed a phenotype consistent with BSP.

Genetic analysis for the PMT cow confirmed the clinical diagnosis, and related calves were identified as carriers. Genetic analyses for the remaining cases, as well as histopathological evaluation from seven necropsied animals, are currently ongoing.

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Conclusion

Although investigations are still ongoing, the study of neuromuscular diseases in cattle demonstrates strong potential as a valuable model for translational research. Continued data collection and phenotypic characterization are expected to enhance our understanding of shared pathological mechanisms and ultimately contribute to improved diagnostic and therapeutic approaches in both veterinary and human medicine.

This study was carried out within PRIN 2022 research project titled “Bovine neuromuscular diseases as a model for translational researches”, Project Code 2022XLJXCZ_001, CUP J53D23003300006. The project was funded under the National Recovery and Resilience Plan (PNRR) – Mission 4 – Component 2 – Investment 1.1 “Fund for the National Research Program and Projects of Significant National Interest (PRIN)” (Call issued by Ministerial Decree No. 104 of 02/02/2022).

32- CLINICAL AND DIAGNOSTIC EVALUATION OF RUMENITIS IN ONE-MONTH-OLD CALF

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Introduction

Ruminal diseases in calves are rarely reported and often underdiagnosed, particularly in the early stages of life. While ruminal acidosis and its consequences are well-documented in adult ruminants, often linked to dietary mismanagement, the occurrence of severe rumenitis in young calves remains uncommon. Clinical signs such as fluid regurgitation, progressive weakness, and neurologic dullness tend to be nonspecific, frequently delaying diagnosis. In such cases, diagnostic tools including venous blood gas analysis and minimally invasive techniques like endoscopy are invaluable for clinical decision-making and assessment of ruminant health and welfare for prolonged productivity.

Objective

To report a rare case of acute necrotizing rumenitis in a neonatal calf, detailing the clinical signs, diagnostic process, and systemic complications, while emphasizing the implications for calf health, prognosis, and long-term productivity

Materials and Methods

A one-month-old male Limousin calf was with persistent regurgitation of fluid, inappetence, and recumbency. Prior to presentation, the farmer had administered antibiotics, an antipyretic/analgesic, and vitamins both orally and parenterally to the animal. A complete clinical evaluation included vital parameters, hydration status, and neurologic reflexes. Jugular blood samples were collected for venous blood gas, serum biochemistry and hematological profiling. Blood gas analysis was used to evaluate pH, bicarbonate, base excess, and electrolytes. Biochemical analysis included urea, creatinine, glucose, and liver function markers. Due to poor clinical status, an endoscopy was performed via the oral cavity after sedation with xylazine. A day following the examination, the calf was found dead. The calf underwent a gross histologic evaluation and rumen tissue samples were collected, fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin.

Results

The calf presented lethargy, dehydration, weak/absent suckling reflex, intermittent jaw movement, and passive regurgitation of ruminal contents. Initial venous blood gas revealed severe metabolic acidosis pH of 7.165 (RI: 7.35-7.45), hypobicarbonatemia at 19.5 mmol/L (RI: 22-27), hypercapnia of 76.1 mmHg (RI: 35-50), and base excess of -4.1 mmol/L (RI: ± 3). Electrolyte analysis showed hyperkalemia at 4.9 mmol/L (RI: 3.5-4.5), and hyponatremia of 131 mmol/L (RI: 135-145). Biochemical profile revealed hypochloremia of 86 mmol/L (RI: 97-111), marked azotemia with 1325 mg/dL (RI: 8-23) and elevated creatinine of 2.04 mg/dL (RI: 0.9-1.3), suggesting renal compromise. Hyperbilirubinemia at 0.23 mg/dL (RI: 0-0.01), and hypocholesterolemia of 57 mg/dL (RI: 80-120) were also noted, reflecting hepatic and systemic involvement. Following intravenous bicarbonate and fluid therapy, a second blood gas analysis was performed, showing partial correction: pH 7.307, HCO_3^- 28.3 mmol/L, pCO_2 69.9 mmHg, and base excess +5.6 mmol/L. Despite this observed improvement, clinical signs persisted, prompting further diagnostic evaluation. The endoscopy showed esophageal mucosa was reddened only in the intrathoracic portion; in the rumen, there was acute necrotizing rumenitis, with mucosal detachment and rounded lesions.

Histopathology confirmed acute necrotizing and suppurative rumenitis with extensive mucosal sloughing, neutrophilic infiltration, and submucosal necrosis.

Conclusion

The death of the calf further describes the clinical severity of rumenitis and its systemic effects, including acid-base imbalance, azotemia, and significant electrolyte disturbances. Such physiological imbalances, if unrecognized, can rapidly compromise calf viability. Early detection and prompt intervention are essential, as delayed management may lead to irreversible organ damage, impaired growth, and increased mortality, ultimately resulting in economic losses within ruminant production systems.

33- DECODING A2 MILK: THE GENETICS BEHIND A HEALTHIER DAIRY ALTERNATIVE

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The A1 and A2 variants of bovine β -casein differ by a single amino acid at position 67, yet this minor structural variation leads to significant biological consequences. A1 β -casein (which has histidine) upon gastrointestinal digestion, releases the opioid peptide β -casomorphin-7 (BCM-7), which has been associated with adverse effects such as gastrointestinal inflammation, oxidative stress, and possible links to non-communicable diseases, including type 1 diabetes, cardiovascular disorders, and neurological conditions. In contrast, A2 β -casein has a proline residue at the same position, making the peptide bond less susceptible to enzymatic cleavage and therefore resulting in significantly lower BCM-7 release. Studies in both humans and animals suggest that consumption of A2 milk may alleviate digestive discomfort, improve lactose tolerance symptoms, and reduce inflammation, although evidence for long-term benefits regarding chronic diseases remains inconclusive. Moreover, A2 milk has shown favorable consumer perception and market growth due to its potential health benefits. From a breeding perspective, the frequency of the A2 allele varies among cattle breeds, and selective breeding programs are being implemented to increase A2 milk production. Nevertheless, techno-functional properties of A2 milk for dairy product manufacturing remain under evaluation, with some studies indicating inferior coagulation and yield traits compared to conventional milk. Despite ongoing debates and the lack of definitive causal relationships, the shift toward A2-only dairy products reflects both consumer demand and a precautionary approach in nutrition. The A2 herd conversion process in animal breeding is a complex undertaking that involves genetic testing of both cows and calves, along with the strategic use of artificial insemination. In Holstein-Friesians, the A1 and A2 beta-casein variants are typically present at similar allele frequencies, or with a slight predominance of A2. Successful conversion requires careful consideration of several key parameters, including the initial allele frequencies, herd size, replacement and involuntary culling rates, and the age structure of the animals within the herd. Continued research, particularly well-designed randomized controlled trials and systematic monitoring, is needed to fully elucidate the scope of One Health impacts and to inform evidence-based recommendations from both human health and animal genetics perspectives.

34- STUDY OF GROWTH RATE OF BARELA DROMEDARY (*CAMELUS DROMEDARIUS*) CALVES SUPPLEMENTED WITH POSTBIOTICS IN SEMI-INTENSIVE MANAGEMENT SYSTEM

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Introduction

The camel represents a prospective asset, significantly contributing to the national economy and food security in several countries globally. It has sustained Bedouins, nomads, and pastoralists for millennia. The demand for camel meat seems to be rising due to health considerations, as it yields carcasses with lower fat content (1.2-1.8% compared to 4-8%) and higher water content (5-8% more) than cattle meat. It contains a higher concentration of polyunsaturated fatty acids (PUFA) compared to beef. It is utilized for therapeutic purposes in the treatment of several disorders. Camel meat is a rich source of high-quality protein in numerous nations, particularly in regions where climatic conditions significantly impact the productivity and performance of other livestock. The camel is essential to the economy of pastoral societies; nonetheless, it is insufficiently examined by researchers and development practitioners in Pakistan. The scientific community is neglecting this important genetic resource; therefore, an effort has been made to showcase its production potential and related traits in natural settings. Thus, this study will augment the fundamental information of camel production and offer direction for the newly developed camel feed by Dairy-lac and the implementation of XPM, both in Pakistan and internationally. Pakistan is the fifth most populous country and faces significant challenges with food security. Investigating new resources presents a significant difficulty. Livestock production necessitates increased focus as it sustains food and livelihood for over 20 million individuals. Significant limitations in cattle production include the insufficiency of feed resources.

Objective

Camels are mostly raised in pastoral systems, especially in the Cholistan and Thal deserts in Pakistan. Literature is deficient in comprehensive data regarding the characteristics and associated criteria of pastoral farming in natural ecosystems. Therefore, it is essential to preserve data on production for the nation's primary database development. This study highlights the consequences of concentrated feed and postbiotics supplementation on the growth profile of Barela camel calves. This study was designed to assess its growth potential in grazing/browsing with stall-feeding systems.

Materials and methods

This investigation was carried out in Chak 81M, at Jalalpur Pirwala. About twelve 1-2 years old camel calves participated in the forty-five days trial. Camel calves number were split up into four groups at random, with respective group averages of ≈ 929 , ≈ 936 , ≈ 977 , and ≈ 969 kg. Camel calves were housed under conditions with an average temperature (AAT) of 24.6°C to 8°C varying from the day to night, an average relative humidity (ARH) of 26%, and an average day length (ADL) of 10 hours and 18 minutes during the shortest days of the year, which began at 6:52 am and ended at 5:26 pm (PKT). To ensure their good health, all camel calves were treated for endo and ecto parasites before the trial. The trial lasted for 45 days with first 15 days as acclimation period. Twice a day, clean water was given to the camel calves. As a control group, the first group was permitted to graze for eight hours. The second group was fed 2 kg of concentrate in addition to eight hours of grazing. The third group was offered 2 kg of concentrate and 6 grams of XPM (a postbiotic supplement) along with eight hours of grazing. The fourth group was provided 2 kg of concentrate and 15 grams of XPM along with eight hours of grazing. Each group's concentrate and XPM feeding allowance was measured by weighing scale and supplied every day to ensure accuracy in supplementation as strict adherence to feeding schedules was maintained. Regarding growth rate the body weight in Kg was recorded at day0 and day45 as initial and final readings with Formula method and daily weight gain (DWG) was derived. The data collected was analyzed statistically on SPSS software, the design used was completely randomized design, One Way ANOVA with independent factors as (Feed and XPM supplementation); and dependent factors as (daily weight gain), was applied, least significant differences was used as post-hoc test to compare the differences among the treatment means.

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Results

The daily weight gain (DWG) of experimental calves attained in this trial were found to be significantly varied ($P<0.05$) among treatment groups. The first group which was considered as control expressed DWG as ≈ 80 , the second group as ≈ 259 , the third as ≈ 261 and fourth as ≈ 274 grams, respectively. The growth rates of groups 2nd and 3rd seem differed slightly, although they do differ significantly from the control group while the group 4th achieves a maximum growth rate.

Conclusion

The growth rate of Barela camel calves are strongly influenced by dietary supplements which include their feed and XPM. According to the data provided, Groups 2, 3, and 4, showed a significantly higher daily weight gain (DWG) than the control group. With a growth rate of ≈ 274 g/day, G4 outperformed the control group by a considerable margin (≈ 80 g/day). The statistical significance ($P<0.05$) showed that the supplementation greatly impacted the performance of calves' growth, particularly in G4 in semi-intensive management system. The study concludes by confirming that feeding Barela camel calves XPM and feed supplements increases growth performance. The results imply that adding postbiotics and customized feed formulations can help camel calves acquire more weight and be healthier overall.

Keywords: Camel calves, Growth rate, Postbiotics, Semi-intensive

35- COMPARISON OF TWO DIFFERENT METHODS OF ADMINISTRATION BETA-CAROTENE TO CALVES

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Introduction

In ruminant nutrition, beta-carotene is mostly recognized as a pro-vitamin that improves reproductive parameters in dairy cows. However, it appears that it may also be beneficial for calf health, especially in terms of antioxidant protection and immunity. Beta-carotene can be supplemented to calves in colostrum from their own mothers or administered directly, parenterally.

Objective

Two experiments were performed in our work. In the first experiment, beta-carotene (Carofertin 10 mg/ml, Alvetra & Werfft GmbH, *Betacarotenum* 10 mg/ml, 20 ml/cow) was administered subcutaneously to cows several weeks before calving. In the second experiment, beta-carotene (Dalmavital Fatro S.P.A, *Betacarotenum* 40 mg/ml, 1 ml/40 kg of live weight) was directly administered intramuscularly to 7-day-old calves. The aim was to compare which of these two ways of beta- carotene administrations (through their mothers or directly to calves) is more effective in terms of beta-carotene concentration in the serum of calves and other selected laboratory parameters.

Materials and methods

In experiment 1, 10 cows were subcutaneously given mentioned beta-carotene preparation for about 3-4 weeks before calving. Another 10 cows were selected as a control group. Blood samples were taken from their own calves at the following times: before colostrum intake, 3 hours after colostrum intake, 24 hours after colostrum intake, and 7 days after colostrum intake. The calves were fed colostrum exclusively from their own mothers. In the second experiment, calves were given beta-carotene at 7 days of their age. The control group of calves in this experiment did not receive any vitamin preparation. Blood samples were taken 7 (before beta- carotene application) and 35 days of their age. Beta- carotene, vitamin A, total antioxidant capacity and other selected laboratory parameters were determined in the blood of calves. The results were statistically evaluated using the ANOVA method.

Results

Calves from the first experiment had significantly ($P<0.05$) increased beta-carotene concentration before colostrum administration. Also, an increased concentration ($P<0.01$) of total antioxidant capacity (TAC) was observed in their serum compared to the control group. In the second experiment, calves that were given parenteral beta-carotene had significantly increased its concentration in serum compared to the control group ($P<0.001$) at 35 days of age. However, the TAC concentration was not increased as it was in the calves from the first experiment.

Conclusion

In terms of increasing TAC in calves, parenteral administration of beta-carotene to their mothers before parturition has proven to be the most effective. However, this method of administration in connection with subsequent feeding of calves with colostrum from their own mother is not always possible from the point of view of technology and organization of breeding system in dairy farms. However, regarding the beta-carotene concentration itself, but without increasing the TAC, direct application of beta-carotene to calves has proven to be effective in this work. More details and other laboratory parameters will be discussed at the congress.

36- DIETARY SUPPLEMENTATION WITH A NUCLEOTIDE-RICH YEAST EXTRACT ENHANCES THE CONTROL OF NEONATAL DIARRHEA SYNDROME IN LAMBS

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Introduction

Neonatal Diarrhea Syndrome (NDS) is recognized as a primary cause of mortality in lambs under 20 days of age. It typically has a multifactorial origin, commonly involving *Cryptosporidium* spp., rotavirus, and certain strains of *Escherichia coli*. Nucleotides, essential components of DNA and RNA, play a key role in biochemical and cellular functions associated with rapid genetic replication, protein synthesis, cell division, and tissue development. These functions are especially important in neonates and during challenging periods for the immune system. Given that nucleotides modulate the immune response and support cells with high turnover rates—such as those in the immune system and intestinal lining— their dietary supplementation may help reduce the impact of NDS in lambs.

Objective

In the present study we evaluated the potential effects of dietary supplementation with a nucleotide-rich yeast extract on the control of NDS in lambs.

Materials and methods

A total of 96 newborn lambs were included in the study and divided into two equal groups. The treatment group (NG, $n = 48$) received 1 g of a nucleotide-rich yeast extract (Nucleoforce Livestock™, Bioiberica S.A.U., Palafolls, Spain) orally once daily for the first 15 days of life. The control group (CG, $n = 48$) received no nucleotide supplementation. Throughout the study, all lambs underwent a daily clinical examination for the first 20 days of life and were evaluated for the presence of diarrhea using a three-point fecal scoring system (0 = normal, 1 = intermediate and 2 = watery feces) and the score was recorded. Lambs with a fecal score ≥ 1 were classified as having diarrhea. Diarrhea was considered resolved when the fecal score returned and remained back to 0 for 24 hours. Based on these records, the number of days with diarrhea (fecal score ≥ 1) and the average fecal score on days with diarrhea were determined. Diarrhea index (DI) was calculated as follows: $DI = \text{number of days with diarrhea} \times \text{average fecal score on those days}$. Lambs showing signs of diarrhea were subjected to a daily clinical examination and received an oral electrolyte mixture once per day until recovery. In cases of clinical signs of sepsis (fever/hypothermia, inappetence, depression, weakness, scleral vascularization) treatment with non-steroidal anti-inflammatory drugs (NSAID) and antimicrobials was initiated. Antimicrobials were also administered in cases of diarrhea lasting for ≥ 5 days. The antimicrobial agents used were a combination of ampicillin-dicloxacillin, selected based on the results of the antimicrobial susceptibility testing. The NSAID used was meloxicam.

Results

During the study, a total of 83 lambs developed diarrhea (86.46%); 39 in the NG and 44 in the CG. Despite the lower diarrhea incidence in the NG in comparison with the CG, there was no significant difference between groups ($p = 0.136$). As for the duration of diarrhea, lambs in NG experienced significantly fewer days with diarrhea (3.4) than CG (4.6) ($p = 0.005$). Although the average fecal score during diarrhea was not significantly different between groups, the DI was significantly lower in NG (5.3) compared to CG (7.8) ($p = 0.001$). The number of lambs requiring antimicrobial treatment was significantly lower ($p = 0.004$) in the NG (7/38; 18.42%) than in the CG (21/44; 47.73%).

Conclusion

Dietary supplementation with a nucleotide-rich yeast extract demonstrated beneficial effects in the control of NDS in lambs. Specifically, it significantly reduced the duration of diarrhea, the diarrhea index (DI), and the need for antimicrobial treatment. Additionally, although not significantly, the diarrhea incidence was lower in the supplemented group. These results point towards the usefulness of dietary nucleotides as an effective approach for mitigating the severity of NDS in neonatal lambs. This approach not only contributes to improved animal welfare but also aligns with efforts to reduce antimicrobial usage in livestock production.

37- THE INFLUENCE OF SERUM STORAGE TEMPERATURE ON THE STABILITY OF BIOCHEMICAL PARAMETERS IN COW BLOOD

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Introduction

The pre-analytical phase is the greatest source of errors in laboratory medicine. The distance between farms and laboratories, the method of storing separated serum, as well as the workflow dynamics within the laboratory, can all influence the stability of metabolic parameters in a sample, potentially leading to falsely elevated or decreased values.

Objective

The aim of this study is to determine the stability of key parameters from the metabolic profile of cows using serum samples stored at room temperature or under refrigeration, and to establish the number of days these samples can be stored without compromising the stability of the parameters, thereby ensuring valid results.

Materials and methods

Thirty serum samples were collected from dairy cows at different lactation stages, with blood drawn from the v. coccygea using appropriate serum separation tubes. After centrifugation, approximately 5–6 mL of serum was obtained per sample and divided into two equal aliquots, one stored at room temperature (+20°C) and the other in a refrigerator (+4°C). Measurements of standard biochemical parameters from the metabolic profile were conducted daily over six days using photometric methods with Rayto RT1904C and Chemray120 analyzers. Diagnostic kits from Biosystem (Spain), Randox (UK), and Pointe Scientific (USA) were utilized.

Stability loss was evaluated based on percentage deviation (PD%), calculated as $PD\% = ((t_k - t_0)/t_0) \times 100$, representing the relative change from day 0; and maximum permissible instability (MPI), calculated as $MPI = 0.5 \times (CV_a + CV_i)$, where CV_a is the analytical coefficient of variation and CV_i is the intra-individual biological variation (data from laboratory). A parameter was considered stable if PD% was less than MPI; if PD% exceeded MPI, the value was deemed unreliable due to instability over time.

Results

Indicators of protein status showed high stability: albumin remained stable for 6 days at +4°C and for 3 days at +20°C, while total protein levels remained stable throughout the entire observation period at both storage temperatures. Urea was stable for 5 days at +4°C and 2 days at +20°C, with a progressive increase in concentration over time. Among energy balance parameters, glucose and NEFA demonstrated low stability, with both remaining stable only for 1 day at +20°C and 2 days at +4°C; notably, glucose levels decreased while NEFA levels increased during storage. In contrast, BHB was much more stable—maintaining acceptable stability across all 6 days at +4°C and for 4 days at +20°C—with a slight upward trend observed over time. Regarding hepatocellular function markers, stability varied: all measured values declined progressively. Cholesterol remained stable for 6 days at +4°C and 3 days at +20°C, while triglycerides were stable for 6 days at +4°C and 4 days at +20°C. Total bilirubin showed good stability for all time points up to 5 days at both temperatures. AST retained stability for 4 days at +4°C, but only for 2 days at +20°C. Calcium showed excellent stability for the full 6-day period at both +4°C and +20°C. Phosphorus also demonstrated good stability, remaining within acceptable limits over 5 days under both temperature conditions.

Conclusion

Based on PD% and MPI values, it can be concluded that different biochemical parameters exhibit varying degrees of stability under the two serum storage conditions. Glucose and NEFA must be analyzed on the same day the blood sample is collected, as they show rapid degradation. In contrast, most other parameters remain stable for 2–4 days at room temperature and 4–6 days under refrigeration. The highest stability was observed for calcium (Ca), phosphorus (P), and beta-hydroxybutyrate (BHB).

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38- SMART FARMING FOR SMALL RUMINANTS:INTEGRATING REMOTE SENSING-BASED AND FIELD-BASED TECHNOLOGY TO IMPROVE PRODUCTIVITY AND WELFARE

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Introduction

Small ruminants, sheep and goats, are vital to rural economies, and ecological balance in diverse farming systems worldwide, especially for marginal agricultural areas. However, their productivity and welfare remain challenged by environmental variability, resource limitations, disease pressures, and the constraints of traditional management practices. In recent years, smart farming technologies have emerged as promising tools to address these challenges, yet their application in small ruminant systems remains underexplored compared to other livestock sectors.

Objective

This presentation provides a comprehensive review of current knowledge on smart farming applications in small ruminant production. It aims to explore how the integration of remote sensing-based and field-based technologies can support more efficient, welfare-oriented, and sustainable management practices.

Materials and methods

A narrative literature review was conducted, focusing on scientific publications, technical reports, and applied case studies from the past decade. The review was structured around two main pillars: (1) remote sensing technologies, including satellite imagery, UAVs (uncrewed aerial systems - drones), and climate-monitoring tools used for pasture and environmental assessment; and (2) field-based technologies such as wearable sensors (e.g., GPS, accelerometers, RFID), automated health and behavior monitoring systems, and on-farm data collection platforms. Emphasis was placed on integrative approaches, challenges in adoption, and examples from extensive and semi-intensive systems.

Results

The literature shows that remote sensing provides critical insights into forage availability, grazing suitability, and early warning signals for heat stress or drought, supporting better pasture and resource management. Field-based technologies enable continuous monitoring of animal activity, health, and behavior, contributing to timely interventions and improved welfare outcomes. Integration of both systems through decision support platforms facilitates more precise management, reduces reliance on labor-intensive practices, and enhances resilience against environmental and economic challenges. However, barriers such as high initial costs, lack of infrastructure, limited digital literacy among farmers, and the need for context-specific solutions remain significant.

Conclusion

Smart farming technologies represent a transformative opportunity for small ruminant systems, particularly when remote- and field-based tools are used in synergy. While technological potential is clear, broader adoption will depend on multidisciplinary collaboration, farmer training, policy support, and cost-effective, user-friendly solutions tailored to diverse production contexts. This review highlights the path forward for leveraging digital innovation to sustainably improve productivity and welfare in small ruminant farming.

39- PRECISION TECHNOLOGIES FOR THE DIAGNOSIS AND CONTROL OF PARASITIC DISEASES IN SMALL RUMINANTS

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Parasitic infections may influence animal health, welfare and productivity, in terms of reduced growth rate, meat, milk and wool quantity and quality, and reduced fertility. In recent years, technological innovations have been rapidly developed and applied also to parasitology field aiming to perform accurate epidemiological studies, diagnosis, as well as efficient control approaches and treatment strategies (Vercruysse et al., 2018. Parasitology; Charlier et al., 2022. Adv Parasitol).

Indeed, thanks to use of new electronic devices, e.g. biometric sensors, Global Positioning System (GPS), dataloggers, camera traps (CT) and drones, a continuous real-time monitoring and data collection related to macro and micro epidemiology of parasites in pet and farm animals can be performed, rationalizing the control of parasitic infections and reducing the risks of drug abuse and environmental contamination. In this way, Geographical Information Systems (GIS) and Disease Mapping tools are indispensable to manage all the spatiotemporal data, allowing to plan an integrated approach of precision veterinary medicine from sampling strategies to prevention and prediction of parasitic diseases (Rinaldi et al., 2006. Geospat Health). In addition, the use of dataloggers can help to know the epidemiology of helminth infection in small ruminants through monitoring of climate condition as temperature and relative humidity of pasture. As demonstrated by a recent study of the PARALT Project (PRIN 2022 PNRR) which highlighted how monitoring the climatic conditions of pastures is important to know the seasonal trend of gastrointestinal nematodes in extensive and semi-extensive sheep farming systems. Understanding how climate and management strategies influence GIN infections and their trend is essential for developing predictive models and sustainable parasite control strategies.

Moreover, remarkable progress has been performed in diagnostic field thanks to development of new digitized tools (semi-) automated for a rapid, user-friendly, standardized and accurate recognition of parasitic elements and assessment of Faecal Egg Count/Faecal Egg Count Reduction (FEC/FECR) for both pen-side and laboratory use. This new generation of smartphone-based methods and digital microscopes (e.g. Kubic FLOTAC Microscope) combined with use of artificial intelligence (AI) algorithms, including machine learning and deep learning, has been developed to overcome gaps and limitations i.e., human errors and time for analysis, of the traditional microscopy (Cringoli et al., 2021. Parasitology; Rinaldi et al., 2022. Adv Parasitol).

Finally, innovative approaches, e.g. App, immersive virtual reality, can be used also for the dissemination and training activities to stakeholders and end-users (Cringoli et al., 2021. Vet Parasitol). Therefore, all the abovementioned strategies will be very important to assist a new generation of operators (i.e. technicians, physicians, veterinarians and farmers) in veterinary parasitology.

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40- CAN AGROINDUSTRIAL BY-PRODUCTS MODIFY CHEESES NUTRITIONAL AND NUTRACEUTICAL CHARACTERISTICS?

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Introduction

In the Mediterranean basin, where dairy sheep are widespread, the use of by-products in their diet is a frequent practice. The presence of bioactive compounds in these by-products, such as polyphenols, and the high levels of unsaturated fatty acids, confers added value to these feeds, that could modify the ruminal metabolism, animal health, and the quality of derived products (milk and cheese). The expected beneficial effects of bioactive compounds in milk and cheese obtained from small ruminants have not always been observed due to their complex and variable matrices and their dosage and mode of use in the diet of dairy sheep.

Objective

The aim of the Biometric and Protein projects was to evaluate the effects of supplementation with prickly pear by-products or olive pomace by-products on the performance of lactating ewes and on the quality of milk and cheese.

Materials and methods

The Biometric project evaluated the dietary effects of two ensiled prickly pear by-products (PPB) on the performance of 12 lactating ewes (60 days in milk), divided into three experimental groups homogeneous for parity, weight and milk yield, following a Latin square design (3 groups $\times$ 3 diets). Diets with the same crude protein and NDF were different for the PPB supplementation: 1.5 kg/day/head of PPP silage (prickly pear peels + 12% wheat bran) was added to the PPP group, 1.0 kg/day/head of PPS silage (prickly pear peels, pulp, seeds + 12% wheat bran) was added to the PPS group, while only hay and concentrate were fed to the CTR group. Individual milk production and composition and bulk milk composition for each group were determined every two weeks. Bulk milk was processed into fresh Pecorino Siciliano cheese, which was chemically and physically analyzed after 7 days of ripening.

The Protein project investigated the dietary effects of partially destoned olive cake (OC) incorporation on sheep milk yield and milk and cheese composition. A total of 124 ewes (homogeneous for parity and milk production) were selected and randomly divided into control (CTR) and experimental (EXP) groups of 62 each. For 12 weeks, both groups received 0.5 kg/head/day of isoenergetic concentrate (with 17% OC in EXP) and 6 hours of natural grazing. Individual milk yield and composition, and bulk milk composition for each group were determined every fortnight. Furthermore, bulk milk was cheese-making according to the Pecorino Siciliano DOP protocol and, after 6 months of ripening, the cheeses were evaluated for chemical, physical and sensory parameters.

Results

The effect of PPB on milk composition was not significant, except for the urea level, which was significantly ($P<0.01$) lower in the milk produced from PPP and PPS diets than CTR diet. No significant differences were observed between the composition of cheese, while the highest TPC levels of milk and cheese were observed in PPP, followed by PPS, while CTR presented the lowest levels ($P<0.01$). CTR milk showed a significantly ($P<0.01$) higher milk SFA % than PPP and PPS. Moreover, PUFA % was significantly lower in CTR than PPP and PPS milk. Consequently, the UFA/SFA ratio was significantly higher ($P<0.01$) in PPP and PPS than CTR milk. The same trend in the fatty acid composition was observed in the cheeses.

The effect of OC by-product showed that the daily milk yield did not change among the groups probably because the concentrates were isoenergetic. In terms of composition, the milk obtained from the EXP group showed higher somatic cell counts ($p<0.05$) and lower milk urea levels ($p=0.07$), no difference were found in terms of chemical composition and the cheese fatty acids, probably due to the major effect of pasture. Panelists noted differences in the sensory profile of the cheese but did not express any preference between the cheeses.

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Conclusion

The use of by-products in sheep's feed can generate differences in the quality of milk and cheese, but these depend on how these by-products are used: storage methods, dose used and associative effect with other feeds.

41- HYGIENIC AND SANITARY REQUIREMENTS FOR RAW MILK AND PRODUCTION

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Introduction

The hygienic and sanitary quality of raw milk is crucial for the safety and technological suitability of dairy products, particularly cheeses. Implementing Good Manufacturing Practices (GMP) at the farm level and across the entire food supply chain is crucial for minimizing the risk and extent of microbiological contamination. This ensures the attainment of satisfactory hygienic standards, thereby safeguarding consumers from potential foodborne hazards linked to raw milk sheep dairy products.

Objective

This study investigating the relationships between key hygienic (Total Bacterial Count - TBC) and sanitary (Somatic Cell Count - SCC) parameters in ovine and caprine raw milk, and their consequential impact on milk's technological characteristics relevant for cheesemaking. Furthermore, this study aims to provide a detailed analysis of the food safety risks associated with small ruminant products, as well as the relevant mitigation strategies applicable throughout the dairy supply chain. Our focus is on highlighting the critical role of stringent quality controls from the farm through to final processing. We pay particular attention to the crucial role of strict quality controls, from the dairy farm to the final product.

Materials and methods

An overview of hygienic standard requirements will be presented. The analysis was based on a thorough consultation of existing literature and current legislation (Regulation EC 853/04), corroborated by two years of analytical data obtained from ovine and caprine bulk tank milk samples. The study specifically examined ovine (n=4,719 for TBC; n=9,987 for SCC) and caprine (n=518 for TBC; n=576 for SCC) milk samples. The milk coagulation properties were also evaluated in relation with the hygienic and sanitary parameters (lactodynamographic method was used as the reference method). In addition, details were shared about foodborne pathogens found in small ruminant dairy products, drawing upon epidemiological evidence from monitoring and surveillance plans, outbreak reports, and recent literature. Our research utilized predictive microbiology and risk assessment methodologies to evaluate the prevalence and survival of critical pathogens such as *Listeria monocytogenes* and Shiga-toxin producing *Escherichia coli* (STEC) throughout the entire cheese production process, from initial milking to the final product. We also examined the relevance of different critical factors affecting risk and assessed the efficacy of various proposed control measures in reducing risk using sensitivity and scenario analysis.

Results

The findings revealed a significant inverse relationship between elevated values of hygienic and sanitary parameters and the technological suitability of raw milk. High TBC values have consistently been associated with a worsening cheese-making properties, particularly with regard to the impact on coagulation time (RCT). Probabilistic risk assessments have quantified the risk for human listeriosis from raw sheep milk cheeses as extremely low at an average of $\sim 2 \times 10^{-14}$ per random portion for adults. In contrast, the risk of STEC infection has been estimated at an average of $\sim 4 \times 10^{-4}$ per random portion, representing a non-negligible risk.

Conclusion

Maintaining stringent hygienic and sanitary standards for ovine and caprine raw milk is of critical importance for ensuring both product safety and optimal technological performance in cheesemaking. High TBC and SCC milk values directly compromise milk quality, leading to adverse effects on coagulation properties and overall cheese yield. Several foodborne hazards can be associated to raw milk and dairy products from small ruminants. Among these, *Listeria monocytogenes* is often subjected to control plans, although the consumer risk for listeriosis, in type of cheese that does not support the microorganism's growth, appears significant only under specific conditions. In contrast, the risk of infection with STEC warrants careful consideration by both

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farmers and manufacturers. Various measures, particularly those implemented on-farm, could be adopted to reduce the probability and magnitude of contamination, thereby limiting consumer exposure.

42- TWENTY YEARS OF BLUETONGUE IN SARDINIA: UNCOVERING FACTS, TRACKING SCIENTIFIC DEVELOPMENTS, AND DEBUNKING MYTHS

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Bluetongue (BT) is a non-contagious infectious disease of ruminants caused by *Bluetongue virus* (BTV), a double-stranded RNA virus belonging to the genus *Orbivirus* within the family *Reoviridae*.

BTV is classified as a disease under category C according to the European Animal Health Law, enquiring specific control and prevention measures to be implemented by member states.

The primary mode of transmission of BTV is via **hematophagous midges** of the genus *Culicoides*. To date, 24 “classical” serotypes of BTV have been identified. In recent years, several “**atypical**” serotypes have emerged, characterized by distinct biological properties, including apparent **vector-independent transmission** mechanisms.

The **first documented outbreak** of Bluetongue in Sardinia occurred on August 18, 2000, in the municipality of Pula (Province of Cagliari) and was caused by serotype 2 (BTV-2). Among the various hypotheses regarding the introduction of the virus, the most widely accepted is that infected *Culicoides* midges were passively transported to the island by **sirocco winds** originating from North Africa, particularly Tunisia. This epidemiological pattern highlights the challenges in **preventing and controlling** the introduction and spread of the disease. As such, traditional control measures—such as restrictions on the movement of susceptible animals—have proven only partially effective.

Indeed, in 2000, despite the immediate implementation of **movement bans**, BT rapidly disseminated across the island. Within three months, nearly the entire territory had been affected, with the exception of some eastern coastal areas. The lack of both direct (e.g., vaccination) and indirect (e.g., vector control) strategies facilitated the **recrudescence** of the disease in 2001, originating from the eastern regions that had been relatively spared during the previous year.

Following the implementation of **vaccination campaigns** targeting BTV-2, the disease did not reappear in epidemic form for several years. However, in 2004, the emergence of serotype 4 (BTV-4) necessitated a new vaccination effort in 2005, which included both BTV-2 and BTV-4. Thanks to these immunization campaigns, no major epidemics occurred until 2013. Additionally, in 2007, the vaccination protocol was extended to include serotype 1 (BTV-1), following the detection of outbreaks in late autumn 2006.

Nonetheless, the **lack of consistent and sustained control strategies**, combined with reluctance among some livestock producers to participate in vaccination programs, enabled the continued circulation of BTV during the second decade of the 2000s. This culminated in a **major epidemic in 2013**, primarily driven by BTV-1. In subsequent years, new serotypes emerged, including BTV-3, initially restricted to southern Sardinia.

In 2024, a **severe epidemic** involving the **simultaneous circulation** of BTV-3, BTV-4, and BTV-8 confirmed that Bluetongue has become **endemic** to the region. This scenario underscores the urgent need for **annual planning and implementation of integrated control strategies**, guided by comprehensive and evidence-based **risk assessments**.

Decades of Bluetongue endemicity in Sardinia have resulted in a substantial body of scientific research and epidemiological data that has significantly contributed to the understanding of this disease. Notable findings include:

- the elucidation of **infertility mechanisms** in rams infected with BTV,
- the demonstration of **transplacental transmission** of BTV-8 in sheep, and
- the identification of **pathogenic determinants** responsible for the wide range of clinical manifestations observed in infected ovine hosts.

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43- APPLICATION OF A LCA MODEL TO ASSESS THE ENVIRONMENTAL IMPACTS OF DAIRY CATTLE FARMS IN ITALY

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Introduction

Life Cycle Assessment (LCA) is the most widely accepted method to assess the potential environmental impacts of products, processes, and systems, and it is regulated by ISO standards. In the agricultural sector, LCA can be particularly complex due to the variability in farming practices. Currently, no harmonised or standardised model exists for dairy cattle farms, which limits comparability and the development of effective mitigation strategies. Moreover, livestock systems are associated with a range of environmental pressures, including greenhouse gas emissions, land use, water consumption, and nutrient runoff, making a robust assessment framework essential.

Objective

This study aims to develop a model tailored to assess the environmental impact of dairy cattle farms in Italy, adopting a cradle-to-farm gate approach.

Materials and methods

During the 2023–2024 period, a survey was conducted on 20 dairy cattle farms in Northern Italy to collect data necessary for the LCA study, referring to the years 2021 and 2022. The main farm characteristics were as follows (mean; min-max): total herd size 396 (50-907); lactating cows 222 (24-525); milk production (tons FPCM/year) 2,209.4 (153.8-4,718.5); milk yield (kg/cow/day) 31.9 (21.9-38.6); milk fat content (%) 4.0 (3.6-4.3); milk protein content (%) 3.5 (3.4-3.6). The input variables collected included fuel, fertilizers, pesticides, electricity, purchased feed and bedding material, water and chemicals. The outputs were referred to milk yield and waste generation. Meat production from male calves and culled dairy cows was also considered. Allocation procedures were also extended to other co-products, including self-produced bedding materials, surplus crop sales, and sold photovoltaic energy. In cases where primary data were unavailable, selected secondary data sources from relevant databases were used. For detergents and pesticides, a 10% cut-off criterion was applied to determine the appropriate product categories within the database framework. LCA was conducted following a cradle-to-farm-gate system boundary. The analysis was carried out using SimaPro v9.6 (PRé Sustainability, Netherlands), integrating datasets from Ecoinvent v3.10 and Agri-footprint v6.3. Environmental impact assessment was performed using the ReCiPe 2016 Midpoint (H) method. To ensure consistency and comparability across studies, the functional unit was defined as 1 kilogram of Fat-and Protein-Corrected Milk (FPCM).

Results

Results per 1 Kg of FPCM for the 20 dairy cattle farms related to environmental impacts are (mean; min-max): global warming potential (GWP) 1.84 kg CO₂ eq. (0.91-5.84); terrestrial acidification 9.76E-03 kg SO₂ eq. (2.38E-03 – 2.46E-02); freshwater eutrophication 1.74E-04 kg P eq. (3.59E-05 – 5.47E-04); human carcinogenic toxicity 5.92E-04 kg 1.4-DCB (2.26E-04 - 2.36E-03); land use 1.42 kg m²a crop eq. (0.42-3.91); fossil resource scarcity 0.14 kg oil eq. (0.03-0.94); water consumption 0.09 m³ (0.01-0.25). The main contributors to GWP (kg CO₂ eq/kg FPCM), averaged across farms, are: fertilisers (29%), enteric fermentation (26%), purchased feed (24%), self-produced feed (11%), inorganic waste (10%). A thorough analysis of the data revealed that farms with high GWP values were characterised by low production efficiency or a lack of self-sufficiency in cow feed production. Consequently, these farms were obliged to purchase most of their feed from suppliers located distant from the farms.

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Conclusion

The integration of LCA modelling into dairy farming practices facilitates a more rigorous and comprehensive analysis of the environmental impacts associated with milk production. LCA implementation allowed to identify the main environmental hotspots at the farm level. By adopting a cradle-to-farm-gate perspective and by using farm-specific data, the model offers context-relevant insights that can support informed decision-making and guide the implementation of mitigation strategies.

44- IMPACT OF SUBCLINICAL KETOSIS ON GREENHOUSE GAS EMISSIONS AND ULTRASOUND IMAGING IN DAIRY COWS

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Introduction

After calving, cows typically experience a negative energy balance, leading to mobilization of body reserves, especially adipose tissue. Ketosis is a metabolic condition of dairy cows, characterized by increased levels of ketone bodies, particularly β -hydroxybutyrate (BHB). A BHB threshold of 1.0 mmol/L is typically used to diagnose subclinical ketosis. This condition affects up to 56% of cows in early lactation and it is influenced by factors such as body condition at calving. Excessive lipid mobilization is also associated with hepatic fat accumulation and muscle catabolism, both assessable through ultrasonography.

Around 25% of total greenhouse gas (GHG) emissions originate from non-CO₂ sources, with methane (CH₄) representing approximately 80% of emissions from livestock. The CH₄ is the major non-CO₂ greenhouse gas produced in the rumen. Metabolomics analyses have shown that ketotic cows possess higher levels of CH₄ precursors, indicating a potential connection between metabolic disorders and CH₄ output.

Objective

The aim of the study was to determine the impact of subclinical ketosis on GHG emissions and ultrasound imaging of liver, back fat and muscle.

Materials and methods

Study procedures were approved by Ethical Committee for Animal Welfare of University of Padua (protocol n. 103549/2024). A total of 60 Holstein-Friesian multiparous dairy cows were enrolled from a single farm (CERZOO ltd, Piacenza, Italy).

All animals were evaluated at 7, 14, 21, and 28 $\pm$ 2 days in milk (DIM) through field measurement of BHB, ultrasound imaging, and GHG monitoring. BHB was assessed using a portable digital meter and specific blood ketone test strips. Based on BHB level, cows were divided into two groups: control (CTR, BHB < 1.0 mmol/L; n = 43) and subclinical ketosis (KET, BHB $\geq$ 1.0 mmol/L; n = 17). Ultrasound examinations of the liver, back-fat (BFT), and *longissimus dorsi* muscle were performed using a Mylab™ OneVET portable scanner (ESAOTE S.p.A., Genoa, Italy) equipped with a multifrequency convex probe. The following parameters were recorded: portal vein diameter (PVD), depth (DPV), area (PVA), and liver depth (LD). Daily CH₄, CO₂, and H₂ emissions were measured using the GreenFeed system (C-Lock Inc., Rapid City, SD, USA).

Statistical analyses included a mixed model to detect differences between groups. Significance was set at $p \leq 0.05$.

Results

Significant differences in BHB concentrations were observed between groups at all-time points ($p < 0.001$), with higher levels in KET animals. Ultrasound data revealed lower PVD and PVA in KET cows at days 7, 14, and 21, suggesting impaired hepatic perfusion. In contrast, CTR animals showed a time-related decrease in DPV, with the highest value at 7 DIM (122 mm). The LD was higher in KET cows at 14, 21, and 28 DIM (≥ 156 mm), consistently exceeding the 152.6 mm threshold for hepatic lipidosis. The BFT was initially greater in KET cows but declined markedly over time, unlike CTR animals whose BFT remained stable. This pattern suggests accelerated lipolysis in KET cows, which also had higher pre-partum fat reserves. No significant group differences were observed for *longissimus dorsi* thickness, indicating muscle reserves were relatively preserved across both groups.

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Regarding GHG emissions, KET cows emitted significantly more CH₄ (392 ± 27.2 g/day) than CTR (216 ± 16.3 g/day; $p < 0.001$). CO₂ emissions were lower in KET ($10,838 \pm 355.03$ g/day) than CTR ($12,209 \pm 215.35$ g/day; $p = 0.006$), and H₂ was also reduced in KET (0.83 ± 0.52 g/day vs. 2.01 ± 0.51 g/day; $p = 0.023$).

Conclusion

The findings of this study highlight the negative impact of subclinical ketosis on both GHG emissions and ultrasound imaging in dairy cows. In particular, ketosis reduced portal vein diameter and area, and increased CH₄ emissions, liver depth, and back-fat loss. In conclusion, these results emphasize the need for ketosis management to improve both animal welfare and environmental impact.

45- ANIMAL GROWTH AND ULTRASOUND CHARACTERISTICS OF *LONGISSIMUS DORSI* OF BEEF HEIFERS ACCORDING TO TWO STOCKING DENSITY: A PRELIMINARY STUDY

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Introduction

The animal production and welfare in beef fattening livestock may be influenced by space allowance. In fact, beef productivity can be reduced by insufficient space per animal. The marbling score is used on meat to evaluate the amount of fat around and between the muscle bundles, elements influenced by animals' productivity. Consequently, the marbling score can be used to link animal welfare and meat quality. The in vivo ultrasonographic evaluation of *longissimus dorsi* can be used to estimate the amount of fat in muscles through a texture analysis.

Objective

The aim of this preliminary study was to use ultrasound for a field estimation of muscle growth and quality in animals undergoing two different stocking densities.

Materials and methods

Experimental procedures were approved by Ethical Committee for Animal Welfare of University of Padua (protocol n. 11/2024). This study was funded by Research projects of relevant national interest (PRIN; n. 20223H5YPJ) as part of "Next generation EU" project. A single stock of 27 Charolais heifers were enrolled with average age and weight of 13.4±1.2 month and 415±33 Kg at arrival. Animals were randomly divided into three indoor boxes of 6×12m (72m²). Two boxes housed 7 animals per box and represented the low stocking density group (LowD group, 10.3m²/animal), while one box housed 13 animals and represented the high stocking density group (HighD group, 5.5m²/animal). Animals were evaluated at arrival and monthly until the slaughtering (0d, +30, +60, +90, and +120 days) with *longissimus dorsi* ultrasonography and individual fecal samples. Two ultrasound scans, transversal and longitudinal, was performed dorsally between the 12-13 ribs of right side with a linear probe, 5 MHz and 15 cm of image depth. Fecal samples were collected from rectal ampulla for cortisol assessment. Individual weights were assessed on arrival and the day of slaughter to estimate average daily gain (ADG; Kg/d). A section of muscle tissue about 2cm thick was taken at slaughterhouse from the same location as the ultrasound scans to perform a centesimal meat analysis. Differences in *longissimus dorsi* ultrasound evaluations and fecal cortisol levels were assessed by mixed model. The ADG and meat analysis were evaluated by linear model with only group effect. Carcass yield at the slaughter was compared by chi-square test. A post-hoc pairwise comparison was performed using Bonferroni correction. A p-value<0.05 was accepted.

Results

The production cycle length was 158 days for HighD and 136 days for LowD, with no differences in ADG (1.35 and 1.41 Kg/day, respectively). On the contrary, the carcass yield was greater in LowD (59.9% vs. 58.3%, p<0.001). Centesimal analysis of meat revealed no differences in dry matter, ash, protein and fat. The muscle thickness (cm) and intramuscular fat (%) evaluated by *longissimus dorsi* ultrasound was not different between groups. However, ribeye area and backfat thickness were higher in LowD compared to HighD from +30d and +60d, respectively, until the end of the trial (Ribeye +120d: 107 cm² in LowD vs. 94 cm² in HighD; Backfat thickness +120d: 1.3 cm in LowD vs. 1.0 cm in HighD). The fecal cortisol level showed greater concentration in the HighD compared to LowD only at +60d. However, this parameter was found to be almost stable in the LowD (from 32.2 to 44 ng/g), and showed a progressive increase at +30d and +60d in the HighD (from 28.4 to 60.2 ng/g) to decrease thereafter to 31.3 ng/g.

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Conclusion

Preliminary results of this field study revealed that a difference of 5 m²/animal could negatively affect the carcass yield (-1.6%), ribeye area (-13 cm²), and backfat thickness (-3 mm). Moreover, the fecal cortisol levels progressively increase for the first 60d of the stocking period. However, ADG and centesimal analysis did not reveal any additional adverse effects. Further studies with a larger sample size were needed for a more thorough evaluation of the effect of stocking density.

46- MICROBIOLOGICAL EVALUATION OF VACUUM-PACKED DRY AGED BEEF

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Introduction

Dry aging of beef is a technique used to improve the tenderness, flavor, and organoleptic properties of beef. In recent years, the consumption of dry aged meat has increased significantly due to growing consumer interest in high-quality sensory products. Current European regulations provide guidelines on the extrinsic parameters of the maturation process—temperature, humidity, and ventilation—but lack clear indications on post-aging storage, particularly for vacuum-packed dry aged meat. Some studies suggest that vacuum storage at 4 °C for up to 14 days is acceptable from both microbiological and sensory perspectives. However, the stability of the product beyond this period remains uncertain. It is known that for fresh meat, total bacterial counts (TBC) above 6–7 log CFU/g are associated with the onset of spoilage, including off-odors, slime formation, and loss of freshness. Similarly, lactic acid bacteria (LAB) counts above 7 log CFU/g have been linked to sensory degradation.

Objective

The aim of this study was to evaluate the microbiological evolution of dry aged beef at different maturation times (0, 14, 30, 45, and 60 days), followed by vacuum storage at 4 °C for 6, 9, 15, and 18 days. Microbiological analyses included total bacterial count (TBC), lactic acid bacteria, and Enterobacteriaceae, both on the surface (crust) and in the edible inner portion vacuum stored.

Materials and Methods

Three batches of beef knuckle from three different cattle breeds were selected. The cuts were dry aged for 60 days using the Stagionello® Meat Curing Device with the following parameters, temperature 1 ± 2 °C, relative humidity $78 \pm 7\%$, ventilation 2.5 m/s. Samples were collected at aging days 0, 14, 30, 45, and 60, with portions taken from the crust and inner edible part. The parameters analyzed were water activity (aw), pH, total bacterial count (TBC), lactic acid bacteria, and Enterobacteriaceae. For each maturation time, five slices were vacuum-packed and stored at 4 °C for 6, 9, 15, and 18 days. One sample from each group was analyzed at the end of each storage period.

Results

From day 14 of aging, a significant reduction in Enterobacteriaceae was observed, decreasing from an initial mean of 3.56 log CFU/g on the surface and 1.75 log CFU/g internally, to undetectable levels in subsequent samples. Surface TBC remained stable up to T30, where a 1 log increase was noted, followed by stabilization between 6 and 7 log CFU/g. In the internal edible portion, TBC decreased progressively from 5.59 log CFU/g at T0 to a minimum of 2.63 log CFU/g at T45. During vacuum storage, TBC exceeded 7 log CFU/g on day 18 of shelf life for T0 and T45 samples and on day 15 of shelf life for T60 samples, an isolated spike was recorded at day 15 of storage in the T30 group, but not confirmed at day 18. LAB counts exceeded 7 log CFU/g On day 18 of storage for T45 and on day 15 and 18 for T60. Regarding the values of water activity and pH, a slight acidification of the meat was observed during the dry aging process. A decrease in surface water activity was also recorded, whereas internal aw values remained essentially stable throughout the dry aging period.

Conclusion

The results suggest that for beef dry-aged up to 30 days, an 18 day vacuum shelf-life at 4 °C remains microbiologically safe. However, longer aging times (45 and 60 days of dry aging) require further investigation, including sensory evaluation, to confirm edibility. The increase in TBC and LAB on the surface highlights the importance of proper hygiene and handling practices (GHP and GMP) during trimming to minimize contamination of the inside beef and during vacuum packaging.

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47- BUFFALO BREEDING SYSTEM: STATE OF THE ART AND FUTURE PERSPECTIVES

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Buffalo (*Bubalus bubalis*) is the 5th species bred in the world after cows, sheep, goats and pigs with 203,939,158 heads (FAO 2023) with the vast majority located in Asia (97.2%), predominantly in India, China, and Pakistan. Approximately 1.9% are present in Africa (notably Egypt); in Europe, buffalo populations are estimated at 424 thousand head (FAOSTAT 2023), with the remainder distributed across Latin America (Brazil, Venezuela, Colombia, and Argentina). In Italy, the current population comprises 434,773 buffaloes raised on 2,238 farms. Between 2015 and 2025, the buffalo population increased by 15.5%. Approximately 70% of the animals are raised in Campania, across 50% of Italian farms (BDN 2025). Buffaloes are mainly reared for milk production and, globally, in 2021 buffalo milk production comes before cow's milk (15% and 81% of world milk production, respectively; FAO 2023).

The data (BDN) indicate a farm density for Campania of 0.0856 farms per km² and a stock density of 22.4616 head per km², metrics that bear significance for assessing environmental and socio-economic impacts associated with buffalo husbandry. Further analysis of Campania data from 2015 to 2025 shows an expansion in animal numbers concomitant with a reduction in the number of farms. The most prevalent farm-size category is 100–499 head, representing 43% of farms in Italy, corresponding to a 26% decline in farm numbers but an 8.4% increase in head numbers.

In current Italian practice, the primary production objective of buffalo farming is milk for cheese manufacture. The use of buffalo as draught animals has largely been superseded by mechanisation. This transition has been facilitated by proactive management decisions within the buffalo-farming sector, wherein producers have continued to exploit the species' productive potential rather than diversifying into alternative livestock species.

From 2019 to 2025, considering only the first half of the year, production increased by 8.44%, while annual production up to December 2024, in the 2019–2024 period, grew by 8.01%.

Concurrently, the headcount of water buffalo in Italy during 2019–2024 rose by 8.5%. This suggests that production per head is relatively stable: the number of farms has decreased, but the average herd size has increased. This implies the persistence of larger-scale farms, with a loss of smaller operations. The current picture indicates a farming segment around 499 head, with an average annual production of approximately 1,980 kg per head. (tracciabilita' bufala 2025).

Data on brucellosis and tuberculosis, interpreted as disease incidence, provide indications of the effectiveness of eradication plans and the attention to biosafety, despite high livestock density per km². It should be noted that the highest concentration of buffalo is recorded in the Campania region.

Future perspectives: considering the rise in the global human population, the average increase in annual temperatures, and rainfall patterns trending toward tropicalization, buffalo farming could expand into marginal territories. For equal agricultural land use, the buffalo species provides proteins both from milk and meat, contributing to global nutrition.

From a sustainability viewpoint, the longevity of the species represents an additional factor to consider in the analysis of demographic dynamics and biosafety.

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48- APPROACH TO THE MANAGEMENT OF MEDITERRANEAN BUFFALO BREEDING IN ITALY

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Introduction

Since the 12th century, there are reports of the presence of the buffalo in Italy. While evidence of the first breeding of this species seems to date back to the 14th century.

Objective

Objective of this research is to highlight that buffalo farming (Italian Mediterranean Buffalo) in Southern Italy has reached very high production and quality standards, with significant profitability.

Materials and methods

The BuBoVet srl group, composed of 6 professionals, who control approximately 70,000 heads of cattle and buffalo, and approximately 55,000 heads of buffalo, both adults and young, distributed across at least 100 farms. The activity of the group's veterinarians is mainly dedicated to monitoring reproduction (artificial insemination, pregnancy diagnosis, post-partum visits, etc.), clinical assistance, surgery, herd well-being and monitoring udder health and, consequently, milk quality. All the monitored farms have as their main activity the production of milk intended for local dairies to produce buffalo mozzarella. However, there are only a few that dedicate part of their breeding to the breeding of buffalo for meat. It is important to underline that buffalo farms suitable to produce milk for Mozzarella DOP (Consortium for the Protection) must be composed of buffalo heads of the Italian Mediterranean breed only and provide for free or semi-free housing of the heads inside enclosures.

Results

In the 19th century, when Italy was going through a livestock crisis, buffalo breeding was the most profitable thanks to the production of cheese, meat and above all the extraordinary aptitude of the buffalo for work. This species showed a great ability to adapt to living in marshy areas, with adverse weather conditions and to feed foods with low energy levels. Under these conditions, no species of livestock producing income could resist, except the buffalo. Studies by the Royal Faculty of Agriculture of Portici (Na) (1930) showed that the net income per head of buffalo was 20% higher than that obtainable from cattle breeding conducted in satisfactory environmental conditions. During the Second World War and until the early 1950s, the buffalo was considered an endangered species. Because the economic conditions for its survival and the use of its milk were not bearable by a population that had just emerged from the war and in economic difficulty with a country in full economic recession. In fact, in the post-war period, the sale of mozzarella decreased more and more, and the consumption of meat was replaced with the use of other cheaper animal species. With the economic recovery starting in the 1960s, there was a notable and continuous increase in the demand for mozzarella, with the consequent increase in the number of heads and companies, until the birth, in 1981, of the Consortium for the Protection of Mozzarella di Bufala.

Conclusion

In recent years, buffalo farming has undergone significant transformations. We have moved from extensive farming (until the first post-war period) to confined farming which, precisely because it is more controllable, is able to better satisfy the needs of buffaloes and protect the environment. Furthermore, there has been a substantial improvement in the reproductive aspect and feeding. In conclusion, by improving the aspects indicated above, a very high level of profitability of buffalo farming is being achieved. It is important to underline that the profitability of buffalo farming, although generally considered positive, is influenced by various factors, including production costs, market prices and health risks. The buffalo mozzarella supply chain maintains a strong market, both in Italy and abroad, with constant demand. But the raw material, milk, to be sold to dairies must have particularly severe and restrictive requirements to produce a quality mozzarella.

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49- MILK MICROBIOTA VARIABILITY IN THE QUARTER AND FARM BULK MILK IN WATER BUFFALO MILK VALUE CHAIN IN BANGLADESH

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Introduction

In Bangladesh, the indigenous river buffalo population dominates, comprising a small number of swamp-type buffalo raised primarily in the eastern part of the country. Buffaloes are mainly reared in a traditional crop-livestock integrated rearing system by smallholder farmers, whose educational level is low, thus implying poor veterinary preparation for buffalo udder health. Therefore, the buffalo milk chain in Bangladesh tends to be informal and remains underregulated in terms of fundamental hygiene practices. However, to the authors' knowledge, limited information is available to date on the microbiota composition in water buffalo, and it has never been explored in the buffalo milk chain, for example, in bulk milk.

Objective

This study investigated the variability of bacterial taxonomy in relation to udder health status, specifically healthy and subclinical mastitis (SCM), at the individual level and subsequently in bulk milk (BM) from buffalo farms to evaluate milk quality in water buffalo in Bangladesh.

Materials and methods

Fifteen farms met the selection criteria for having at least one CMT-negative culture-negative (HM) and one CMT-positive culture-positive (SCM) quarter. Three types of buffalo milk fraction samples (n = 45) were collected for DNA extraction: i) one healthy and one SCM quarter from each farm, and ii) one BM sample from the farm. Total genomic DNA was extracted using the Maxwell® 16 Cell DNA Purification Kit (Promega, UK). Bacterial DNA was amplified targeting the V3-V4 hypervariable regions of the 16S rRNA gene. Quality and quantity measurements of the amplified PCR products were taken using Agilent Bioanalyzer 2100. The normalized and pooled PCR products (N = 45) were sequenced on the Illumina MiSeq platform (San Diego, CA, USA).

Results

The overall composition and relative abundance of the microbiome always remained higher in SCM milk samples compared to BM and HM (SCM > BM > HM). In total, 251 bacterial genera were identified, of which 152 (~ 60%) genera were found to be shared across three metagenomes. The relative abundances of the top 50 bacterial genera were compared across the SCM, BM, and HM groups. In SCM and HM samples, Staphylococci were the most abundant bacterial genera, with a relative abundance of 64.1% and 41.1%, respectively. However, it remained much lower (14.4%) in BM samples. The SCM milk microbiome was also predominated by Cellulomonas (6.5%), Macrococcus (5.8%), Acinetobacter (4.2%), Pseudomonas (2.4%). In contrast, Macrococcus (15.5%), Cellulomonas (10.6%), Cutibacterium (4.4%), Pseudomonas (1.2%), and Corynebacterium (1.6%) were the top abundant genera in HM samples. Simultaneously, Staphylococcus

Pre-proof

(14.4%), *Macroccoccus* (14.1%), *Chryseobacterium* (10.9%), *Lactococcus* (10.5%), and *Acinetobacter* (9.7%) were the most abundant genera in BM samples. Compared to healthy quarter samples, farm BM and SCM quarter samples exhibited significant differences ($P \leq 0.05$) in four alpha-diversity indices (ACE, Chao1, Fisher, and Observed). In the first two dimensions of the multidimensional scaling of unweighted Unifrac distances, no clear clusters were detected across the three groups.

Conclusion

These results suggest that unique bacterial genera are present in different types of samples, which could help differentiate between healthy and SCM pathogens, indicating mammary dysbiosis and potential bacterial contamination of bulk milk in water buffalo.

50- THE PRESENCE OF STAPHYLOCOCCUS AUREUS, MRSA, AND ENTEROBACTERIA PRODUCING EXTENDED-SPECTRUM BETA-LACTAMASES IN WATER BUFFALO FARMS IN THE SALERNO PROVINCE

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The water buffalo (*Bubalus bubalis*) holds an important economic role in the dairy industry in Italy, the top producer of buffalo milk in Europe, accounting for 97% of the continent's production. With the rise of the global health concern of antimicrobial resistance, our study aimed to highlight the prevalence and genotypic diversity of *Staphylococcus aureus* (*S.aureus*) isolated from milk of water buffaloes sampled in the Campania region in southern Italy. Methicillin-resistant *Staphylococcus* (MRS) and Extended-Spectrum Beta-Lactamase (ESBL) producing *Enterobacteria* have also been assessed. Bulk tank milk samples from 59 different farms were collected between June and November 2023 and subjected microbiological analyses on selective media, with and without enrichment, plus molecular analysis. Results revealed a high presence of *S. aureus* as 71.18% of samples tested positive, indicating variable bacterial load. Up to six colonies for each positive farm were characterized by molecular analyses, totaling 202 isolates. According to the primary results of the RNA template-specific polymerase chain reaction (RS-PCR), the isolates belonged to 8 different genotypes with a genotype B predominance (71.80% of farms). Analysis using selective media demonstrated that 45.76% of samples were positive for MRS. Using MALDI-TOF the most frequently identified species were *S. aureus* (44.44%), *S. epidermidis* (18.51%), *S. sciuri* (18.51%), *S. haemolyticus* (3.70%), *S. saprophyticus* (3.70%). On the other hand, 23.72% tested positive for ESBL-producing enterobacteria. The identified species were *E. coli* (64.28%), *Enterobacter* spp (21.42%), and *Klebsiella pneumoniae* (14.28%). Several isolates were resistant to antibiotics. The results demonstrate the high prevalence of *S. aureus* but also MRS and ESBL bacteria in water buffalo farms. Given their impact on animal and human health, this reaffirms the relevance of monitoring and characterizing antibiotic resistance.

51- CONTAGIOUS BOVINE PLEUROPNEUMONIA

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Contagious Bovine Pleuropneumonia (CBPP) is a highly contagious and economically significant disease affecting cattle, caused by *Mycoplasma mycoides subsp. mycoides*. It is of particular importance due to its severe impact on livestock health, productivity, and international trade. The disease is prevalent in Sahel and sub-Saharan Africa and remains a major constraint on cattle production in affected regions. Early and accurate diagnosis is crucial for the implementation of control measures and the prevention of disease spread.

The risk of introducing CBPP into North Africa is increasing due to the uncontrolled and often illegal movement of livestock across Sahel and North Africa region. Such informal transboundary trade undermines biosecurity protocols and facilitates the silent spread of infectious diseases, including CBPP but also LSD and RVF. This risk is particularly concerning given that the disease remains underreported and underestimated throughout much of the African continent, both in terms of prevalence and economic burden. In several endemic areas, CBPP management is predominantly limited to the use of antibiotics administered by livestock owners, often without veterinary prescription, supervision, or adherence to proper therapeutic protocols. This approach not only compromises effective disease control but may also contribute to the development of antimicrobial resistance. Moreover, the absence of coordinated surveillance systems, preventive vaccination campaigns, and effective regulatory enforcement in many regions significantly hampers the containment and eventual eradication of the disease. A more robust, harmonized approach to CBPP control—including regional cooperation, veterinary capacity building, and stricter regulation of animal movements should be required to mitigate the growing threat to animal health and agricultural sustainability in North and sub-Saharan Africa.

Diagnosis can be supported by the identification of characteristic pathological lesions observed during post-mortem examination.

In many African contexts, it is the only diagnostic activity that can be carried out.

These typically include fibrinous pleuritis, hepatisation of lung tissue, and the presence, in chronic case, of Sequestra, encapsulated necrotic areas within the lungs. Thickening of the pleura and abundant pleural fluid may also be observed. These lesions are often asymmetrically distributed and localized in the caudal lobes of the lungs. The detection of such lesions, in combination with clinical signs and laboratory confirmation (PCR, culture, or serological tests), is essential for a definitive diagnosis of CBPP.

Of course, it is important to be able to make a differential diagnosis with diseases present in North Africa and other countries in the Mediterranean basin, such as *Mannheimia haemolytica* and *Pasteurella multocida* or Bovine Herpesvirus 1 (BoHV-1) – IBR or Bovine Respiratory Syncytial Virus or *Mycoplasma bovis*, etc.

52- CURRENT SITUATION OF BRUCELLOSIS IN THE MAGHREB REGION

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Abstract

Brucellosis is a contagious zoonotic disease affecting livestock and humans, primarily transmitted through direct contact with infected animals or consumption of contaminated animal products. It is an important human disease in many parts of the world especially in the Mediterranean countries and yet it is often unrecognized and frequently goes unreported. While many countries have made progress in controlling the disease, it remains endemic in several Maghreb countries, including Algeria, Tunisia, Morocco and Libya. World Health Organization (WHO) argued that human brucellosis incidence in Maghreb countries was 10 to 25 times under estimated.

In **Algeria**, brucellosis has persisted despite a national control program initiated in 1995. Bovine infection averages 1.26%, based on screening just 6% of the cattle population. Poor enforcement, inadequate farmer awareness, and unregulated animal movement have limited program success. Human brucellosis ranks first among zoonotic diseases, with over 5,000 annual cases and a rising trend. In 2018, 11,428 cases were reported. *Brucella melitensis* bv 3 is the predominant strain in human infections. Molecular studies suggest distinct Algerian *Brucella* lineages, highlighting the need for further research.

In **Tunisia**, brucellosis remains endemic. Between 2008 and 2015, human incidence ranged from 2.9 to 4.35 cases per 100,000. A peak occurred in 2017 with 1,022 reported cases. The disease is largely linked to the consumption of unpasteurized dairy products and contact with livestock. Gafsa, near the Algerian border, reports the highest prevalence. Animal seroprevalence stands at 21%, especially in small ruminants, though cattle also show significant infection in some areas.

Morocco experienced several waves of control efforts since the 1960s, including a failed eradication campaign in the 1980s. Although test-and-slaughter strategies launched in 2007 helped reduce brucellosis in some farms, national herd seroprevalence has remained around 4.9% since the 1970s. Human cases are sporadic (0–27 annually), mostly caused by *Brucella melitensis* biovar 3 through occupational exposure and consumption of raw goat dairy products.

In **Libya**, prior to 2011, bovine brucellosis prevalence was under 0.2%. However, post-2011 political unrest disrupted surveillance and border control, leading to a sharp increase in infection rates. A 2017 study found 21% seropositivity overall, with 5% in cattle. Unregulated animal trade and the breakdown of quarantine services significantly contributed to disease spread.

In conclusion, despite varying degrees of intervention, brucellosis continues to challenge public and animal health systems across the Maghreb, due to weak enforcement of control measures, and practices related to livestock and dairy consumption.

Key words: Brucellosis, Maghreb, Algeria, Tunisia, Morocco, Libya.

53- EMERGING EXOTIC DISEASES IN SMALL RUMINANTS: ARE HUMAN MIGRATIONS PLAYING A ROLE?

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Introduction

Emerging exotic diseases, including zoonoses, can be introduced in non-endemic areas through animal trade and uncontrolled movements during times of conflict and migration. Sheep and Goat Pox (SGPX) and Peste des Petits Ruminants (PPR) were respectively reported in Europe in 2018 and 2010 as a possible consequence of animal uncontrolled movements. EFSA emphasized the risk of spread into Europe through informal trade and unmonitored migration routes that may involve the informal transport of small ruminants. The zoonotic Crimean-Congo Hemorrhagic Fever (CCHF) is a further rising concern with reported travel-associated cases in Europe. Displaced persons, from endemic regions in the Middle East and Central Asia, may pose a risk of introducing or amplifying disease transmission in the Balkans.

Objective

The study aimed to analyze how migratory flows may influence the spread of PPR, SGPX and CCHF, employing the One Health conceptual framework.

Materials and methods

The study focused on the countries crossed by the Balkan route, a corridor for displaced populations seeking entry into Europe. PPR, SGPX and CCHF events analysis was performed to map disease occurrence and identify possible overlaps between outbreaks and migratory corridors. A discourse analysis of policies governing health in migration was performed to understand how the presence of animals in camps is conceptualized in institutional narratives. Interviews were carried out with experienced professionals in the Balkans' migratory context to assess the presence of animals in camps, how animals are perceived by humanitarian professionals and to what extent the presence of animals can affect the health and welfare of migrants. Informal conversations were conducted with migrants who travelled the route to explore the roles of animals during migration journeys.

Results

The tracks in which diseases had spread during the considered time approximately align with the routes of migrations and the locations of camps. Considering the dynamic animal and by product trade happening in the region, it is not possible to rule out their influence on the transboundary spread of the diseases, but this variable was not included in the study and may constitute a limitation in the analysis. The analysis performed on migrants' health policies highlights how they failed to account for the potential health threats posed by animals in formal and informal settlements.

As a consequence, only human health professionals are involved in humanitarian support, overlooking the interconnectedness of human, animal, and environmental health in these contexts. The presence of animals in formal and informal settings throughout the Balkan route was confirmed by interviews and informal discussions. Particularly, small ruminants represent food sources and are ritually sacrificed during Islamic feasts, even in migratory contexts. No evidence of animals migrating with people was reported, instead informal trade networks, following human migrations paths cannot be excluded.

Conclusion

Our findings seem to suggest that human migration along the Balkan routes is not directly responsible for the spread of emerging exotic diseases of small ruminants as there is no evidence of significant small ruminants' movements accompanying migrants. However, overlaps in the diseases spread along the Balkan routes were identified suggesting a possible role of informal trade. Finally, our study underscores the importance of animals

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in migration contexts with risks associated with their presence in settlements in the absence of a proper veterinary management.

54- LUMPY SKIN DISEASE: CLINICAL ASPECTS

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Lumpy Skin Disease (LSD) is a highly contagious viral disease of cattle caused by a Capripoxvirus in the family *Poxviridae*. Clinically, it is characterized by fever, enlargement of superficial lymph nodes, and the appearance of multiple cutaneous nodules (2–5 cm diameter) on the skin and mucous membranes. Additional signs include oedema of limbs, ocular and nasal discharge, lameness, abortion, decreased milk yield, poor growth, infertility, and occasional mortality (typically <10 %). The disease has major economic implications due to hide damage, production losses, and trade restrictions.

Transmission occurs primarily via mechanical vectors—blood-feeding insects such as *Stomoxys*, *Tabanidae*, *Culicoides*, mosquitoes, and ticks. Long-distance spread is typically associated with cattle trade and transport, while local expansion follows vector-mediated dispersal.

Originally endemic to sub-Saharan Africa, LSD was first reported north of the Sahara in Israel in 1989, thought to have arrived via windborne *Stomoxys* flies from Egypt. Thereafter, the virus spread into the Middle East and Turkey in November 2013, marking the gateway for further expansion into Southeast Europe.

By August 2015, LSD had reached Greece, with outbreaks recorded in northern Greece and Limnos island. From May 2015 to August 2016, more than 1,092 outbreaks were reported across western Turkey and eight Balkan countries (Greece, Bulgaria, North Macedonia, Serbia, Kosovo, Albania).

A coordinated vaccination program using live homologous vaccines was launched across Southeast Europe. Between 2015 and 2018, over 2.5 million cattle were vaccinated, achieving >70 % coverage. This led to a dramatic drop in outbreaks from approximately 7,483 in 2016 to only 385 in 2017, and zero reported outbreaks in the Balkans in 2018.

After several years of apparent freedom from LSD in the EU, new outbreaks were officially confirmed in mid-2025. On June 21, 2025, the first outbreak in Italy was detected on Sardinia (province of Nuoro), followed by cases in Mantua (Lombardy) and additional outbreaks in Sardinia. On June 29, 2025, France reported its first-ever outbreak, in Savoie. These represent the first occurrences in mainland Italy and France and underscore renewed vulnerability during vector-favourable summer months. Containment measures (protection and surveillance zones, movement restrictions, quarantines, culling) were promptly activated.

The Mediterranean region is at heightened risk owing to vector-friendly climates, impressive movements of ships and other modes of transport

And, in Europe, livestock trade connectivity across borders.

Diagnosis can be supported by the identification of characteristic skin lesions.

The detection of such lesions, in combination with clinical signs and laboratory confirmation (PCR, culture, or serological tests), is essential for a definitive diagnosis of LSD.

Of course, it is important to be able to make a differential diagnosis with diseases present in North Africa and other countries in the Mediterranean basin, such as Pseudo-lumpy skin disease, Herpetic Mammillite, Dermatophilosis, Besnoitiosis, Hypodermosis, Bovine papillomatosis, Malignant catarrhal fever. Cutaneous lymphoma, etc.

55- ONE HEALTH-BASED APPROACHES IN BOVINE NUTRITION: EXAMPLES FROM THE EVALUATION OF TRACE ELEMENTS IN FEEDS BY THE EFSA FEEDAP PANEL

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Safety and sustainability of animal feeds is a pillar of the safety of the entire food chain. Feed additive assessment incorporates consumer safety as well as animal health and welfare, which, in turn, can affect productivity and hence food security; the safety of feed users and the environment are also essential components. The assessment process, therefore, builds on a One Health perspective, integrating contributions from different expertise and viewpoints. In particular the assessment of trace elements added to feeds as nutritional additives is a telling example. Essential trace elements may have a significant (eco)toxicity at excess dose levels, hence, the assessor should balance the prevention of deficiencies in animals and that of undesirable high exposures of humans and/or environment.

Four examples of assessments by the EFSA FEEDAP Panel and relevant to bovine nutrition are presented. Through multidisciplinary approach and the identification of the main questions, these assessments led to changes in the regulation of trace elements in bovine feeds in the European Union. The presented examples are:

inorganic cobalt (main issue: high human toxicity)

copper (main issue; environmental exposure)

iodine (main issue: transfer into milk)

organic selenium from selenized yeast (main issue: higher deposition in edible tissues).

These case studies indicate that the safety of additives and ingredients in bovine feeds may call for a benefit-to-risk assessment within a One Health perspective. A consistent identification of both the question to be answered and the benefits and risks (animals, human, environment) to be balanced, supports a robust, science-based process.

56- IDENTIFICATION OF ZOONOTIC AGENTS IN BIOAEROSOLS IN SHEEP AND GOAT FARMS: A ONE HEALTH APPROACH (ZOOAIR)

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Introduction

Bioaerosol is a vector for biological particles including viruses, bacteria and fungi, potential pathogens whose inhalation can have adverse effects on human health. The main sources of bioaerosol in livestock farms are animals, bedding and feedstuffs. *Coxiella burnetii* and *Chlamydia* spp. are widespread in sheep and goat farms and represent environmentally resistant zoonotic agents capable of surviving in the air.

Objective

The aim of this work is to acquire scientific evidence on the potential role of bioaerosol in the transmission of some zoonotic agents in sheep and goat farms. Environmental sampling as an approach to identify zoonotic agents can contribute, according to One-Health approach, to monitor exposure within risk categories. In addition, the project aims to validate an innovative diagnostic system in the airborne environment, useful for better understanding the epidemiology and route of transmission of zoonotic agents in order to implement control strategies.

Materials and methods

Enrollment: Three small-medium sized farms in north-eastern Italy were enrolled on the basis of biomolecular positivity for at least one of the two pathogens (*Chlamydia* spp./*Coxiella burnetii*), in aborted foetuses/placentas/vaginal swabs during the current lambing season.

Sample collection: During an on-site visit to each enrolled farm to submit a farm management questionnaire to the farmer, the air sampling was carried out. Bioaerosol was sampled once at two different sites inside the farm using a Coriolis Compact sampler at a flow rate of 50L/min for 30 minutes. In symptomatic animals nasal, eye and/or vaginal swabs were also taken, as well as fetuses or dead born lambs/kids.

Molecular analysis: *Chlamydiaceae* family was detected with an rPCR protocol following the WOA method, validated by the laboratory. Characterisation of the detected *Chlamydiaceae* is carried out using another rPCR, with species-specific probes.

Results

Farm A was recruited for the detection of *Chlamydia pecorum* (*C. pecorum*) in two dead kids during the lambing season. The air was sampled at two different locations inside the farm: one closer to the new born animals and the other one closer to adults. At both sites, air resulted positive for *C. pecorum*. In addition, two vaginal swabs were taken from two post-partum goats and analysed with negative results; four eye swabs were taken from newborn goats, of which three out of four were positive for *C. pecorum*.

Farm B was enrolled for the presence of *C. pecorum* in one stillborn kid. The air was sampled at two places as described in Farm A. Only kids site was positive for *C. pecorum*. In addition, seven vaginal swabs were taken from adult goats, all negative; eight ocular swabs were taken from kids, of which six out of eight were positive for *C. pecorum*; 11 nasal swabs were taken from kids, of which six out of eleven were positive for *C. pecorum*.

Farm C (mixed cow and goat farm) was recruited for the presence of *Chlamydia abortus* (*C. abortus*) in two abortions. The air was sampled at two sites: 1) in front of the goat site, 2) between the goat site and the cow one. At both locations the air was positive for *C. abortus* and *C. pecorum*. In addition, six vaginal swabs were taken from adult goats, of which two were positive for both *C. abortus* and *C. pecorum*, two were positive only for *C. abortus*, and two were negative. Four nasal swabs were taken from kids, of which three were positive for both *C. abortus* and *C. pecorum* and one were negative; another two nasal swabs were taken from two male breeders, one was positive only for *C. pecorum* and one only for *C. abortus*.

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Conclusion

Our results show the presence of zoonotic agents in the air, demonstrating that the use of an air sampler can be a successful and innovative strategy to monitor these pathogens in the farms. In this way, it is possible to improve management strategies and decrease the potential impact of such agents on human and animal health.

57- DIAGNOSTIC IMAGING IN THE PRESERVATION OF ANIMAL BIODIVERSITY: THE TERAMANA GOAT UNDER X-RAYS

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Introduction

Teramana Goat is a local variety from the center of Italy now at risk of extinction. Nowadays the urgent need to preserve local biodiversity, leads the researchers to assess conservational strategies to increase the subjects of this variety. For that purpose, authors focused on the reproductive aspect, providing a comprehensive imaging evaluation of pelvis of female subjects and then a complete morphological evaluation for oembrios/foetuses during the pregnancy.

Objective

This work was conceived with a dual purpose: first assess the normal pelvis anatomy (pelvimetry) of female goats before the breeding season and then evaluate morphology of oembryos/ foetuses during all the pregnancy to assess eventual factors potentially impairing newborn natality.

Materials and methods

In the context of pelvimetry, radiographic and ultrasonographic pelvimetry were compared in twenty female goats by measuring three pelvic diameters before pregnancies: the narrowest transverse diameter at the acetabula, the distance from the pecten pubis to the sacral promontory, and from the dorsal pubis to the first coccygeal vertebra. Measurements were taken three times by a single observer. Intraclass correlation coefficient (ICC) and Bland-Altman analyses were then performed. During the breeding season, thirteen female goats were monitored during gestation to define fetal growth indices and umbilical artery blood flow parameters. Ultrasonographic evaluations were performed starting from 14 days post-mating (dpm), initially transrectally, then transabdominally from day 36 onward. The following parameters were recorded: biparietal diameter (BD), crown-rump length (CRL), trunk diameter (TD), placentome size, and fetal heart rate (HR). Umbilical artery Doppler metrics included arterial pulse, peak systolic velocity (PSV), end diastolic velocity (EDV), mean velocity (MV), pulsatility index (PI), and resistance index (RI).

Results

Pelvimetry results showed excellent intraobserver agreement. The transverse pelvic diameter showed excellent correlation (ICC 0.96) between the two modalities, while the other two measurements had poor agreement. Concurrently, the reproductive evaluation of the Teramana goat—a native Italian breed at risk of extinction—was conducted. Nine goats were pregnant (eight single, one double gestation), with one abortion at 140 dpm showing no morphological abnormalities. BD, TD, and placentome dimensions showed a strong correlation with gestational age ($R^2 > 0.87$), while HR showed moderate correlation ($R^2 = 0.66$). Doppler values showed varying trends: AP, PI, and RI decreased, while PSV, EDV, and MV increased with gestation, though with moderate to low correlation ($0.13 < R^2 < 0.6$). No correlation was found between biometric or Doppler parameters and abortion.

Conclusion

This integrated study highlights the selective utility of ultrasonographic pelvimetry for specific pelvic dimensions and provides, for the first time, baseline ultrasonographic growth and vascular data in Teramana goats, contributing valuable information for the conservation and reproductive management of this endangered breed.

58- CONSERVATION AGRICULTURE'S IMPACT ON THE DIGESTIVE AND THE RESPIRATORY PARASITISM OF SHEEP

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Introduction

In a context of climate change and natural resource degradation, the transition to sustainable agricultural systems represents a major challenge for food security, particularly in semi-arid areas. This work is part of this dynamic by comparing two agricultural systems in Tunisia: Conservation Agriculture (CONS A) and Conventional Agriculture (CONV A), with respect to their impact on digestive and respiratory parasitism.

Objective

The objective of this study is to investigate the short-term impact of A CONS on digestive and respiratory parasitism across various age groups and occupations of sheep, and to explore opportunities for medium-term parasitological monitoring of animals. This aims to encourage farmers to adopt this sustainable eco-agriculture approach alongside livestock farming

Materials and methods

The study focused on the comparative assessment of the impact of these two systems on sheep health, through three main components: monitoring of live weight and hematological parameters (red blood cells, hemoglobin, hematocrit), coproscopic monitoring using two techniques: the McMaster technique (gastro-intestinal nematodes GIN, coccidia, *Moniezia* sp.), helminthological necropsy, and monitoring of respiratory parasitism by identifying respiratory strongyles using the McKenna technique, over two successive years during the winter-spring season. The observations were carried out over a first period of five bi-monthly visits on two groups of batches of lambs kept successively for two months of pasture and a second part on a sample of female sheep monitored over three and a half months during seven successive visits.

Results

The results revealed that during the first grazing season, CONS A had no negative impact on growth, carcass yield, or digestive parasitism in fattening lambs compared to lambs monitored in CONV A, with high prevalence rates of *Eimeria* oocysts in both types of agricultures (from 33.33% to 100 % on CONS A and from 100 % to 66.66 % on CONV A respectively), moderate infestation rates in GIN and *Moniezia*. Helminthological necropsy revealed a predominance of *Ostertagia* sp in both groups of lambs (94.25 % of *Total worms count*).

During the second year of monitoring, the results revealed the presence of significant variation between the two groups of sheep, and that those maintained in CONS A had a lower body condition than those maintained in CONV A. A statistically significant difference was also found when comparing the hematological parameters of the two groups of animals at some visits. No significant difference was noted in infestation by coccidia oocysts and *Moniezia*, however, the prevalence rate by GIN showed a significant variation at the last visit.

Likewise, no statistically significant differences were revealed when comparing respiratory strongyle infestation rates between the two groups of sheep across all visits.

Botanical and chemical analyses of plant cover samples from both farming systems revealed no significant differences in dry matter (DM), mineral matter (MM), crude fiber (CF), and crude protein (CP) content of the two types of grass samples, and there was no correlation between chemical composition and the measured parasitological parameters. Botanical composition, in turn, revealed the abundance of weeds which have an

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hemolytic effect in CONS A pastures, which could explain the slight alteration in the blood count of animals monitored in this farming system without the presence of major health changes.

Conclusion

This study provides new insights to guide the shift toward integrated agroecological models tailored to semi-arid contexts.

Additional studies over a longer period and incorporating other parasites could improve our understanding of the interaction of this type of sustainable agriculture with parasitism in sheep.

59- CERCANDO UNA PECORA: VOCI DIVERSE DA E SULLA CULTURA PASTORALE DELLE ANTICHE PROVINCE ESTENSI, QUASI VENT'ANNI DOPO

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“La prova che il piccolo principe è esistito, sta nel fatto che era bellissimo e che voleva una pecora. Quando uno vuole una pecora, è la prova che esiste.” (Antoine Marie Roger de Saint-Exupéry, “Il piccolo Principe”)

Le ricerche rigorose hanno schemi precisi, materiali e metodi codificati, grande rigore scientifico, ma nel nostro caso, confessiamo che le cose sono andate diversamente.

Forse è per questo, o addirittura per le inconfessabili motivazioni suggerite da Antoine Marie Roger de Saint-Exupéry nel brano sopra citato, che stiamo trovando qualcosa di molto diverso da quanto pensavamo all'inizio.

Siamo partiti per cercare la perduta Cornella Bianca (un tempo la più diffusa razza di ovini della provincia di Reggio Emilia e nel 2004, quando abbiamo iniziato la ricerca, considerata estinta) e l'abbiamo trovata, non tanto in centri per la conservazione della biodiversità o in parchi zoologici, bensì esattamente nello stesso posto di sempre.....coi pastori.

In questo modo però, abbiamo sì trovato la Cornella, ma abbiamo trovato anche il mondo dei pastori, la loro cultura, il modo di vivere, le difficoltà di ogni giorno, sotto il sole o con la pioggia.

Così noi, forse senza accorgercene, ci siamo addentrati in un mondo che poi si è rivelato per niente lontano o dimenticato, ma solo nascosto.

Un mondo popolato di uomini che restano tenacemente legati al lavoro, all'ambiente, agli animali e alla loro storia; tanto tenacemente da rimanere in fondo isolati da un altro mondo, che si muove al ritmo di suonerie di telefonini, week end, happy hour, brunch e party, pur distando, i due, poche centinaia di metri.

Alla fine, il centro della ricerca si è spostato dalla pecora a questi uomini e alla loro attività, perché sono loro l'unica alchimia in grado di evitare l'estinzione della nostra pecora, come di qualsiasi altro animale da reddito.

Di qui si è creato un altro stranissimo fenomeno: sono riemersi (anzi non visto che anche loro sono sempre rimasti lì dove li abbiamo trovati) prodotti come il Pecorino dell'Appennino Reggiano, le barzigole, i salami ed i violini di pecora, il brodo ed il lesso di pecora tipici della zona ed infine la cultura della lana.

Il Pecorino dell'Appennino Reggiano è un prodotto rubricato nel catalogo dei prodotti tradizionali dell'Emilia Romagna: grazie all'eccellente lavoro svolto da alcuni professionisti, in collaborazione con Comunità Montana, GAL e Provincia di Reggio Emilia, sta vivendo un momento di grande rilancio al punto che è quasi impossibile reperirne una forma.

La barzigola è una particolare preparazione di carne di pecora tipica delle valli del Tresinaro e del Secchia, tanto in particolare dei comuni di Carpineti e Baiso; probabilmente nasce come metodo per recuperare alcuni muscoli addominali di pecore a fine carriera produttiva e per conservarli, con uso di sale, in damigiane a bocca larga o vasi di coccio, sotto concce gelosamente tenute segrete.

Oggi si utilizzano animali più giovani e tagli più nobili (ad esempio, di coscia) e la conservazione è più breve, con prodotto finale meno salato.

Il Coshet o violino si ottiene dalla coscia di pecora salata e stagionata.

Questi prodotti sono stati inseriti nell'ambito del progetto, nell'elenco dei prodotti tradizionali dell'Emilia Romagna e stanno riscuotendo un lusinghiero successo tra i consumatori.

Assieme a salami, violino e bresaola, brodo e bollito, sono tipici delle zone di mezza montagna dove probabilmente i pastori transumanti del crinale facevano tappa e, anche in cambio dell'ospitalità ricevuta, commerciavano, oltre a ricotta, formaggio e agnelli, anche le pecore non in grado di proseguire il viaggio.

Gli ovini fornivano un importante contributo alla dieta carnea familiare: nella versione più semplice le carni venivano conservate sotto sale in barili o mastelli di legno, fino a giugno, e consumate addirittura in brodo, o sotto forma di più sofisticati salami, cosciotti o violini.

L'ultimo prodotto, fondamentale per l'economia pastorale, era la lana: il reddito che ne derivava tradizionalmente si diceva pagasse l'intero mantenimento dell'animale, trasformando gli altri prodotti in guadagno netto.

Questo tipo di economia, oggi quasi dimenticato, non era limitato alle zone montane. Anche i contadini delle zone pedemontane e di pianura tenevano alcune pecore, soprattutto per la produzione di lana per usi domestici: produzione di calze, maglie, maglioni, ma anche coperte, trapunte e materassi.

Negli ultimi quattro anni si è fatto il tentativo di recuperare la lana, lavarla, cardarla e filarla realizzando gilet, calze, poncho, borsette e sciarpe oltre ad oggetti in feltro come pantofole, portachiavi e bisacce.

Il fascino di questi ricordi ritrovati ha fatto nascere una rete di appassionati che irresistibilmente ne attira nuovi e che sta rendendo sostenibile una operazione altrimenti impensabile.

Il tutto era partito con il progetto "Cornella Bianca" dell'AUSL di Reggio Emilia, coordinato dal Dr. Carlo Alberto Alberti, inserito nel piano di lavoro 2006, e non si sarebbe potuto svolgere senza la preziosa collaborazione dei veterinari delle AUSL dell'Emilia orientale (in particolare Bologna, Ferrara, Imola, Modena, Rovigo), della Romagna e del Veneto.

Ricordi dunque, alcuni affioranti tra i greggi, nei racconti dei pastori, altri forse rinchiusi nei cassetti di qualche armadio o comodino oppure negli angoli della memoria, magari legati a qualche oggetto di uso quotidiano o fotografia conservati in qualche soffitta.

Sarebbe formidabile poter raccogliere queste testimonianze, servono però almeno due cose: la disponibilità di chi le conserva a condividerle, ed un sistema per poterle registrare senza comprometterne integrità e legittima proprietà.

Due novità importanti sono da segnalare nel 2022 per un gregge aderente al progetto: il pascolo invernale nei vigneti di pianura dell'azienda agricola Iotti Egidio di Correggio (che ha soddisfatto praticamente tutto il fabbisogno alimentare durante tutto l'inverno 2022/23) e l'invito del Consorzio Forestale a pascolare su terreni e boschi del seminario di Marola per il periodo primaverile-estivo. Si tratta di un ritorno all'antico (transumanza periodica), ma nel nostro caso anche di una interessante evoluzione, anche perché una delle ipotesi su cui si basa il progetto è l'utilizzo di risorse foraggere che altrimenti verrebbero perdute o addirittura sarebbero da eliminare periodicamente con mezzi meccanici e relativi costi.

Nell'autunno inverno 2023/24 la rete dei vigneti messi a disposizione per il pascolamento nel comune di Correggio si è ampliata con l'adesione un'altra decina tra viticoltori e coltivatori tra cui uno che ha chiesto di stipulare un contratto di pasco pascolo, per un totale di un centinaio di ettari che ha soddisfatto completamente il fabbisogno del gregge nel frattempo cresciuto a quasi 100 adulti. Le circa 300 balle di fieno tradizionali, prodotte nell'estate 2023, sono state utilizzate per le pecore con agnelli nei primi giorni dopo il parto.

Tra l'autunno 2024 e la primavera 2025 si sono moltiplicate le richieste di pascolamento invernale in vigneto rendendo difficile soddisfarle tutte, coprendo completamente il fabbisogno di foraggio.

La razza attualmente ha una numerosità di circa 900 capi, la metà iscritta al libro genealogico, distribuito in una dozzina di allevamenti nelle province di Reggio Emilia, Modena, Piacenza, Ferrara, Bologna, e Udine.



Fiumalbo (MO) anni 40 “Mignan” Nardini nel bosco con un ariete di cornella bianca

60- SURGICAL TREATMENT OF COENUROSIS IN SMALL RUMINANTS

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Introduction

Coenurosis is a neurological disease that occurs mainly in sheep, with a lesser prevalence in goats. It is caused by *Coenurus cerebralis*, the larval stage of *Taenia multiceps*, a tapeworm that infests the small intestine of carnivores. The most common clinical manifestation is the chronic form, which occurs in animals aged between 6 and 18 months. It is characterized by progressive neurological dysfunction due to slowly increasing size of the cyst in the central nervous system. The cyst is most often located in one cerebral hemisphere but may be also located in cerebellum or might affect multiple locations in the brain. The treatment is based on surgical removal of the cyst.

Objective

The objective of this presentation is to provide a comprehensive description of the surgical technique employed at our clinic for the treatment of chronic coenurosis and to discuss the reasons for possible failure.

Materials and methods

Prior to undergoing surgical intervention, the probable location of the cyst is determined based on clinical signs and the findings of the neurological examination. The surgical procedure is performed under intravenous general anesthesia, with the administration of xylazine and ketamine. Dexamethasone and beta-lactam antibiotics are applied preoperatively. The animal is placed in sternal recumbency, the head is secured, and the surgical field is aseptically prepared. After local infiltration with lidocaine 2% s.c., the skin of the skull is semi-circularly incised. The incision line is commenced at one horn bud, extended caudally, and terminated at the other. Subsequently, the skin is detached from the skull, and the subcutaneous tissue and periosteum are removed with a bone curette. Following comparative acoustic percussion at the respective opposite sides of the skull, the thinner part of the bone is located, and it is selected as the trephine (osteotomy) site. A 10 mm trephine hole is created at this point, and following the removal of the bone core, the dura mater is incised along the longitudinal axis. When a superficial cyst beneath the dura mater is present, it protrudes directly through the trephine site. A deeper located cyst could be identified using a 20-gauge intravenous catheter that is inserted vertically, at first and in different directions afterwards. The presence of fluid egressing from the catheter serves as an indication of the cyst's location. Once the cyst has been located, an 18-gauge catheter attached to a 20-ml syringe is inserted through the same canal to facilitate further drainage of the cyst and to remove a part of the cyst wall to the trephine hole. The cyst wall is then grasped with mosquito forceps and is gradually removed. The skin is closed with horizontal mattress sutures, and the wound is covered with an antimicrobial spray. Following surgery, dexamethasone and beta-lactam antibiotics administration continues for two and four days respectively.

Results

The success rate of this technique in our clinic is nearly 88%, as shown by the records from the last three years. The most common reason for failure is the presence of multiple cysts that cannot be identified intraoperatively. Further reasons are the inability to locate or remove the cyst, especially if it is located in the cerebellum, severe intraoperative hemorrhage and post-operative abscessation.

Conclusion

The applied surgical technique has a high success rate especially when applied in animals with good clinical condition and a single cyst, providing an effective treatment of chronic coenurosis.

61- RECENT GREEK EXPERIENCE RELATED TO PESTE DES PETITS RUMINANTS

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Introduction

Peste des petits ruminants (PPR) is a highly contagious viral disease affecting sheep and goats. Greece had maintained its official PPR-free status for years, but a field outbreak in July 2024 marked the first-ever detection of the virus in the country. This unexpected event provided an opportunity to assess national capacities for early detection, rapid response, and disease control in the context of a transboundary animal disease.

Objective

This report aims to summarize the recent Greek experience with PPR, focusing on the epidemiological profile, clinical signs, and lessons learned from the 2024 outbreak, which led to the suspension of Greece's PPR-free status.

Materials and methods

Following the notification of clinical signs in sheep in central Greece in July 2024, the veterinary services conducted sampling and laboratory confirmation using RT-PCR. Immediate response measures included culling, movement restrictions, disinfection, and active surveillance within protection and surveillance zones. Official data on morbidity, mortality, and outbreak spread were collected from national sources and WOA (WAHIS) reports.

Results

The first outbreak was confirmed on 8 July 2024. To date, 86 outbreaks have been reported to WOA, affecting both mainland Greece and several islands. No new cases have been reported since 31 October 2024. In a press statement, the Minister of Agriculture attributed the introduction of the virus to the importation of live animals from Romania into the Thessaly region. However, many farmers reportedly restocked their herds through unregulated channels, including illegal imports from neighboring countries such as Turkey, where PPR is endemic and routine vaccination with a live vaccine is practiced. This may explain certain outbreaks in which only RT-PCR-positive animals were detected without exhibiting clinical signs—likely indicating prior vaccination rather than active infection. This scenario was particularly suspected in parts of Thessaly and other regions.

Clinically, the most consistent sign was diarrhea, followed by reduced appetite. Sudden deaths, mainly in young animals, were often reported before the onset of gastrointestinal signs. Nasal, ocular, or oral discharges were observed in a small proportion of animals. Fever (body temperature $>39.5^{\circ}\text{C}$) was observed in only a few cases, likely due to delayed detection after the fever had already subsided. Thoracic auscultation was unremarkable in early stages but wheezing and bubbling sounds were noted in moribund animals. No ulcers or lesions were observed in the oral cavity of any symptomatic or deceased animal. Post-mortem findings in acutely deceased sheep consistently included severe pulmonary oedema and acute congestion, usually accompanied by congestion of the abomasal and intestinal serosa.

Conclusion

The 2024 PPR outbreak in Greece highlights the urgent need to reassess transboundary movement controls between EU member states and to implement stricter regulations on livestock imports from third countries such as Turkey. Enhanced surveillance, strengthened biosecurity, and coordinated regional response mechanisms are essential to prevent future incursions.

62- EVIDENCE-BASED CLINICAL GUIDELINE FOR TARGETED ANTIBACTERIAL TREATMENT OF NEONATAL DIARRHEA IN LAMBS

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Introduction

Neonatal diarrhea syndrome (NDS) is the leading cause of death in lambs younger than 20 days of age. Most outbreaks are complex in origin and commonly involve pathogens such as *Cryptosporidium spp.*, rotaviruses, and certain *E. coli* strains. Although antibacterial agents are often used to treat NDS, they should be reserved for cases in which lambs exhibit signs of systemic illness, and their use must be supported by evidence-based decision-making. Therefore, there is an urgent need to develop specific, clinically driven guidelines for the use of antibacterials in the treatment of NDS in lambs.

Objective

The objective of this study was to propose an evidence-based clinical guideline for the selective administration of antibacterials to lambs with NDS as well as to evaluate its efficacy under field conditions.

Materials and methods

According to the proposed treatment protocol, all lambs with diarrhea were to be allowed to suckle voluntarily and received an oral drench of an electrolyte solution once daily starting from the first day of diarrhea until recovery. Antibacterials and nonsteroidal anti-inflammatory drugs (NSAIDs) were only to be administered in cases suggestive of bacteremia, presenting at least two of the following clinical signs: pyrexia, hypothermia, anorexia, depression, and scleral congestion. Lambs showing only pyrexia were to be treated with NSAIDs alone. Lambs with persistent diarrhea lasting more than five days and lacking the aforementioned symptoms were to be administered antibacterials alone, without NSAIDs. The selection of antibacterial agents was guided by susceptibility testing.

This protocol was implemented and assessed on a dairy sheep farm, where 82 out of 92 lambs born over a forty-days period developed NDS. All lambs were clinically monitored daily from birth, and diarrhea was evaluated using a three-point fecal scoring system: 0 = normal, 1 = intermediate, and 2 = watery feces. Lambs scoring 1 or higher were classified as having diarrhea. Diarrhea was considered resolved once a fecal score of 0 was maintained for at least 24 hours. Diarrheic lambs underwent thorough daily clinical examinations until they recovered.

When antibacterial treatment was indicated, a combination of ampicillin and dicloxacillin was administered based on the results of susceptibility testing obtained using the Vitek 2 system and interpreted according to CLSI guidelines. Meloxicam was the NSAID used in the protocol.

Results

Pyrexia alone was detected in approximately 26% of the animals with diarrhea. In about 15% of the diarrheic lambs, pyrexia was initially detected, and subsequently, further symptoms indicative of bacteremia developed in the following days. Antibacterials alone or in combination with NSAIDs were administered in about 35% of lambs. From the lambs of the last category, 24% received only antibacterials due to prolonged diarrhea and 76% were treated with antibacterials and NSAIDs due to signs of bacteremia. More specifically, the latter percentage is analyzed as follows: 31% had scleral congestion – pyrexia, approximately 17% had scleral congestion – pyrexia – depression – anorexia, 10% had scleral congestion – hypothermia – depression, 7% had scleral congestion – depression, and 3.5% for each one of the following combinations pyrexia – depression, hypothermia – depression and depression – anorexia. The mortality rate was 3.7%.

Conclusion

The proposed protocol, which is based on clinical evidence, proved effective in treating lambs affected by NDS. The presence of pyrexia alone did not constitute a strong indication for initiating antibacterial treatment.

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Therefore, the decision for the administration of antibacterials should not rely exclusively on pyrexia but rather on the presence of at least two clinical signs that suggest bacteremia.

63- STUDY OF DIFFERENT VIRAL INFECTIONS IN SOME DROMEDARY AND CATTLE HERDS IN TUNISIA: PRELIMINARY RESULTS

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Introduction

In Tunisia, cattle and dromedary herds represent two important activities in terms of local transportation of people, meat and milk production. Since the international literature is poor on the spread of the Bovine alphaherpesvirus 1 (BoAHV-1), Bovine Viral Diarrhea Virus (BVDV) and Enzootic Bovine Leukemia (EBL) in Tunisia, in this preliminary study, we hypothesized that some dromedary and cattle herds could be infected with BoAHV-1, BVDV and EBL.

Objective

The objective of this study was to assess the spread of BoAHV-1, BVDV and EBL in several dromedary and cattle herds in the southern region of Tunisia.

Materials and methods

The study was conducted in the Tataouine Governorate, located in southern Tunisia, during March 2024. In this area, there are several intensive dairy cattle herds and extensive dromedary farms. For this preliminary study, four farms were selected in an area free of foot-and-mouth disease outbreaks. Specifically, the first two were extensive dromedary farms (No. 1 and No. 2), while the other two were intensive dairy cattle farms (No. 3 and No. 4). Each farm included 50 animals and was enrolled in this study. The farms were georeferenced for precise location identification.

Blood samples were collected from the jugular vein and transported under refrigeration to the Regional Agriculture Development Commissariat laboratory of the Tataouine. After centrifugation at $850 \times g$ for 30 minutes at 4°C, the serum samples were tested for antibodies against BoAHV-1, BVDV, and EBL. Specifically, the following ELISA tests were used: 1) IDEXX IBR gB X3 and IDEXX IBR gE for detecting antibodies against glycoprotein B (gB) and glycoprotein E (gE) of BoAHV-1; 2) IDEXX BVDV Total Ab X3 test for detecting total antibodies against BVDV; 3) IDEXX Leukosis Blocking Ab (EBL) for detecting antibodies against glycoprotein 51 (gp51) of Enzootic Bovine Leukosis. The kits were used according to the manufacturer's instructions. In addition, all serum samples were tested using a virus neutralization test for BoAHV-1 following the protocol described in the Manual of the World Organization for Animal Health.

Results

The dromedary sera tested negative for all viruses except for farm No. 1, which showed a 2% positivity rate for EBL. In contrast, the cattle sera showed positivity for BoAHV-1, BVDV, and EBL. Specifically, farm No. 3 exhibited a variable positivity rate for BoAHV-1 ranging from 4% to 10%, with 40% positivity for BVDV and 14% for EBL. Meanwhile, farm No. 4 showed a variable BoAHV-1 positivity ranging from 6% to 10%, with 36% positivity for BVDV and 4% for EBL.

Conclusion

In conclusion, the preliminary results obtained in this study did not reveal the presence of the targeted viruses in dromedary farms, except for a low percentage (2%) of positivity for EBL. Conversely, a moderate positivity for BVDV was confirmed in cattle farms (36%–40%), with lower positivity rates for BoAHV-1 (6%–10%) and EBL (4%–14%). For these reasons, the hypothesis was confirmed only for cattle herds and partially for the dromedary's farms. As a result, this study highlights that the viruses investigated are present in cattle in

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the southern region of Tunisia. Therefore, understanding the epidemiology of BoAHV-1, BVDV, and EBL in different animal species will help improve control measures and mitigate the impact of these diseases on animal health and local livestock production. For these reasons, further studies are needed to understand better the prevalence of the viral infections considered in this study throughout the entire Tunisian territory.

64- CONTAMINATION OF ALPINE PASTURES BY *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS*: EVALUATION OF CATTLE AND RED DEER CONTRIBUTION THROUGH ENVIRONMENTAL FECAL SAMPLES

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Introduction

Paratuberculosis (PTB) is a chronic enteritis of both domestic and wild ruminants caused by *Mycobacterium avium subsp. paratuberculosis* (MAP). The disease globally causes significant economic losses in livestock industry and compromises animal health and welfare. MAP transmission primarily occurs via the fecal-oral route. Animals eliminate MAP mainly into feces and since MAP does not multiply outside the host, the prevalence of shedding animals and the load eliminated by feces are crucial for the environmental biocontamination. Semi-extensive and extensive livestock production may determine exposure to MAP infection and intra- and inter-species transmission on pasture.

Objective

Based on fecal sampling of the environment, the contribution of cattle and red deer to the contamination of pastures by MAP was quantified, and risk areas were identified within alpine grazing areas.

Materials and methods

Samples were collected during 2020 and 2021 summer seasons. In 2020 the sampling covered 21 pasture areas classified as “sharing” or “non-sharing” by red deer and cattle. In 2021 because of the low prevalence obtained in 2020, the sampling areas were extended to 6 macro-areas, encompassing the previous ones and fecal sampling was increased. The sampling was carried out on red deer and cattle mixed pasture areas. Fecal samples were analyzed in two replicates by IS900-qPCR. PCR positive samples were submitted to culture according to double incubation method by WOAHP manual. Isolates were typed for the assignment to type S or C and further subtyped analysing a panel of VNTR/SSR loci. To explore spatial variation in the probability of detecting MAP-positive feces, we used a generalized additive modeling approach. The model included the presence/absence of MAP as the response variable and a smooth two-dimensional surface term $s(x, y)$ – where x and y represent the sample coordinates – as the predictor. Data were analyzed using R.

Results

In 2020, out of 470 red deer feces collected, 12 tested positive (2.6 %); out of 287 bovine feces, 3 tested positive (1%). MAP contamination by red deer and cattle feces was detected in six and three pasture areas, respectively. Contamination by both species simultaneously was observed in one shared area only. In 2021, out of the 1338 red deer feces, 33 were positive (2.5 %), while out of 662 bovine feces, 16 gave positive result (2.4 %). MAP was isolated in eight out of 45 red deer feces: six from the sampling of 2020, and two from the sampling of 2021. In contrast, out of 19 positives bovine feces by qPCR, none showed growth by cultural methods. All the positive cultures showed a low number of colonies (1-10 CFU/slant). All MAP isolates resulted as type C and the profile obtained was the INMV profile 1, a profile previously described in Italy. The smoothed spatial trend surface revealed broad-scale spatial heterogeneity in the probability of detecting MAP-positive faeces across the study area. Overall, a low but widespread percentage of MAP-positive feces in both species was observed, indicating a low proportion of grazing MAP-shedders. The highest contamination was reported mainly in overlapping areas for both species.

Conclusion

In the perspective of biosecurity management of multi-host grazing areas and in order to achieve the goal of MAP control, it would be important to carry out surveillance targeted at reservoir hosts, both domestic and wild. The present study supports the utility and reliability of environmental fecal sampling as a non-invasive sampling approach to assess paratuberculosis shedding in grazing animals. Environmental MAP contamination in the study area resulted low and should be considered as a negligible route of exposure to MAP infection, although the sub-typing profile of red deer MAP isolates suggests a possible interspecies transmission.

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65- USING ARTIFICIAL INTELLIGENCE TO DETECT SIGNS OF WELLBEING IN MEDITERRANEAN DAIRY CATTLE: A PILOT STUDY

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Introduction

In recent years, assessing animal welfare has become increasingly important in the Mediterranean dairy industry (Tsiokos et al., 2024). Current methods for assessing welfare mainly rely on manual observation, which can be subjective and time-consuming (Aubé et al., 2022). Advances in computer vision and artificial intelligence offer the possibility to quantify visible signs of wellbeing in cattle automatically (Tassinari et al., 2021).

Objective

The objective of this study is to investigate whether simple computer vision techniques can differentiate healthy (presumably happier) cows from sick or stressed cows based on their posture and activity, using easily obtainable video data.

Materials and methods

We analyzed 20 publicly available annotated videos (10 healthy, 10 unhealthy) from Mediterranean dairy farms using OpenCV, DeepLabCut (Mathis et al., 2018) and MediaPipe to extract skeletal keypoints and derive behavioral metrics such as time spent lying, head movement frequency, and spatial activity. These were analyzed using lightweight statistical classifiers.

Results

Healthy cows were observed to spend a higher proportion of time ruminating while lying with relaxed head movements, and to show broader spatial activity patterns compared to cows with clinical signs of illness. A simple classifier achieved 80% accuracy in differentiating healthy from unhealthy cattle, indicating that visible wellbeing traits can be effectively quantified from video.

Conclusion

This study provides first evidence that accessible AI methods can move beyond disease detection toward proactive wellbeing monitoring. Our results open a path toward affordable, real-time welfare tracking in extensive Mediterranean systems, bridging veterinary science, AI, and sustainable agriculture.

66- BUYING AND SELLING GOATS IN SARDINIA: FORENSIC OBSERVATIONS ON A SPECIFIC CASE

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The authors (party's technical advisors), in an expert's report on the purchase and sale of goats in the province of Cagliari, highlight the interpretative difficulties in the application of the Civil Code rules on the termination of the contract due to redhibitory defects. They emphasise these issues also and especially in the presence of actions (property deeds) that might exclude the guarantee for defects. Following an old-fashioned, "bulk buy" a confrontation arises between seller and buyer. Through the various interlocutory stages consideration is given to contract termination, property deeds and animal welfare regulations. The authors especially examine Caprine Arthritis -Encephalitis Virus (CAEV) and Caprine Paratuberculosis, subject of the dispute between seller and buyer. At the end they propose a series of corrective measures to better define these cases, also considering the current legislation on animal protection and welfare.

Introduction

Question: CAEV and Caprine Paratuberculosis are "redhibitory vices."

We will evaluate the legislative, medicolegal and animal welfare implications following the sale of goats in the province of Cagliari and the interpretative difficulties in light of the old RPV and EU Regulation No. 429/2016. Positive serological results (often asymptomatic) pose a problem for institutional and non-institutional bodies (compensation, etc.).

Objectives

The authors propose optimal solutions to these disputes, both to safeguard animal welfare and to resolve these disputes.

Materials and Methods

June 2023, in Villanovatulo, we viewed some goats for purchase. Two Malaguna breed animals were very thin, but they were excellent milk producers. The seller was opposed to the sale, but the buyer purchased them in bulk. Two other goats had carpal swellings. On August 15th, the buyer purchased additional goats at the beginning of lactation. The transfer of 110 animals (70 five-month-old and 40 fully grown and pregnant) took place on September 1st, to the buyer's farm. 68 days after the purchase, the buyer sent a letter (per WhatsApp) requesting: "Cancellation of the contract," the return of the deposit, the return of two promissory notes and the maintenance of a 0,60 cents fee per animal from September 1st to the date of the message. On December 15th 2023 the buyer sent a certified e-mail to the seller with a formal notice of default. He had inspected the goats purchased in September suspecting CAEV. Samples of 46 animals were sent for testing to the Lombardy Institute for Animal Health (IZS). The same letter reported easy miscarriages, interstitial mastitis and death within three to six months.

Results

35 animals tested positive for CAEV, 2 animals tested positive for PTBC and 11 animals tested positive for both diseases. Regulatory framework for CAEV and PTBC The two diseases are considered non-dangerous (health and livestock/market).

Forensic veterinary medicine

The warranty for defects (Article 1496 of the Italian Civil Code) is governed by Special Laws, customs or the preceding provisions (Articles C.C. 1490, 1491, 1492, 1493, 1494, 1495). Serious, hidden, and pre-existing defects must be reported within eight days of discovery, provided that one year has not passed since delivery. After reporting, retired animals are subject to a Property Deed.

The request of withdrawing from the contract was incorrect because there wasn't any "state of danger". Thus future events are uncertain (serological positivity not concomitant with clinical signs). Thus, the contract is not

affect the milk processing capacity of sick goats. There is no reduction in fertility, weight, or mortality of kids. CAEV and PTBC are defects, but not redhibitory defects. The goats do not lack the characteristics that would make them unsuitable for their intended use (parturition and milk production). The diseases are endemic and the goats live with seropositivity without a decreasing production. Among the 40 adult and pregnant goats (parturitions at the buyer's farm), no difficulties were found in calving or in the number of kids born; no abortions or interstitial mastitis.

Second question: the buyer, after the complaint, milked the goats, using the milk for his own personal use (processing and sale). The buyer performed a positive ownership act. By leaving the adult goats with the kids, he exercised a positive ownership act, transmitting seropositivity to the young kids. By feeding the kids colostrum from HIV-positive mothers, the virus is transmitted to the newborns ("spread of infectious diseases" art. 500 of the Criminal Code). The death (?) of three of the 110 goats sold, likely Blue Tongue goats, constitutes a negative ownership act (lack of care). Why was the IZS Lombardia consulted (the name of the requesting veterinarian is not listed)? Test report no. 203/462387: 35 animals tested positive for CAEV, 11 remaining samples (out of 46) were negative. Of these 11, only 2 were positive for CAEV. Samples were identified with prime numbers, not with company code and registration number. Furthermore, IZS Piacenza is not certified for CAEV while IZS of Sardinia would have been licensed. The formal notice letter reported: "After just twenty days, my client noticed weight loss in the animals and contacted a veterinarian and a nutritionist". Before delivery, the same animals were receiving proper nutrition on the farm of origin, in compliance with animal welfare parameters (2.5 kg/head/day in 4 administrations based on Unifeed, plus wheat straw at the end of the day). The sudden weight loss was evidence of insufficient nutrition. On September 1st the goats were in perfect clinical and BCS conditions; request for intervention on September 20th. Suspected CAEV on the same day; samples were shipped two months later. Too many unclear points are detected: IZS Piacenza instead of IZS Sardinia (more convenient). The buyer wanted the deposit back and did not honor the contractual obligations.

Conclusions

This is a clear example of justice difficulties. A judge should be supported by one or more experts. Still, technical consultants should be Oregistered (Law No. 24/2017, Gelli -Bianco). "Special laws" would facilitate the work of judges. Finally, the preliminary technical assessment (Article 696 of the Code of Civil Procedure), with conciliatory purposes, cannot be assimilated "quoad effectum" to the technical consultant's report, which is delayed. These corrective measures would at least fill these serious legislative gaps, to the benefit of justice and animal welfare. Correct use of the Gelli - Bianco Law.

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67- OFFICIAL CONTROL OF GOAT WELFARE AT FARM LEVEL: A COMPARISON OF CLASSYFARM RESULTS AT LOCAL, REGIONAL AND NATIONAL LEVEL

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Introduction

Animal welfare (AW) is a key aspect of livestock management, with implications on ethics, production, health and economics. However, there is limited specific legislation for goats, for which only general welfare rules are applied. Within this context, in Italy the ClassyFarm system has emerged as a national tool for risk classification on farms and for monitoring AW conditions, both through self-assessment and official controls.

Objective

The aim of this study was to analyze and compare welfare data from goat farms at local (ATS Insubria), regional (Lombardy) and national level, to identify strengths and weaknesses and propose strategies for improvement.

Materials and methods

In Italy, official controls for goats, in compliance with Directive 98/58/EC, are carried out through a specific check list concerning both resource- and management-based indicators as well as animal-based indicators (ABMs). This new tool was developed by the Italian Ministry of Health in collaboration with the regions and the National Reference Centre for Animal Welfare. The results presented in this study refer to data collected in 2024. National data (n=884) were extracted from Vetinfo at a general level. Regional (n=104) and local (n=16) data were more detailed. Local data were directly obtained from ATS Insubria, whereas regional data were formally requested by ATS Insubria to the Lombardy Region and then extracted from the ClassyFarm system. All data were treated anonymously.

A comparison was made between the regional and local levels based on optimal/adequate vs insufficient results for ABMs, and on compliance vs non compliance with current legislation (non-conformities of type A, B, and C) for all the other items. The "Not Applicable" (NA) option was also included for those items that could not be evaluated on a given farm.

Results

At national level, over 94% of the farms received a compliant overall judgment, demonstrating a high level of attention towards the minimum legal requirements in goat farms. Some criticisms arose about housing and facilities (insufficient freedom of movement); management (e.g., feeding, watering and animal inspection); health (e.g., insufficient farm records on drugs and treatments). However, ABMs were favourable in more than 90% of the inspected farms, in spite of some problems for the mortality of adult animals and kids. This suggests that overall some deficiencies are still present, especially with regard to health surveillance and individual attention towards the animals.

In ATS Insubria, traditional and familiar goat farms are often present. This model has strengths as to the quality of the human-animal relationship, and the access to pasture; however, some structural (e.g., lack of adequate shelter, of sufficient space for rest) and management (e.g., insufficient feeding and watering, lack of farm records) limitations are present, which require targeted interventions. Furthermore, access to pasture involves a potential risk of parasitosis and other problems linked to the difficulty of a close control.

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Compared to the ATS Insubria, the Lombardy Region, shows a greater variety of production models and a high capacity for innovation, with a higher technological level. Such an intensification of farming techniques can lead to risks for animal welfare, if not balanced by adequate attention to ethological needs.

Conclusion

The findings confirm the effectiveness of this new tool for Official Control as a means for checking the enforcement of the current legislation on animal welfare. The comparison between the local and the regional context showed substantial alignment, while the national overview revealed greater variability due to heterogeneous territorial, climatic and structural characteristics, which significantly influence farming systems and, consequently, animal welfare conditions. This emphasizes the importance of a context-sensitive approach that takes local specificities into account.

68- MISSING THE SIGNS? VETERINARY FORENSICS AS A GAME-CHANGER IN RUMINANT WELFARE

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Introduction

Veterinary forensic medicine is emerging as a potential game-changer in livestock health and welfare management. While its application is well established in companion animal and wildlife contexts, its use in ruminant farming remains limited and largely under-recognized. This underutilization risks overlooking early or subtle signs of welfare violations, chronic neglect, or agro-zootechnical crimes affecting cattle, sheep, and goats, issues that standard clinical approaches may fail to detect. By applying objective, science-based methodologies, veterinary forensics offers a unique opportunity to bridge gaps between clinical evidence, regulatory frameworks, and legal accountability. In this context, it can reveal hidden patterns of suffering, document complex cases, and support evidence-based interventions that enhance both animal protection and transparency. When integrated into structured surveillance systems such as ClassyFarm - the platform established by the Italian Ministry of Health for livestock risk categorization - forensic tools could redefine how welfare risks are identified, managed, and prosecuted across the livestock sector.

Aims and approach

This study aims to: (i) identify key pathological and environmental conditions in ruminants that may constitute criminal offences or serious welfare violations; (ii) propose a tailored forensic protocol for rural settings, including scene investigation, evidence collection, chain-of-custody procedures, and legal reporting; and (iii) explore the interoperability between veterinary forensic medicine and surveillance systems such as ClassyFarm, to support veterinary jurisprudence.

Strategic Priorities and Discussion

To consolidate the role of veterinary forensic medicine in protecting ruminant welfare, the following strategic priorities are proposed:

- Standardization of Protocols. Develop validated forensic protocols adapted to livestock settings. Harmonized guidelines would improve consistency, legal strength, and training outcomes.
- Integration with Surveillance Systems. Incorporate forensic data into national platforms like ClassyFarm to support risk assessment, early warning, and prevention.
- Interdisciplinary Training. Promote training for veterinarians, legal professionals, and inspectors on pathology, animal welfare legislation, and forensic documentation.
- Research and Technological Innovation. Invest in diagnostics (e.g., molecular autopsy, toxicogenomics), imaging (e.g., post-mortem CT), and digital tools (e.g., AI, blockchain). Further Research should identify biomarkers for chronic suffering or neglect.
- Legal and Regulatory Development. Advocate for legal recognition of forensic activities, defining admissibility of evidence and inclusion in welfare audits.
- Evidence-Based Policy and Communication. Use forensic data to guide policy and optimize resource allocation. Anonymized data sharing can enhance transparency and stakeholder trust.
- Ethical and Social Engagement. Uphold ethical standards, ensuring proportionality and respect for animal dignity.
- International Collaboration. Strengthen global networks and promote joint research to harmonize and advance veterinary forensic practice.

Conclusions

Veterinary forensic medicine has the potential to be a true game-changer in how animal welfare is monitored and enforced in livestock systems. As a transformative discipline, it can fill diagnostic gaps, support legal action, and bring long-overdue accountability to complex welfare violations, ensuring that critical warning signs are no longer missed. When integrated into structured surveillance platforms such as ClassyFarm, veterinary forensics enhances risk-based monitoring and enables more targeted, evidence-driven, and transparent interventions in ruminant welfare protection.

69- ANALYSIS OF THE EFFECTIVENESS OF THE VETERINARY CONTROL SYSTEM ON RUMINANT FARMS: A RETROSPECTIVE STUDY IN THE METROPOLITAN AREA OF MILAN (ITALY)

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Introduction

Official veterinary controls in ruminant farms represent a cornerstone of animal health management and food safety assurance. The Milan Metropolitan Area, characterized by intensive dairy and beef cattle, provides an optimal setting for evaluating veterinary surveillance effectiveness.

Objective

To evaluate the effectiveness of the official veterinary control system specifically applied to ruminant farms in the Milan Metropolitan Area through comprehensive analysis of 2024 surveillance data, assessing compliance rates, control distribution patterns, and identifying sector-specific performance indicators across different categories of veterinary inspections.

Materials and Methods

A retrospective observational study was conducted analyzing official veterinary control data from the ATS Milan Metropolitan City for 2024. The study population included all ruminant farms (cattle, buffalo, sheep, goats) subjected to official controls. Data were extracted from the veterinary control information system, focusing on five main control categories: Identification & Registration (CGO 6-7-8), Animal Welfare with specific attention to calves (CGO 11-12), Animal Welfare for other species including adult cattle (CGO 13), Food Safety (CGO 4), Prohibited Substances (CGO 5), and Disease Prevention and Eradication (CGO 9). Variables analyzed included control distribution by species, compliance rates, and ratios between performed and minimum required controls. Statistical analysis calculated frequencies and percentages for categorical variables, with compliance rates determined as the ratio of favorable outcomes to total controls.

Results

A total of 147 controls were performed on ruminant farms, achieving an overall compliance rate of 95.2% (140/147 controls), with only 26 instances of non-compliance detected. Cattle and buffalo dominated control activities across all categories, reflecting their economic importance in the regional livestock sector. Identification & Registration controls (CGO 7-8) showed the highest volume, with cattle and buffalo receiving the most intensive surveillance, followed by targeted controls on sheep and goats. Animal Welfare controls demonstrated a differentiated approach: controls on calves (CGO 11-12) revealed specific focus on vulnerable populations, while CGO 13 controls for other species, including adult cattle, showed balanced distribution among different ruminant categories, emphasizing welfare attention throughout the entire production cycle. Food Safety controls demonstrated concentrated attention on dairy operations, particularly bovine milk production for regional DOP cheese manufacturing. Disease Prevention controls emphasized bovine tuberculosis and brucellosis surveillance programs. The distribution pattern indicated a risk-based surveillance approach, with resource allocation proportional to economic impact and health risk assessment.

Conclusion

The veterinary control system for ruminants in the Milan Metropolitan Area demonstrates exceptional effectiveness with a 95.2% compliance rate, significantly exceeding national averages. The strategic distribution of controls reflects appropriate risk-based surveillance, with justified emphasis on dairy cattle operations given their economic significance and integration with the Lombardy dairy processing industry. The systematic inclusion of animal welfare controls for all age categories and species highlights an approach to animal protection covering the entire production cycle. The high compliance rates indicate successful implementation of professional management practices and effective regulatory compliance.

represents a benchmark for ruminant control systems, demonstrating how scientific, risk-based approaches can achieve optimal animal health and food safety standards while supporting economic sustainability in the livestock sector.

70- LIVESTOCK TRANSPORT: SAFEGUARDING ANIMAL HEALTH AND WELFARE THROUGH THE 'ONE HEALTH' APPROACH

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Introduction

The international transport of live animals, especially within and beyond the European Union, is subject to complex regulations due to the use of different transport modes, the involvement of various stakeholders, and the coexistence of national and international rules. Excluding domestic movements within EU Member States, around 1.6 billion live animals are transported annually in the EU, including 1.4 billion poultry, 31 million pigs, 4.3 million cattle, and 3 million sheep (ECA, 2023). Maritime transport, especially in the Mediterranean region, presents specific animal welfare challenges that are often underestimated. Long journey times, heat stress, overcrowding, and limited veterinary oversight on livestock vessels can seriously compromise animal health and welfare. From a One Health perspective, addressing these issues is crucial, not only to improve animal welfare standards, but also to help prevent the spread of zoonotic diseases and antimicrobial resistance, which can impact human and environmental health.

Objective

This study aims to identify the main challenges concerning animal welfare during maritime transport, particularly in the Mediterranean context, and to propose strategies grounded in the One Health approach aimed at enhancing animal protection standards. Furthermore, it intends to contribute to the discourse on the integration of the One Health perspective into the existing legal framework governing animal transport.

Materials and methods

Conduct a literature review on the impact of international livestock transport from the European Union across the Mediterranean Sea with a focus on the potential of incorporating the One Health approach in the regulation on the protection of animals during transport.

Results

Scientific opinions confirm that animal transport is a complex, multivariate stressor, where exposure level and duration significantly affect welfare. Prolonged hunger is a major concern and may cause physiological alterations. Several studies report that long journeys lead to measurable pathophysiological changes (Giangaspero et al., 2024). According to Boada-Saña et al. (2021), leading causes of cattle deaths are heat stroke, trauma, and respiratory disease, while starvation and salmonellosis are major contributors to sheep mortality.

Maritime transport presents critical issues, including non-compliance by national authorities, incomplete or incorrect documentation, undefined responsibilities, lack of emergency planning, and structural vessel deficiencies (e.g. poor water supply, ventilation, lighting, drainage, alarms). According to a 2017 report by the Swiss Tierschutzbund, 54% of vessels transporting animals from EU ports were blacklisted in 2015 for safety risks under the Paris MoU. Boada-Saña et al. (2021) further report that livestock vessels remain the top category for detentions due to serious deficiencies.

Proper assessment of animals' fitness is essential before transport. Shorter journeys, appropriate temperatures, and more space are needed to improve animal welfare. Despite improvements under Regulation (EC) No 1/2005, long-distance transport remains a major welfare concern. Animal welfare problems have transboundary implications, including risks to public health such as zoonotic diseases and antimicrobial resistance and environmental risk in a One Health perspective. About 60% of human pathogens are zoonotic; for emerging infectious diseases, the figure rises to 75% (OIE, 2013). Boada-Saña et al. (2021) also raise concerns over illegal carcass disposal and manure discharge in protected marine areas, harming ecosystems.

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Conclusion

Integrating animal health and welfare into transport rules supports EU goals on public health, environmental protection, and disease prevention. It can also boost consumer trust and the market value of EU livestock products. However, vague legal definitions cause inconsistent enforcement across Member States, weakening both animal protection and fair competition. Recent EFSA opinions and EU-level audits clearly indicate the need to revise Regulation (EC) No 1/2005 in line with current scientific evidence.

71- CLONAL DISSEMINATION OF BACTERIA ANTIBIOMULTIRESISTANTE IN THE HUMAN–ANIMAL–ENVIRONMENT INTERFACE IN TUNISIA

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Introduction

Antibiotic resistance (ABR) is a global health concern that has been described which humans, animals, and environmental factors. One Health's interconnected domains contribute to the emergence, evolution, and spread of antibiotic-resistant microorganisms on a local and global scale.

Objective

We have undertaken same investigation with the aim of study the dissemination of antibiotic-resistant bacteria in the human-animal-environment interface in Tunisia.

Material and methods

In total, 156 samples (112 milk samples from cows with mastitis, 32 manure samples and 12 human nasal swabs) were collected from four farms (I, II, III and IV) in North-east Tunisia. Usual tests for Bacteria isolation and identification were applied. Antimicrobial susceptibility testing was performed using the disc diffusion method in accordance with the recommendations of the Clinical and Laboratory Standards Institute. The presence of seven antimicrobial resistance genes (ARGs) conferring resistance to penicillin (*bla* Z), tetracycline [*tet* (K) and *tet* (M)], erythromycin [*msr* (A)] and macrolide–lincosamide streptogramin B [*erm* (A) and *erm* (B)] was investigated by specific polymerase chain reaction (PCR) assay using specific primers and conditions. Detection of virulence and slim production encoding genes were conducted by PCR. PFGE was performed to compare genetic relatedness between all strains isolates (n = 49).

Results

In the first study, similar gene (*mecC*) and SCCmec types and PFGE patterns were found in meticillin-resistant *Cogulase Negative Staphylococci* (CNS) within different farms and origins, showing the potential of SCCmec interspecies exchange and circulation of the same clones of meticillin-resistant CNS in the human–animal–environment interface. In the second study, Molecular typing by PFGE showed that *E. coli* isolates harbouring ESBL-mcr-1 genes were clonally related. MLST revealed the presence of different ST lineages among ESBL- and mcr-1-carrying *E. coli* with clonal dissemination of *E. coli* ST4187 between two of the farms. Also in the other study Multidrug resistant and pathogenic *Mammaliicoccus sciuri* were frequently disseminated between different animal species within the farm environment. *mecA* and *mecC* can be disseminated by both frequent acquisition of the SCCmec element and clonal dissemination. Furthermore, molecular typing by ERIC-PCR showed high genetic diversity of CREC and ESBL *E. coli* isolates from the three animal diseases (Mastitis, diarrhea, colibacillocis) and gave evidence of their clonal dissemination within farms in Tunisia. Finally, recently our study show the highlight the potential role of stagnant water in both urban and rural areas as a reservoir of ESBL-producing *Enterobacteriaceae*.

Conclusion

Global interdisciplinary and unified approaches based on One Health principles are required to limit the ABR dissemination cycle.

Keywords: Bacterial clone, Antibioresistance, Dissemination, human–animal–environment interface, Tunisia

72- OCCURRENCE AND ANTIMICROBIAL RESISTANCE PROFILES OF ESKAPE BACTERIA IN DAIRY CATTLE POPULATION: A PROSPECTIVE OBSERVATIONAL STUDY AND ZOO NOTIC RISK EVALUATION

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Introduction

The ESKAPE group, *Enterococcus faecium/faecalis* (EF), *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP), *Acinetobacter baumannii* (AB), *Pseudomonas aeruginosa* (PA), *Enterobacter* species (Es), and *E. coli* (EC), include pathogens recognizes as “critical priority microorganisms” for their multidrug resistance (MDR) and capacity to cause severe, often hospital-associated infections in humans. Increasing evidence of ESKAPE bacteria in animals and the environment raises concerns about their zoonotic transmission potential, highlighting the importance of antimicrobial resistance (AMR) surveillance within a One Health framework.

Objective

The study aimed to: - investigate the role of healthy and diseased dairy cattle as carriers of ESKAPE bacteria; - characterize their phenotypic antimicrobial resistance profiles; - assess potential zoonotic transmission risk by comparing strains isolated from animals and human operators.

Materials and methods

A prospective epidemiological study was carried out from July to September 2024 on 20 Friesian dairy cows, including both healthy and clinically diseased animals. Faeces, milk, and swabs from eye, oral, nasal, ear, and skin sites were collected, along with skin swabs from the operator's hands after each sampling. Samples were processed at the Laboratory of Medical Microbiology and Infectious Diseases, University of Camerino, using selective culture media (Liofilchem, Italy). Bacterial identification was performed by MALDI-TOF MS (Bruker Daltonics, Germany-Flex Analysis software 3.4, Biotyper 3.1), and antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method and E-test against 38 veterinary and human antibiotics from 20 classes. Susceptible, resistant and multidrug-resistant (MDR) strains were interpreted according to CLSI VET01S (2024), EUCAST (2025), and Magiorakos et al. (2012). Statistical analysis employed Fisher's exact test, relative risk (RR), and attributable risk (AR), with *P* values<0.05 considered significant.

Results

From 148 samples, 796 microorganisms were isolated, including 133 (16.7%) ESKAPE bacteria. The most frequently detected were *Enterobacter* spp. (36.1%, 48/133), *K. pneumoniae* (30.8%, 41/133), *E. coli* (30.8%, 41/133), *Acinetobacter* spp. (12.8%, 17/133), PA (0.8%, 1/133), and *S. aureus* (0.8%, 1/133). ESKAPE isolation was significantly higher in Autumn (20.8% vs. 14.6%, *P*=0.025) and in diseased animals (21.2% vs. 10.7%, *P*=0.0001). No significant difference was observed between animal and operator hands isolates (17% vs. 12%, *P*=0.134). Isolation frequencies were similar across different animal body sites (1<RR<1.38, AR=0.05). Among ESKAPE isolates, 56.6% (30/53) resulted MDR: KP (42.9%), showing 100% resistance to ampicillin and fosfomycin; Es (78%); EC (15.4%); *Acinetobacter* spp. (including *A. baumannii*) (70.6%), demonstrated resistance to third-generation cephalosporins, carbapenems, and monobactams; *Pseudomonas* spp. (100%), with PA resistant to tetracyclines, penicillins, first- to fourth-generation cephalosporins, and critically important antimicrobials (carbapenems, macrolides, monobactams). The same phenotypic resistance profile was observed for KP, EC, *Enterococcus casseliflavus*, *Acinetobacter* spp. and *Pseudomonas* spp. strains isolated from animals and operator hands.

Conclusion

This study highlights the role of dairy cattle, particularly diseased individuals, as reservoirs of ESKAPE bacteria, with over half of the isolates showing multidrug resistance to both veterinary and human antibiotics.

The detection of similar antimicrobial resistance patterns in bacteria isolated from cattle and human hands suggests a potential zoonotic transmission risk. These findings reinforce the necessity for integrated AMR surveillance within a One Health framework. While valuable, the study's conclusions are limited by the small sample size and short sampling period, underscoring the need for expanded molecular investigations.

73- EFFECT OF NITROOXYPROPANOL (3-NOP) SUPPLEMENTATION IN HIGH-PRODUCING DAIRY COWS: A PILOT STUDY

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Introduction

Feed additives can suppress methanogenic archaea directly or indirectly, leading to reduced CH₄ emissions. A notable example is nitrooxypropanol (3-NOP), a synthetic molecule similar to *methyl-coenzyme M*, identified as a potential methane inhibitor in the intestines.

Objective

This study aims to assess the effects of replacing calcium soaps (based on palm oil) with 3-NOP on enteric methane emissions and evaluate the quality of milk produced.

Materials and Methods

One Holstein Frisian dairy farm located in Galicia (N.W Spain) was evaluated, with an average of 75 cows. The farm was carefully selected based on the 2019 Refinement to the 2006 International Panel of Climate Change (IPCC) Guidelines for high-producing dairy cattle (>8500 kg/head/year) that receive diets with over 70% feed quality digestibility (DE%) according to the formula DE (MJ/kg)/GE (MJ/kg) x100 and less than 35% NDF (DMI basis). These high-producing cows have calved at least once and are primarily utilized for milk production.

In this study, 75 multiparous cows were divided into two groups. group 1 (the 3-NOP group, n=10) received 3-NOP (Bovaer®, DSM-Firmenich, Germany) with the ration (60 mg/kg feed DM), while group 2 (the CSPO group, n=65) received the same basal diet, supplemented with 150 g of calcium soap based on palm oil.

The CH₄-EF for each dairy farm (G-A and G-B) was developed using the TIER-2 approach based on gross energy (GE) and Y_m (%) as follows: EF = [GE x (Y_m/100) x 365 days]/55.65 MJ/kg CH₄.

Results

When assessing statistical differences, the *t*-statistic value indicates a significant difference in CH₄ emissions between the two groups, with 3-NOP showing lower values. Concerning milk yield, there was a significant difference in milk production, although it was smaller than methane emissions. These results suggest that 3-NOP is not only more effective in reducing methane emissions but also has a minimal impact on milk production.

A comparison of the 3-NOP and CSPO groups in terms of milk fat and milk protein reveals a statistically significant difference in fat content between the two groups, with cows supplemented with CSPO showing slightly higher fat content.

The decrease in milk fat observed in dairy cows following 3-NOP supplementation can be attributed to changes in ruminal fermentation patterns. Although 3-NOP improves overall feed efficiency and reduces methane emissions, the change in rumen VFA production can lead to a slight decrease in milk fat percentage.

Conclusion

In the search for new supplemental fat sources to minimize enteric methane emissions, the use of nitrooxypropanol (3-NOP) as a substitute for soybean oil reduces around 25% of enteric methane emissions compared to supplementation with calcium soap oils, the traditional and commonly used supplement in high-producing dairy cattle. Nevertheless, this supplement, under the conditions of our study, caused a slight milk yield, fat, and protein decrease, attributable to diet composition and management of the farm, although not at the same magnitude as enteric methane emissions.

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74- *ESCHERICHIA COLI* FROM CALF FECES ON DAIRY FARMS: PATHOVARS AND ANTIMICROBIAL RESISTANCE GENES

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Introduction

Neonatal calf diarrhea (NCD) remains a significant production disease in dairy systems, contributing to early-life morbidity, mortality, and long-term productivity losses. *Escherichia coli* is a major bacterial pathogen associated with NCD, particularly in the first week of life. Several *E. coli* pathovars – such as enterotoxigenic (ETEC), enteropathogenic (EPEC) and Shiga toxin-producing (STEC) – carry virulence factors that enable intestinal colonization and disruption of gut function. In addition, the increasing prevalence of antimicrobial resistance (AMR) in *E. coli* isolates presents a growing challenge for the treatment and disease control at the farm level.

Objective

This study aimed to characterize *E. coli* isolates from feces of diarrheic and non-diarrheic calves raised on dairy farms and to evaluate the presence of pathogenic *E. coli* pathovars and AMR patterns.

Material and methods

Fecal samples were collected from 148 calves aged 2–7 days on 15 dairy farms: nine smaller farms (20–50 cows; 3–7 calves sampled) and six larger farms (> 100 cows; 10–30 calves sampled). From these, 542 morphologically distinct *E. coli* isolates (2–3 per calf) and morphologically distinct resistant *E. coli* isolates (1–2 per calf) were obtained. A total of 363 isolates were subjected to whole-genome sequencing (WGS) with Illumina paired-end technology.

Blood agar and Drigalski agar were used for isolation of *E. coli*, whereas resistant *E. coli* strains were grown on selective solid media (MacConkey agar with cefotaxime, CHROMID Carba Smart Agar) after overnight enrichment in peptone water and CHROMID Colistin R Agar was used for detection of colistin resistant strains. Species confirmation was performed by MALDI-TOF mass spectrometry (Bruker Daltonics). WGS was used for the detection of virulence and antimicrobial resistance genes.

Results

Surprisingly, only 24/363 (6.6 %) isolates harbored classical pathovar-associated virulence genes (EPEC: 9, STEC: 11, ETEC: 4). ETEC genes were the only virulence genes that were found exclusively in isolates from diarrheic calves. Extended-spectrum beta-lactamase (ESBL)/AmpC-producing isolates were widespread, found on all but three small farms. The most common ESBL resistance genes detected were *bla*_{CTX-M1}, *bla*_{CTX-M-15} and *bla*_{CTX-M-3}, respectively. Colistin-resistant *E. coli* was isolated from a calf on a large farm, but no genetic background was identified. Carbapenem-resistant *E. coli* was not detected.

Conclusion

These findings highlight the limited role of classical *E. coli* pathovars in calf diarrhea within the studied population. In addition, they show a broad distribution of antimicrobial resistance genes in *E. coli* from calves, including the ESBL-encoding genes. The results underscore the need for integrated herd health management and antimicrobial stewardship to mitigate production losses, promote animal welfare and address antimicrobial resistance risks in livestock systems.

Keywords: calf diarrhea, cattle, *E. coli*, virulence gene, extended-spectrum beta-lactamase, whole-genome sequencing

75- ARE IMPROVED BIOSECURITY AND WELFARE LEVELS SUFFICIENT DRIVERS TO REDUCE ANTIMICROBIAL USE IN RUMINANTS? CASE STUDY OF ITALIAN BUFFALO FARMS

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Introduction

Biosecurity in ruminant is often an overlooked area, but it is a key aspect in a One Health approach. Future scenario models aimed at reducing multi-drug-resistant bacteria pose a challenge for AMU control systems. Animal welfare (AW) and biosecurity are also essential tools to achieve this goal. ClassyFarm (CF) is an integrated system for categorizing farms according to the risk assessment methodology. CF collects and processes data related to AMU, AW and biosecurity. It can be applied to several livestock species, including water buffalo.

Objective

The aim is to describe the correlation between Biosecurity, AW and AMU.

Materials and methods

In CF, AMU is estimated using the Defined Daily Dose Animal for Italy (DDDAit). The AW assessment for water buffalo includes 62 non-animal-based measures (N-ABMs) and 17 animal-based measures (ABMs). The N-ABMs were divided into 'management' (32 items) and 'housing' (30 items). Biosecurity is assessed with 15 items. AW and Biosecurity scores are expressed as percentages.

Results

DDDAit, AW and Biosecurity were assessed on 382 buffalo farms with an average size of 374 head. The overall mean DDDAit was 0.40 ± 0.04 , with 43% of the farms scoring DDDAit =0, while the mean of the only farms with DDDAit $\neq 0$ was 0.7 ± 0.07 . The AW score was $76.9 \pm 9.8\%$ and the Biosecurity score was $68.9 \pm 15.3\%$. Statistical analysis was performed using Spearman's rank correlation in GraphPad Prism 8.0.1. Biosecurity and AW were positively correlated ($\rho=0.42$; $p<0.001$). A positive correlation was also observed between Biosecurity and both Management ($\rho=0.61$; $p<0.001$) and Housing scores ($\rho=0.36$; $p<0.001$). No significant correlation was found between AMU, Biosecurity and AW, probably due to the low levels of AMU found.

Conclusion

Contrary to the linear narrative often suggested between AMU, biosecurity and AW, the dynamic system we described is far more complex and articulated. Knowing the level of biosecurity and AW, their relationship and critical points on livestock farms is a tool for planning strategic priorities in human resources and investments.

It also helps to improve farmer awareness and animal health. However, while these tools are necessary, they are not sufficient to significantly reduce AMU; other socio-economic factors must be considered to achieve this goal. Achieving good levels of biosecurity and AW reduces exposure to pathogens and improves the natural immunity of the host. We believe it is essential to integrate mitigation measures with decision-making processes for appropriate diagnostic and treatment protocols involving breeders, veterinarians and farm workers.

76- VALIDATION OF THE BOVINE BLOOD CALCIUM CHECKER AS A RAPID AND SIMPLE MEASURING TOOL FOR THE IONIZED CALCIUM CONCENTRATION IN SHEEP

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Introduction

Calcium deficiency is common during the peri-parturient period, when it can cause parturient paresis, more commonly referred to as hypocalcaemia (Mavrogianni and Brozos, 2008).

There are three forms of serum calcium: ionized (iCa), complexed (cCa), and protein-bound (pCa). Physiologically, pCa, cCa, and iCa account for approximately 40%, 8%, and 52% of total serum calcium (tCa) concentration, respectively (Endres and Rude, 1999). Even though iCa is biologically active, clinicians rely on serum tCa measurements to predict iCa status (Schenck et al., 2005). In almost all laboratories, only total calcium is routinely measured (Gidenne et al., 2003).

An ion-selective electrode handheld meter, the bovine blood iCa checker (LAQUAtwin Ca-11C Compact Bovine Blood iCa Checker, HORIBA), recently became available for measurement of the iCa concentration in whole blood. This handheld meter can facilitate cow-side diagnosis and treatment of clinical and subclinical hypocalcemia (Suzuki et al., 2021).

Objective

The objective of this study was to evaluate LAQUAtwin CA-11C, as a sheep-side device for iCa measurements. The accuracy of LAQUAtwin CA-11C was evaluated using the handheld gas analyzer Epoc® Blood Analysis System (Siemens Healthineers), as a gold standard method.

Materials and methods

The LAQUAtwin CA-11C was used in direct mode, without diluting the sample. The device was calibrated using a 2-point calibration with standard Ca²⁺ solutions of 1.25 mmol/L and 2.5 mmol/L.

Precision determination (Whitin-run repeatability (CVw) and Between-day repeatability (CVb)) was performed independently at two concentration levels (1.25 mmol/l and a 2.50 mmol/l).

The study was carried out between July 2019 and July 2020. 118 Sarda sheep from 16 different commercial dairy farms (300±100 sheep) in northern Sardinia (Italy) were enrolled in the study. All the subjects were multiparous. Blood samples were obtained from animals in different physiological phases. Systematic errors were minimized by sampling 6-8 subjects for each farm on different days. Whole blood was collected and immediately analysed. To avoid the variability due to the exposure of the blood to air, a portion of the samples was first used for immediate determination of [iCa] using Epoc®. Immediately thereafter, the whole-blood [iCa] was measured in the same sample, using LAQUAtwin CA-11C.

Data were analysed using MEDCALC software (Version 23.2.7) for simple linear regression, the Bland-Altman plot and the Passing-Bablok regression. The performance of LAQUAtwin CA-11C was assessed using Sigma metrics and Method Decision Chart.

Results

The between-day repeatability with standard concentration of 1.25 mmol/L was CVb=3.08%; with standard concentration of 2.50 mmol/L was CVb=2.85%.

Whitin-run repeatability with concentration of 1.25 mmol/L was CVw=2.91%; with concentration of 2.50 mmol/L was CVw=2.21%. The Bland-Altman plot indicated that the bias for the LAQUAtwin CA-11C was -5.40% (P < 0.0001) when compared with the EPOC®.

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Passing Bablock's comparison between iCa^{2+} concentrations using EPOC® and LAQUAtwin CA-11C showed a linear relationship with a slope of 1.0 and an Intercept -0.06. Passing Bablock indicated a significant systematic bias and non-significant proportional bias.

Using clinical decision level at 1.25 mmol/L (Rosol and Capen, 1997) and closest reference interval boundary at 1.06 mmol/L (Belonse, 1976), the quality requirement for this test using the formula from Ettinger and Feldman (2000) was 15.2% (TEa).

For TEa=15.2%, CV=3.08% and bias=-5.40%, the accuracy acceptability levels of Sigma metrics were: TE/2=7.60%, TE/3=5.07%, TE/4=3.80%, TE/5=3.04%, TE/6=2.53%.

The operating point using x coordinates CV%=3.08 and y coordinates the Bias%=-5.40, was classified as marginal ($\geq 3 \sigma$ and $< 4 \sigma$).

Conclusion

This novel ion-selective electrode Ca-module could become a rapid low-cost tool for assessing iCa sheep-side, qualitatively allowing classification of subclinical hypocalcemia on-farm.

77- USE OF KIT PORTA SCC AS A POTENTIAL ON-FARM SCREENING TOOL FOR SUBCLINICAL MASTITIS IN DAIRY SHEEP

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Introduction

Mastitis is a disease of lactating ewes that occurs worldwide (Jones and Watkins, 2000). While the clinical form of mastitis can be readily diagnosed by inspection or palpation, subclinical mastitis requires Somatic cell count (SCC) measurement for diagnosis (Keisler et al., 1992). Although accurate tests are available, such as PCR or bacterial culture for the diagnosis of IMI or the measure of the electrical conductivity of milk, they are not suitable for on-farm use. Researchers are currently looking for new diagnostic biomarkers of mastitis – acute phase proteins produced by the liver, unique genetic sequences of mRNA, antimicrobial peptides produced by immune cells (Libera et al., 2021) but they also require laboratory equipment.

SCC is widely used to diagnose this condition considering its high sensitivity; however, milk samples need to be brought to the laboratory for analysis. The California Mastitis test (CMT) has long been used as an on-farm screening test for the detection of subclinical mastitis. Despite this, the relationship between results of electronic SCC and CMT is not straightforward due to the variability in SCC values within each CMT score (Ruegg, 2003). To overcome this situation, indirect on-farm tests that produce a numeric value for SCC have been created (Barratt et al., 2003; Ruegg et al., 2005; Moon et al., 2007). The PortaSCC (PortaScience, Portland, OR) is a qualitative on-farm test that uses an algorithm to convert an enzymatic reaction into an estimated SCC.

Objective

The study aimed to evaluate the performance of PortaSCC® in detecting subclinical mastitis in sheep's milk, compared to the laboratory-based Somatic cell counter (Fossomatic®).

Materials and methods

265 milk samples were collected between November 2022 and June 2023 from half-udders of Sarda ewes at different stages of lactation, belonging to 15 different semi-intensive dairy flocks located in northern Sardinia (Italy).

Milk samples were immediately processed for somatic cell count using PortaSCC® at the collection site. Using a pipette, 35–40 µL of milk was transferred from each sample tube to the sample window of the PortaSCC® milk test strip, along with 100µL of activator solution. Evaluation was done after 45 minutes using Digital reader PortaSCC®. The same milk samples were used for somatic cell count using the Fossomatic® machine in the laboratory. Samples were kept in a cooler prior to transport to the laboratory for analysis.

A case of subclinical mastitis was defined as the presence of more than 500.000 somatic cells per mL of milk (Berthelot et al., 2005; Fragkou et al., 2014; Gelasakis et al., 2015). The results of the Fossomatic® were used as the reference in the calculation of the test properties of the PortaSCC®.

The agreement between the results of the two tests was assessed through the Passing-Bablok regression and the Bland Altman plot, using MedCalc Statistical Software (version 23.1.7).

Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value of PortaSCC® were calculated using Microsoft®-Excel (Version 2504).

Results

Passing–Bablok regression analysis for the agreement between the Fossomatic® and the PortaSCC® indicated a proportional bias (intercept 1.07, 95% CI, 1.05 1.09), and no constant bias (slope 1071, 95% CI, -5588.2 to 7894.7).

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Bland–Altman analysis revealed a mean positive bias of 7.8% that was different ($P < 0.0001$) from zero.

Sensitivity, specificity, positive predictive value, and negative predictive value of PortaSCC® were respectively 100%, 93%, 83%, 100%.

Conclusion

Our results indicate that the PortaSCC® could be used as an effective on-farm tool for determining udder health in sheep and for differentiating between a healthy status and the presence of subclinical mastitis, as an alternative to the laboratory-based cell counter.

The PortaSCC® provides reliable, rapid and cost effective SCC values in field conditions, even when operating in remote areas.

78- EVALUATION OF FIVE DIFFERENT ANTICOAGULANTS AND A STABILIZER ON TOTAL LEUKOCYTE, T LYMPHOCYTE AND MONOCYTE SUBSET COUNTS IN CATTLE AND BUFFALOES USING FLOW CYTOMETRY

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Introduction

Veterinary flow cytometry, while a powerful diagnostic and research tool, has historically been limited by a scarcity of specific reagents and insufficient understanding of pre-analytical variables that can alter the stability of cellular markers. Anticoagulant choice is crucial, especially when analyses cannot be performed immediately.

Objective

This study aimed to evaluate the effect of five different anticoagulants (Lithium Heparin, Sodium Heparin, K₂EDTA, K₃EDTA, ACD) and one stabilizer/anticoagulant (Cyto-Chex) on the identification and quantification of total leukocytes (CD18⁺), T lymphocytes (CD3⁺), and monocyte subsets (classical, intermediate, and non-classical) in cattle and buffaloes.

Materials and Methods

Whole blood samples were collected from four clinically healthy cattle and four buffaloes via jugular venipuncture, using vacuum tubes containing the following anticoagulants: K₂EDTA, K₃EDTA, lithium heparin (LiHepa), sodium heparin (NaHepa), acid citrate dextrose (ACD), and Cyto-Chex[®] (Streck Laboratories). Each sample was analyzed in duplicate or triplicate to ensure technical reproducibility.

Flow cytometric analysis was performed using two multicolor antibody panels optimized for the identification of total leukocytes, T lymphocytes, and monocyte subsets including classical (cMo), intermediate (intMo), and non-classical monocytes (ncMo).

Absolute quantification of leukocyte populations was carried out using a “single-platform” approach based on the addition of fluorescent counting beads (e.g., Flow-Count[™] Fluorospheres, Beckman Coulter) directly into each tube prior to acquisition on Cytoflex flow cytometer.

All data were normalized to lithium heparin (LiHepa), selected as the reference anticoagulant due to its widespread use in veterinary immunophenotyping, particularly when sample processing exceeds 4 hours from collection. A p-value < 0.05 was considered statistically significant.

Results

Statistical analysis of total absolute leukocyte counts revealed no significant differences among anticoagulants overall, except within the monocyte compartment. ACD consistently underestimated leukocyte counts across all populations and in both species, with an average reduction of approximately 36% in total leukocyte counts compared to lithium heparin. In both cattle and buffaloes, ACD also led to a marked decrease in CD3⁺ T lymphocyte counts.

Species-specific effects were particularly evident with the Cyto-Chex stabilizing reagent. In cattle, Cyto-Chex produced results comparable to EDTA and heparin. In buffaloes, however, Cyto-Chex caused a severe loss of fluorescence signal for both CD3 and CD14, resulting in unreliable gating and poor discrimination of lymphocyte and monocyte populations. In addition, the Friedman test revealed statistically significant

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differences among anticoagulants for non-classical ($p = 0.0064$) and classical monocytes ($p = 0.013$). However, post-hoc pairwise comparisons (Wilcoxon test) did not reach statistical significance, likely due to the small sample size ($n = 4$). Although post-hoc comparisons were not significant, descriptive analysis showed that ACD and Cyto-Chex® yielded altered monocyte profiles, with Cyto-Chex showing reduced CD14 expression and poor subset discrimination, especially compared to LiHepa and K2EDTA

Conclusions

This is the first study evaluating anticoagulant effects on total leukocytes, T lymphocytes, and monocyte subsets in buffaloes. Although confirmation in larger cohorts is needed, these findings provide useful guidance for anticoagulant selection. Lithium heparin appears most suitable, while Cyto-Chex® is not recommended due to poor marker preservation. Further research in a larger cohorts is needed to confirm these observations and to evaluate anticoagulants suitable for delayed processing (24–72 h) in buffalo immunophenotyping.

Acknowledgements

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79- DIFFERENTIAL IMMUNE RESPONSES TO BOVINE TUBERCULOSIS IN WATER BUFFALO AND CATTLE: A FLOW CYTOMETRY AND TRANSCRIPTOMICS ANALYSIS

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Introduction

Bovine tuberculosis (BTB), caused by members of the *Mycobacterium tuberculosis* complex (MTBC), primarily *M. bovis*, is a major zoonotic and economic concern, especially in endemic areas where both cattle and water buffaloes (*Bubalus bubalis*) are affected. While the disease in cattle has been extensively studied, in buffaloes remain underexplored despite their growing global relevance. Understanding species-specific immune responses is crucial to improve BTB diagnostics and control in both hosts.

Objective

This study aimed to decode the immune response to *M. bovis* infection in water buffaloes using two complementary approaches: (i) multiparametric flow cytometry to characterize CD4⁺ T cell responses, and (ii) whole-blood gene expression profiling to identify infection-associated transcriptional signatures. Comparative analysis with cattle was included to contextualize species-specific immune features.

Materials and Methods

Flow cytometry analysis (i), was performed on peripheral blood collected from 35 buffaloes and 10 cattle. Buffaloes were grouped by interferon-gamma release assay (IGRA) status: IGRA-positive (n=17, from BTB outbreak herds) and IGRA-negative (n=18, including 13 from outbreak herds and 5 from Officially Tuberculosis-Free farm). Cattle were divided in 6 IGRA-positive and 4 IGRA-negative animals. Whole blood was stimulated with PPD-B or PBS (negative control) and incubated at 37 °C and 5% CO₂ for 2 hours, followed by brefeldin A addition and a further 4-hour incubation. Intracellular cytokine staining was then performed using cross-reactive anti-bovine monoclonal antibodies to detect IFN- γ , TNF- α , and IL-17A in CD4⁺ T cells. Frequencies of cytokine-producing subsets were analysed by IGRA status. Factor Analysis of Mixed Data (FAMD) was applied to identify immune profiles distinguishing infected from uninfected animals across species.

In the transcriptomic analysis (ii), 33 buffaloes and 13 cattle were classified based on IGRA test. Whole blood was stimulated for 24 h with PBS or PPD-B, then processed in TempusTM tubes. RNA was extracted and analysed via high-throughput qPCR (Fluidigm Biomark HD) using a custom panel of 45 immune-related genes. The panel included genes of Th1 responses (IFNG, IL12B, TNFA), apoptosis and inflammasome (CASP1, CASP8, CASP13), chemokines (CXCL1, CCL4), and innate immunity (IL1B, COX2, CD14, IFNAR1) and vitamin D metabolic pathway (CYP27A1 and VDR). Canonical Discriminant Analysis (CDA) was used to differentiate infection status and detect species-specific transcriptional signatures.

Results

Flow cytometry revealed that IGRA-positive buffaloes had significantly higher frequencies of IFN- γ ⁺ and IFN- γ ⁺TNF- α ⁺ T helper cells (CD4⁺) than uninfected controls, indicating BTB-specific immune activation. IL-17A⁺ cells were detectable but not statistically different. Cattle showed greater polyfunctionality in some subsets. FAMD identified cytokine profiles distinguishing IGRA-positive buffaloes.

Transcriptomic profiling revealed broader gene modulation with 21 differentially expressed genes in buffaloes compared to 16 in cattle, including upregulation of IL1B, CXCL1, IL17A, COX2 and down regulation of CD14, IFNAR1, CYP27A1 and VDR. CDA identified buffalo-specific immune signatures distinct from cattle.

Conclusion

This study reveals distinct immune responses to *M. bovis* in water buffaloes compared to cattle. Combined flow cytometry and transcriptomic profiling showed robust IFN- γ ⁺TNF- α ⁺ T helper cells response and broader gene modulation in buffaloes, with 21 differentially expressed genes. Upregulation of pro-inflammatory mediators like IL1B and CXCL1, alongside downregulation of immune regulatory genes, such as VDR and CYP27A1, suggests heightened inflammation and impaired vitamin D-mediated immune control, potentially contributing to disease progression in buffaloes. These findings highlight unique immune regulation in buffaloes and support development of species-specific BTB diagnostics and eradication strategies.

Acknowledgements

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80- SURVEILLANCE ON ZOONOTIC AGENTS IN WILD RUMINANTS OF SICILY: MOLECULAR INVESTIGATION ON *TOXOPLASMA GONDII*, *LEPTOSPIRA* SPP. AND *COXIELLA BURNETII*

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Introduction

Wild ruminants play a crucial role in the transmission and maintenance of zoonotic agents. In Sicily, populations of fallow deer (*Dama dama*), have been rapidly increasing in an uncontrolled manner. Extensive farming is a widespread practice in the region, facilitating interactions between wild and domestic animals. This scenario may promote the spread of neglected or emerging zoonoses, thereby increasing human infection risk.

Objective

This study aims to assess the presence of zoonotic agents, including *Toxoplasma gondii*, *Leptospira* spp. and *Coxiella burnetii* among fallow deers in environments characterized by high interspecific interactions at the human-livestock-wildlife-environment interface.

Materials and methods

The study involved post-mortem examinations of fallow deer (*Dama dama*) carcasses, aimed at detecting pathological lesions and identifying the presence of ectoparasites and endoparasites. External assessments were conducted to determine the animals' nutritional status, document any superficial injuries, and evaluate ectoparasitic infestation levels. Necropsy procedures focused on the assessment of internal organ positioning, identification of anatomical abnormalities, and the presence of effusions within body cavities.

A total of 53 fallow deer carcasses were analyzed. The study involved the health inspection of fallow deer carcasses, with clinical assessments documenting pathological lesions and ecto-/endoparasite presence. External examinations evaluated nutritional status, external injuries, and ectoparasite burden. Necropsy focused on internal organ topography and cavity effusions, with detailed characterization of any pathological lesions and associated parasitic findings. Ticks, when present, were collected and identified using morphological keys.

A total of 360 organ samples, including heart, spleen, liver, lung, intestine, kidney, testicle, mammary gland, tongue, and lymph nodes, were collected from 53 fallow deer carcasses from regional culling plans for wild ungulate population control.

Detection of pathogen DNA was performed through molecular analysis. In particular, *C. burnetii* DNA was detected by real-time PCR targeting the IS1111 region, *T. gondii* DNA was identified using a nested PCR targeting the B1 gene. *Leptospira* spp. DNA was identified through a multiplex real-time PCR on 16S ribosomal RNA gene and LipL32 gene (and characterized through multilocus sequence typing (MLST)).

Results

None of the analyzed cases exhibited pathological lesions indicative of the targeted pathogens. *Toxoplasma gondii* DNA was detected in 7 fallow deer (13.2%). DNA of *Leptospira* spp. was detected in a single fallow deer (1.9%) and was classified as *Leptospira interrogans*. Multilocus sequence typing identified allele [3] at the *glmU* locus and allele [3] at the *sucA* locus. The remaining loci either failed to amplify or generated sequences too short for reliable allele identification, thus preventing the accurate characterization of a sequence type. All samples were negative for *C. burnetii*.

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Conclusions

These findings highlight the possible role of wild ruminants in the epidemiology of zoonotic agents, such as *T. gondii* and pathogenic *Leptospira* serogroups, within Sicilian wildlife. The increasing interaction between wild and domestic ruminants, coupled with continuous changes in wildlife habitats and human lifestyle, may enhance the risk of zoonotic transmission to domestic animals and humans. These results emphasize the need for large-scale surveillance programs to further investigate these neglected and re-emerging zoonoses. This research has been funded by Ministero della Salute GR-2021-12373930.

81- HISTOPATHOLOGY, IMMUNOHISTOCHEMISTRY AND IN SITU HYBRIDIZATION FINDINGS IN MASTITIS CAUSED BY *MYCOPLASMA AGALACTIAE*

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Introduction

Mycoplasma agalactiae (*Ma*) is considered the primary causative agent of Contagious Agalactia (CA) in small ruminants [1]. CA is a multiorgan syndrome, characterized by monolateral or bilateral mastitis and less frequently keratoconjunctivitis, arthritis, and abortion both in sheep and goats [2].

Objective

This work reports the diagnostic investigations carried out during mammary infection in some Sicilian sheep farms, to investigate the localization of *Ma* in udder and characterize the immunoinflammatory response to *Ma* infection.

Materials and methods

Sheep were subjected to a complete necropsy. During the necropsy, tissue specimens were collected from the right and left halves of each mammary gland for microbiology, molecular analysis, histology, immunohistochemistry and in situ hybridization.

Results

Clinical investigations highlighted: swelling of one or two mammary halves, pain and tenderness during milking, a sudden drop in milk production and alteration of secretion with separation into two phases and presence of flocs. Based on clinical, pathological, and histological data, sheep, testing positive for *Ma* PCR, were divided into four stages: acute, sub-acute, chronic and recovery stage. Microbiological investigations performed on milk samples confirmed the positivity to *Mycoplasma* spp. In addition, the real-time PCR confirmed *Ma* in all isolated strains. Histological findings showed an interstitial mononuclear cell infiltrate in the acute phase, that persist in advanced stages with a slight reduction of secretory tissue. In severe cases, a marked atrophy of secretory tissue associated with mononuclear cell infiltration and interstitial fibrosis was observed. Immunohistochemistry for *Ma* was strongly positive in the exudate present in ducts, while interstitial mononuclear cells and macrophages showed an intracytoplasmic immunoreaction. Activated T-lymphocytes, label by CD3, were mainly observed in the intralobular connective tissue around acini and intralobular ducts and in the intraepithelial infiltration. Lymphocytes B (CD79) were sparsely present, markedly in the sub-acute stage. MHC-II+ cells were observed in central areas of multifocal interstitial mononuclear cell infiltrations.

Conclusion

IHC was useful to detect and localize *Ma* in udder, confirmed by isolation and PCR. Histological mammary lesions progressed from a diffuse, interstitial mastitis limited to the glandular acini and intralobular ducts in acute stage, to a galactophoritis with intense, diffuse subepithelial and intralobular mononuclear cell infiltration in sub-acute stage. In chronic stage, there was severe acinar atrophy, fibrosis, and chronic galactophoritis. Our histological study describes different histopathological patterns relating to *Mycoplasma agalactiae* infection in sheep. In the acute phase, the main histological lesion is an interstitial mastitis characterized by lymphocytes, macrophages and dendritic cells; while in the chronic phase, we observed a reduction of secretory tissue, associated with fibrosis and accompanied by a clinical reduction in milk production. ISH for the detection of *Ma* p-40 rRNA in FPPE tissues showed high efficacy and specificity in order to detect the pathogen in target cells. In conclusion, immunohistochemical characterization of *Ma* mastitis suggests the ability of this microorganism to induce changes in the inflammatory cell subsets during the mammary infection process.

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82- SOCIAL ASPECTS RELATED TO ANIMAL HUSBANDRY IN TRADITIONAL AND IMPROVED STABLES IN EASTERN BURUNDI

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Introduction

Burundi is a country where more than 90% of the population lives of agriculture. Livestock farming was extensive and contributed around 4.6% of GDP. In addition, the multiplication of the number of agricultural operations on an already limited space causes the reduction of community pastures, thus making cattle animal husbandry difficult. Law No1/21 of October 4, 2018 was promulgated on zero grazing and the prohibition of straying of domestic animals and farmyard.

Our team of researchers was involved in a project aiming to spread, among farmers, improved stables providing adequate repair, feed and water supply and husbandry management.

Objective

A study was carried out with the general objective to evaluate the contribution of improved stables in increasing domestic animal production and safeguarding animal health in zero grazing system.

In particular, this communication aims to report some social aspects which have been analysed through the comparison of improved and traditional stables.

Materials and methods

This study was carried out from June to December 2023, in the Rutana, and Ruyigi Provinces (Eastern Burundi). The study was carried out in the form of a survey using a questionnaire containing firm and open questions and by physical observation of livestock in households. The animal survey and observation operation was carried out in four rounds to clearly define the variables.

The results of the survey were entered using Excel software and descriptive statistics were processed using SPSS version 21 software.

Results

16 rural households were surveyed: 8 with traditional and 8 with improved stables. The report is mainly based on result obtained from cattle.

- Proportion of Women taking care of animals

Women's participation in livestock activities is more than men's in non-commercial activities like grazing, feeding of animals, cleaning of sheds, etc. In contrast, their role in marketing and revenue-generating activities is less.

- Situation of conflict between human and wildlife

Conflicts between humans and wildlife exist in Ruyigi and Rutana provinces. These conflicts include wild animals that come to devour domestic animals (examples: rapacious birds that devour chicks, jackals that devour small ruminants).

- Animal species raised by population

In traditional animal husbandry, the species that are raised together are cattle, pigs, sheep and chickens at a rate of 17.1%. In zero grazing type of livestock farming, the species that are raised together are cattle, pigs, sheep and chickens at a rate of 17.1%.

are cattle, goats, pigs, rabbits and chickens (26.5%). In both types of animal husbandry, the species raised together do not represent a significant difference.

- Animal movement

According to the results of the study, in both types of animal husbandry systems, the purchase or other movement of animals is rarely done by farmers in Ruyigi and Rutana provinces.

Conclusion

The social aspects related to animal husbandry in Eastern Burundi do not differ significantly from traditional and improved stables, confirming that they are associated to Burundian tradition and that the different systems analyzed are not influencing ancestral behavior of the population taking care of animals.

83- EFFECT OF PREPARTUM SHEARING ON SERUM THYROID HORMONE CONCENTRATIONS IN AUSTRALIAN MERINO SHEEP UNDER EXTENSIVE GRAZING CONDITIONS

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Introduction

Homeothermic animals must maintain a stable body temperature in order to perform their vital functions. One of the first responses of animals to low temperatures is to reduce heat loss. Cold exposure also triggers shivering and the release of catecholamines, which initiate adaptive thermogenesis. Subsequently, adrenaline secretion increases, promoting hepatic glycogenolysis. In parallel, thyroid hormone secretion rises, enhancing basal metabolic rate and oxygen consumption—mechanisms essential for sustaining productive performance. Shearing is both an acute and chronic stress factor. The chronic stress factor is caused by the cold when wool is removed in winter.

Objective

The objective of this study was to evaluate the effects of early (85–90 days of gestation) and late (115–120 days) prepartum shearing on short-term thyroid hormone concentrations in Australian Merino sheep managed under extensive grazing conditions.

Materials and methods

The study was conducted at Experimental Station N°. 1 of the Faculty of Veterinary of Udelar, Uruguay, with 230 multiparous ewes. Half of the animals were artificially inseminated with fresh semen for 6 days between March 8 and 13, and the other half between April 8 and 13. Forty-five days after each insemination, a diagnostic ultrasound was performed, and 120 ewes with single pregnancies were selected. They were randomly assigned to four treatments according to the stage of gestation (early Te or late Ta) and prepartum shearing (with or without). Shearing was performed on July 20 in the shorn groups so that the ewes were shorn at two gestational stages. The recorded ambient temperature ranged from 7°C to 19.6°C during the first 72 hours after shearing. Blood samples were collected to analyze free triiodothyronine (T3) and thyroxine (T4) levels using radioimmunoassay (RIA). Samples were taken at -72, -48, and -24 hours before shearing and at +1, +4, +24, +48, and +72 hours after shearing. The originality of the design lies in the fact that both experimental groups underwent the shearing procedure simultaneously, which allowed for standardization of the environmental challenge and ensured that the differences observed could be attributed primarily to the gestational stage rather than to variations in external conditions.

Results

The concentration of T3 was higher in shorn sheep than in unshorn sheep ($1.19 \text{ nmol/l} \pm 0.02$ vs $1.10 \text{ nmol/l} \pm 0.02$; $P = 0.0065$) and was also higher in sheep in the Ta group than in those in the Te group ($1.18 \text{ nmol/l} \pm 0.02$ vs $1.10 \text{ nmol/l} \pm 0.02$; $P = 0.0103$). T3 levels also varied with the time of sampling ($P < 0.0001$), with a significant interaction between shearing and the time of blood sampling ($P = 0.0092$). Shorn ewes had higher T3 concentrations at 24 hours ($1.23 \text{ nmol/l} \pm 0.04$ vs. $1.03 \text{ nmol/l} \pm 0.05$; $P = 0.0042$) and at 72 hours ($1.24 \text{ nmol/l} \pm 0.04$ vs. $1.02 \text{ nmol/l} \pm 0.05$; $P = 0.0015$) compared to unshorn sheep. The T4 concentration was higher in the group of shorn sheep ($95.56 \text{ nmol/l} \pm 1.56$ vs. $80.88 \text{ nmol/l} \pm 2.14$; $P < 0.0001$) and was also higher in the Ta group than in the Te group ($91.00 \text{ nmol/l} \pm 1.90$ vs. $85.44 \text{ nmol/l} \pm 1.81$; $P = 0.0355$). The T4 concentration also varied depending on when the samples were taken ($P = 0.013$). No interactions between the effects were observed.

Conclusion

In conclusion, prepartum shearing increased maternal thyroid hormone concentrations, indicating a physiological response to cold thermal stress induced by shearing.

84- JOINT EFFORTS FOR SUSTAINABLE RESILIENT FARMING OF LOCAL APULIAN SHEEP AND GOAT POPULATIONS: THE CONTRIBUTION OF THE “LOCAL”, “AGRITECH”, “BIOZOOCARE” AND “WOOLLY” PROJECTS

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Introduction

The LOCAL project focuses on dairy production, optimizing selection and conservation decisions for local goat and sheep breeds to enhance milk yield and quality. BioZooCare is dedicated to meat production systems, promoting animal welfare and best management practices, particularly in extensive environments, to ensure sustainable and ethical meat production. WOOLLY advances the genetic improvement of wool quality in European Merino sheep through cutting-edge basic research and its direct application in breeding programs. Agritech provides transversal technical and digital support, offering data analysis tools, digital innovation, and technical accompaniment to empower the other three initiatives.

Objective

The goal of this study is to disseminate the key activities included in the four mentioned projects (LOCAL, BioZooCare, WOOLLY and Agritech).

Materials and methods

Following the project’s implementation plans, we give here an overview of the key activities and the main outcomes of the four considered projects.

Results

The analyses highlighted the strong synergistic nature of the four projects. Also, the relevance of empowering exchange and cooperation within and among different stakeholders’ categories emerged as a fundamental process toward sustainable resilient farming of local Apulian sheep and goat populations in marginal areas.

Conclusion

The experience gathered within the frame of the considered initiatives reinforces the awareness concerning power and the leverage effect of jointly co-designed and co-implemented efforts involving the quadruple-helix actors in the small ruminant sector and the connected downstream industrial sectors (dairy, meat, textile, etc).

85- INVESTIGATING THE ORIGINS OF THE "MURCIUNARA" GOAT: GENETIC INSIGHTS FROM THE VALBIOVICAP PROJECT

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Introduction

Southern Italy, particularly the Basilicata region, hosts a variety of local goat populations reflecting a complex history of animal exchanges and genetic introgressions. Among these, the hornless Black Goat ("Capra Nera Acorne", NA), locally called "Murciunara", echoes a name reminiscent of the Spanish Murciano-Granadina breed.

Objective

Within the frame of the regional ValBiOviCap project, our objective was to explore the genetic relationships between the NA and other local breeds from Continental Southern Italy, and Spanish breeds from the southern Iberian Peninsula (i.e., Malagueña and Murciano-Granadina), along with the cosmopolitan Saanen breed. We aimed to test the hypothesis that the NA may derive from the Murciano-Granadina breed, currently expanding across the Mediterranean thanks to intensive selection for dairy production.

Materials and methods

We genotyped approximately 20 samples per breed using the Illumina Goat SNP 50K BeadChip. Italian breeds included populations from the ValBiOviCap project: Nera Acorne, Nera dalle Corna a Sciabola, Grigia di Potenza, Garganica Lucana, Facciuta Lucana (Basilicata); Garganica and Jonica (Puglia); and Valfortorina (Campania). Spanish breeds (Malagueña and Murciano-Granadina) and Saanen were included using data from the international ADAPTMAP project. After quality control, genetic diversity and population structure were analyzed using MDS (Multidimensional Scaling), ADMIXTURE, NeighborNet, and NetView.

Results

Analyses revealed a high degree of genetic proximity between the NA and other Basilicata breeds, particularly Garganica Lucana, Nera dalle Corna a Sciabola, and Grigia di Potenza. In contrast, breeds such as Jonica and Facciuta Lucana clustered further apart possibly as a consequence of introgression from other breeds (Jonica) and inbreeding bias (Facciuta Lucana). Importantly, the data support the hypothesis of Spanish genetic introgression into Basilicata goat populations. However, the genetic signature was more pronounced in NA. Moreover, this introgression appears to have been mediated through the Malagueña breed rather than the Murciano-Granadina. This suggests that today's Murciano-Granadina, officially recognized only since 1975 following the merge of the two breeds (i.e., Murciana and Granadina) and the subsequent intensive selection, may have significantly diverged, over the last five decades, from the pre-fusion Murciana genetic stock. Consequently, breeds from southern Iberian Peninsula, such as Malagueña, may currently appear more genetically similar to the original genetic material that contributed to the development of the NA.

Conclusion

Our findings provide evidence of historical connections between southern Iberian Peninsula and southern Italian goat populations, reflecting centuries of livestock movement and exchange. While the NA does show traces of Spanish ancestry, its genetic profile is distinct and more closely aligned with regional Basilicata breeds. These results underline the importance of further genomic investigations to trace the evolutionary history of Mediterranean goat populations and support the recognition and conservation of unique local genetic resources such as the NA.

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86- EFFECTS OF THE MANAGEMENT SYSTEM ON THE MAIN MILK TRAITS AND CLOTTING ABILITY IN SHEEP FARMS IN THE LAZIO AND TUSCANY REGIONS

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Introduction

Traditional sheep breeds raised in Central Italy (Sarda, Massese, Comisana and Lacaune) are considered to be adapted to Mediterranean climatic conditions and grazing systems. Extensive and semi-extensive farming systems exert a positive influence on animal health, longevity and metabolism as well as on milk quality.

Objective This study aimed to evaluate how the production system (extensive, semi-extensive or intensive) affects the chemical, cytological and coagulation properties of bulk sheep's milk.

Materials and methods

A total of 215 bulk milk samples, were collected during 2024, from 17 farms located in central Italy (Lazio and Toscana regions). After collection, milk samples were maintained at 4°C and analyzed for milk quality traits at the Istituto Zooprofilattico Sperimentale Lazio and Toscana "M. Aleandri" laboratories. The main chemical milk composition (fat %, protein%, lactose% and freezing depression point (**FDP**) m°C) was performed through FTIR spectra method with the MilkoScanTM 7 RM (Foss Electric, Hillerød, Denmark), while the somatic cell count (**SCC**) was performed through flow-cytometry method using the FossomaticTM 7 DC instrument (Foss Electric, Hillerød, Denmark). Milk Coagulation Properties (**MCP**) – rennet clotting time (**RCT**), curd-firming time (k20) and curd firmness (a30) – were measured at 30 and 60 minutes, using a Formagraph LDG 2.0 device (Ma.Pe System srl, Firenze, Italy), following the Zannoni and Annibaldi methods (10 mL of milk at 36 °C, followed by the addition of calf rennet (200 µL) – composed of 75% chymosin and 25% pepsin (175 international milk clotting units/mL diluted at 1.6%). For the statistical analysis, the farms were classified according to the farming system as follows: intensive (**In**), semi-extensive (**SE**) or extensive (**E**), depending on the availability of grazing access. The effects of the management system on the main milk quality traits and **MCP** were evaluated using one-way ANOVA (p<0.05) with Bonferroni adjustment. The results are presented as the mean ± SEM.

Results

Regarding milk quality traits, the mean values of fat, protein, lactose, **FDP** and solids non fat were 6.89±0.06%, 5.88±0.04%, 4.47±0.02%, - 561±0.9 m°C and 11.58±0.04%, respectively. The mean **SCC** was 1,099±47 cells/ml. Regarding **MCP**, 75.8% (163/215) of samples coagulated within 30 minutes, 19.5% (42/215) between 31 and 60 minutes, and only 4.7% (10/215) did not coagulate. In the present study **RCT** and k20 averaged 21.82±0.34 min and 2.94±0.16 min, while a30 averaged 32.12±1.08 mm.

When analysing the impact of farms management on milk quality traits, it was found that milk from farms **E** had a significantly higher fat content (7.35%) than milk from **In** and **SE** farms (6.94% and 6.68%, respectively). The **SCC** was significantly higher in farms **In** (1,323 cells/ml) than in **E** and **SE** farms (900 and 1,026 cells/ml, respectively).

The **MCP** was better in milk from **E** and **SE** farms than from **In** farms. In fact, the **RCT** time was less than 22 minutes for **E** and **SE** farms and more than 23 minutes for **In** farms. In contrast, the average a30 time was 33.8 mm longer for **E** and **SE** farms than for **In**, which was 27 mm.

Conclusions

fat composition, cytological values, and lactodinamographic characteristics.

Farms with an intensive production system showed worse **SCC** and **MCP** values. In contrast, farms with an extensive production system had a higher fat content in milk; although the composition of fatty acids and the

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quality index of lipids have not yet been evaluated, this could be due to grazing as a substantial part of the diet. This nutritional information could add value to dairy products and differentiate them in terms of animal welfare as well.

This project was founded by the Italian Ministry of Health (RC IZSLT 04/22).

87- GREENHOUSE GAS EMISSION FROM LIVESTOCK FARMING. THE HEALTH STATUS OF THE HERD

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Introduction

It is widely recognized that greenhouse gas (GHG) emissions from livestock farming have a significant environmental impact and contribute to climate change. The agricultural sector is responsible for a significant proportion of GHG emissions in Europe and worldwide. According to EEA data, agriculture contributed 10.3% to total GHG emissions in the EU in 2019 (70% of which came from livestock farming, primarily due to methane (CH₄) and nitrous oxide (N₂O) emissions from enteric fermentation in ruminant animals and manure management). In Italy, according to ISTAT, agriculture accounted for 7.8% of total GHG emissions in 2019 (63% of which came from livestock farming). However, in 2021, agriculture was the main source of CH₄ (45.1%) and N₂O (64.2%) emissions.

Improving animal welfare and performance, minimizing dysmetabolism to improve the efficiency of the animal production system offers the possibility of reducing CH₄ and N₂O emissions from digestive fermentation and animal manure. Therefore, the negative environmental impacts associated with livestock management, GHG emissions require careful monitoring.

Objective

This project focuses on semi-extensive dairy cattle farms in Sicily. In terms of extensive and semi-extensive farming, Sicily ranks first in Italy in terms of the percentage of farms (24.05%) and second in terms of the number of livestock raised (approximately 21.5%).

This project proposal therefore aims to monitor greenhouse gases (GHG) on livestock farms using multi-temporal and multi-scale approaches with the development of low-cost IoT sensors and specific software support and to correlate GHG with the health and metabolic status of the herd.

Materials and methods

The monitoring of the greatest GHG emissions of interest will involve 3 dairy farms that are representative of the situation in Sicily (two intensive and one semi-extensive systems). These measurements will be combined with environmental measurements of temperature, humidity and air velocity to fully define the environmental context, but also with the genetic type bred.

Results

This proposal could lead to the identification of animals/genetic types with lower greenhouse gas (GHG) emissions, enabling the implementation of livestock strategies to mitigate the environmental impact of farming.

Conclusion

By highlighting the interdependency between animal, human and ecosystem health, it becomes clear that investing in cow health has wider implications for the environment and society, as well as benefiting the animals themselves. The One Health approach, which provides a comprehensive understanding of the broader impact of cattle diseases on ecosystems, can inform the development of effective management methods and production systems, paving the way for a more sustainable future.

MITigA project. Monitoring and technological innovation to reduce the environmental impact of livestock farming.

88- THE EFFECT OF LYING TIME ON FATTENING PERFORMANCE IN LAMBS*

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As in other countries, the rapid population growth in Turkey has led to an increased demand for animal protein and meat. Sheep and goats play an important role in meeting this demand, with a population of approximately 45 million head. Due to variations in geographical and socio-economic conditions, small ruminant farms are numerous and diverse. These farms are predominantly operated under extensive conditions within family-based farming systems. Animal behavior, particularly lying behavior, is considered a key factor influencing production efficiency in these farming systems. The objective of this study was to evaluate the effect of lying time on fattening performance in Merino lambs.

The study was carried out male lambs born to Merino sheep housed in a closed sheepfold system at the Sheep Unit of the Animal Health and Animal Production Application and Research Centre, Faculty of Veterinary Medicine, Bursa Uludag University. A total of 12 male lambs were included in the study. The body weights of the lambs were recorded at birth and on days 30, 60, 90, and 120. And their daily live weight gains were calculated. To monitor lying behavior, two cameras were installed at different angles above the pen. Behaviors were recorded via the cameras during four distinct time periods throughout the day. Observations began when the lambs reached 60 days of age and continued for a total of 30 days (10 days each at 60, 90, and 120 days of age). At the end of the study, the relationship between the number of lying behaviors and live weight was evaluated. The effect of weights on days 30, 60, and 90 and daily live weight gain on lying behavior was examined. Data were analyzed using IBM SPSS software, and a statistical model was applied to assess the correlation between lying time and fattening performance.

Birth weight, weight at 60, 90, and 120 days were as 4.62, 19.73, 28.02, and 44.17 kg, respectively. The daily live weight gain calculated on the 60th day was 307.19 g. No statistically significant correlation was found between weights and lying behavior at 60 days. Similarly, no statistically significant correlation was found between daily live weight gain calculated on day 60 and lying behavior.

Lying time is considered one of the key behavioral parameters in lambs; however, there is limited research investigating its association with fattening performance. In this study, the effect of lying time on fattening performance in Merino lambs was investigated. The findings of this study are expected to contribute to future research on lamb behavior and growth performance.

**This research was supported by the Scientific Research Projects Unit of Bursa Uludağ University under the project titled 'The Effect of Lying Time on Fattening Performance in Lambs' (Project No: TLO-2025-2254).*

89- MELATONIN TREATMENT AND HORMONAL AND OXIDATIVE RESPONSES TO SHEARING STRESS IN SARDA SHEEP

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Introduction

Many management practices in livestock farming, such as shearing, can induce an endocrine and metabolic response known as stress. Stress reaction primarily activates the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis, through the secretion of catecholamines and glucocorticoids, respectively. These hormones increase blood glucose levels, and other blood parameters. Furthermore, a state of stress triggers excessive free radicals' production, resulting in oxidative stress, which is involved in several pathological conditions, affecting production, reproduction and animal welfare. Melatonin is a pleiotropic hormone having a direct role in the antioxidant cascade. Through its metabolites (e.g. cyclic 3-hydroxymelatonin) it acts as a scavenger of free radicals - reactive oxygen and nitrogen species (RON/RNS).

Objective

The aim of the study was to highlight how melatonin treatment influences hormonal response and certain blood chemistry parameters affected by shearing stress in Sarda sheep.

Materials and methods

The study was conducted on a farm which raised about 600 Sarda sheep. Traditionally, Sarda sheep are shorn once a year, in late spring. For this study, 30 clinically healthy ewes (aged 4.1 ± 1.1 years) with a body condition score (BCS) 2.5 ± 0.5 , that had lambed before December, were selected. On 10 May, 30 ewes were randomly distributed into three groups of 10 ewes each, based on lambing date, parity, and age. On the same day, one group of 10 sheep received a subcutaneous melatonin implant (18 mg) (Melovine, CEVA Salute Animale), while the other two groups remained untreated. On June 4, the melatonin-treated group and one untreated group were shorn, while the other untreated group remained unshorn, as a control. Shearing was performed with a mechanical shearer, with each ewe restrained on its back for approximately 15 minutes. Blood samples were collected via jugular venipuncture using tubes containing lithium heparin (Becton Dickinson, Plymouth, UK) at four time points. Sampling from the shorn and unshorn groups was carried out in the same way. First sampling the day before shearing; the second sampling at the start of shearing at 9.00 AM, for all groups; the third sampling immediately after shearing; and the fourth sampling the next, at 09:00 AM (same as first sampling). The collected blood was centrifuged, and the plasma was analyzed for cortisol (ELISA), glucose and triglycerides, using commercial kits, with measurements performed on a spectrophotometer. Reactive Oxygen Metabolites (ROMs) and Biological Antioxidant Potential (BAP) were measured in serum by a colorimetric assay kit (d-ROMs test and BAP test Diacron International S.a.S.). The oxidative stress index ($OSI = ROMs / BAP \times 100$) was calculated to evaluate the oxidants-antioxidants balance. The data obtained were subjected to statistical analysis.

Results

Cortisol, glucose and triglyceride levels increased at the start of shearing and returned to physiological levels by the final sampling. Melatonin treated ewes exhibited lower hormonal and blood parameters levels than untreated ewes throughout the study. Even untreated, unshorn ewes exhibited increased parameter levels during separation and positioning on their backs. The oxidative stress index (OSI) was lower in melatonin treated animals, reflecting reduced ROMs and increased BAP.

Conclusion

In conclusion, the data demonstrate that melatonin modulates the stress response, as indicated by reduced cortisol and blood glucose levels in treated animals. Furthermore, melatonin treatment decreased oxidative stress, highlighting its significant role in regulating ROS and BAP. These findings suggest that exogenous melatonin could be utilized to mitigate stress and improve animal welfare.

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90- WELFARE OF FATTENING CATTLE: PRELIMINARY FIELD-BASED COMPARISON BETWEEN A CONDITIONING CENTRE AND A CONVENTIONAL UNIT

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Introduction

Imported fattening cattle undergo long and stressful journeys, and the housing and management practices adopted upon arrival can impact their welfare. They may be housed in a conditioning centre—where health checks, treatments and dietary adaptation occur before transferring to a fattening unit—or directly placed in a conventional fattening unit, where acclimatation, vaccinations and treatments are performed within the pen before starting the fattening phase.

Objective

The study aimed to compare the welfare level of two groups of Limousine beef cattle imported from France to Italy (live weight approx. 400 kg) and housed in different farming systems: a conditioning centre (“conditioned” animals, COND) or a conventional fattening unit (CONV).

Materials and methods

Cattle were observed once a week for five weeks. COND animals (N=58) were housed on straw bedding in pens of 11–12 animals (5.5–6 m²/head) with one water trough. CONV animals (N=60) were housed on slatted floors in pens of 6 animals (3.5 m²/head) with two bowl drinkers per pen. Data collection included nutritional, health, behavioural and housing indicators (animal- and resource-based), assessed according to the Welfare Quality® protocol (2009). Data were analysed by ANOVA, with the pen as the experimental unit. Statistical significance was set at $p \leq 0.05$. No handling or procedures were performed on the animals, and observations were collected during routine farm activities by the same operator.

Results

All animals reached an overall satisfactory welfare level. No differences were observed for the principle of “Good Feeding,” satisfactory in both groups. COND animals scored significantly higher ($p < 0.01$) for “Good Housing” and “Appropriate Behaviour” (72.7 vs. 55.7 and 24.1 vs. 21.2, respectively). Conversely, they scored lower for “Good Health” (50.4 vs. 78.3). For “Good Housing”, better scores in the COND group were linked to greater ease of movement and resting comfort, likely due to more space and solid flooring. For “Appropriate Behaviour”, both groups initially showed fear of humans, but COND animals improved over time, likely due to better handling (dedicated alleys, intranasal vaccination, oral treatments). Although not significant, social behaviours showed opposite trends: COND cattle improved, while CONV animals worsened. It is possible that the less comfortable slatted floors reduced agonistic interactions immediately after arrival, when animals were not yet accustomed to the flooring. Emotional state also favoured COND animals. In the absence of painful procedures or skin lesions, the lower “Good Health” score in COND animals resulted from a higher prevalence of respiratory disease, apparently incidental and unrelated to housing, as no specific factor differentiating the two systems in terms of animal health was identified.

Conclusion

Housing and management practices clearly influenced the welfare of beef cattle. While both systems (CONV and COND) achieved satisfactory results, the conditioned group consistently showed better outcomes in terms of comfort, human-animal relationship, and emotional state. Therefore, conditioning can be regarded as a beneficial practice for improving the welfare of imported cattle in the early rearing phase, following the stress of transport and the abrupt transition to new housing and management conditions.

91- IMPACT OF BASECALLING ALGORITHMS ON VARIANT DETECTION ACCURACY IN ADAPTIVE TARGETED SEQUENCING

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Introduction

Accurate mutation detection from nanopore sequencing data critically depends on basecalling quality, which converts raw electrical signals into nucleotide sequences. Oxford Nanopore Technologies offers multiple basecalling algorithms—Fast (FAT), High Accuracy (HAC), and Super Accuracy (SUP)—each optimized for trade-offs between computational speed and precision. While these algorithms are known to impact general read accuracy, their specific influence on downstream variant calling, particularly in targeted sequencing using adaptive sampling, remains underexplored. Adaptive sampling enables real-time enrichment of regions of interest, adding complexity to sequencing pipelines. Understanding how basecalling algorithms affect mutation detection in this context is essential to optimize diagnostic and research workflows based on nanopore sequencing.

Objective

In this study, we aimed to compare the variant calling metrics obtained using the FAT, HAC, and SUP basecalling algorithms in the context of adaptive sampling-based targeted sequencing.

Materials and methods

A single nanopore sequencing POD5 dataset was basecalled using three Dorado algorithms: FAT, HAC, and SUP. The FAT algorithm was used during adaptive sampling for the real-time read selection. Reads were then re-basecalled with the remaining two algorithms for comparison. Trimmed reads were aligned using the wf-alignment pipeline with Minimap2, applying whole-genome and targeted alignments around the canine PLG gene. Variant calling was performed using Longshot restricting the comparison to the PLG locus. Resulting VCFs were evaluated across algorithms using confusion matrix-derived metrics (accuracy, precision, recall, false discovery rate,) against a high-confidence variant set. Coverage was assessed with the tool mosdepth. Variants were annotated and functionally scored with Ensembl VEP. Finally, the presence of putative and causative variants was definitely confirmed by direct Sanger sequencing.

Results

FAT identified 200 variants, HAC 166, and SUP 162. Among these, 145 variants were shared across all three basecalling outputs, while an additional 10 variants were common to HAC and SUP only. Variant classification revealed a higher number of potentially impactful variants in FAT (2 stop gained, 4 missense), compared to HAC (1 missense, 4 synonymous) and SUP (1 missense, 3 synonymous). Two putative pathogenic variants were detected: a stop-gained variant and a moderate-impact SNV. Sanger sequencing confirmed only the missense variant detected by all methods, excluding all others called by FAT. This confirmed pathogenic variant was consistently identified by all three basecallers. Notably, HAC uniquely detected a synonymous variant within the coding region that was not identified by SUP. These findings highlight both the consistency and the variability introduced by different basecalling algorithms in variant detection using adaptive targeted nanopore sequencing. Using SUP as the reference, HAC showed higher concordance (accuracy 0.96, precision 0.97, recall 0.99) than FAT (accuracy 0.60, precision 0.67, recall 0.83)

Conclusion

These results underscore the importance of basecaller selection in variant detection accuracy. While the FAT basecalling protocol is essential for real-time read enrichment during adaptive sampling, it should be systematically followed by higher-accuracy basecallers such as HAC or SUP to improve variant calling reliability. This approach minimizes the need for extensive, labor-intensive, and costly confirmatory analyses by Sanger sequencing, thus optimizing diagnostic efficiency.

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92- SELECTION OF A DROMEDARY SNP PARENTAGE PANEL

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Introduction

In recent years, global interest in camels and their products has grown rapidly, driving the need for more effective and accurate breeding programs to support production intensification. In this context, parentage testing plays a crucial role, as it enhances the reliability of pedigree records and the accuracy of estimated breeding values.

Objective

To address this need, we designed a panel of SNPs for parentage assignment in dromedaries.

Materials and methods

The panel derives from the already commercially available Illumina® CamelSNP60 BeadChip, developed by our research group within the frame of the eleventh Agricultural Greater Good Initiative. The array was used to genotype a total of 374 dromedary camels worldwide. Subsequently, a multi-step approach was adopted for SNP selection, retaining, for the inclusion in the panel, only those markers that (i) exhibited the highest Minor Allele Frequency (MAF) across the dataset, (ii) exhibited the highest Probability of Identity (PI), (iii) showed no Mendelian errors in verified family trios, and (iv) ensured an even distribution along the genome.

Results

The final panel included SNPs meeting all the above-mentioned selection criteria, providing a reliable resource for parentage testing in dromedaries. The panel's accuracy was assessed using main population genetics and probability parameters, thus providing insights into its reliability and potential applications.

Conclusion

This newly developed SNP parentage panel represents a valuable tool for improving breeding programs in dromedary camels by enhancing pedigree accuracy and supporting production intensification.

93- UNRAVELING GENETIC DETERMINANTS OF TRYPANOSOME INFECTION IN ALGERIAN DROMEDARIES

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Introduction

Trypanosomiasis, caused by protozoans of the genus *Trypanosoma*, is a significant zoonotic disease affecting humans and animals worldwide. Among livestock species, dromedaries are particularly susceptible to trypanosome infections, leading to severe health issues on the affected animals and substantial economic losses, especially in Algeria where the disease due to the infection is endemic. However, the genetic factors underlying susceptibility or resistance to trypanosome infection in dromedaries remain poorly understood.

Objective

This study aimed to explore the genetic basis of trypanosome infection in Algerian dromedaries.

Materials and methods

A Genome-Wide Association Study (GWAS) was conducted using genotyping and phenotypic data available from 80 Algerian dromedaries including 20 *Trypanosome*-positive and 60 *Trypanosome*-negative samples. In addition, an F_{ST} -outlier approach was performed to detect putative signatures of selection. The presence/absence of the parasite was determined based on both PCR and 18S amplicon next-generation sequencing (NGS).

Results

A total of 18 significant loci across 11 genomic regions were identified, harboring 137 candidate genes potentially involved in immune responses and infection-related processes. Key genes, including *TUBB*, *MUC21*, *C2*, *TNF*, and *AIF1*, were implicated in immune modulation and host-pathogen interactions. Gene Ontology analysis revealed associations with immune system processes, cellular responses to stress, and signal transduction.

Conclusion

These preliminary findings provide novel insights into the genetic basis of trypanosome infection in dromedaries, paving the way for further studies to validate the observed signals.

94- DEVELOPMENT OF A SNP PARENTAGE PANEL FOR APULIAN AUTOCHTHONOUS SHEEP BREEDS WITHIN THE FRAME OF THE L.O.C.A.L. PROJECT

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Introduction

In Southern Italy, particularly in the region of Puglia, several valuable sheep populations are reared, representing an important source of the region's agricultural heritage and genetic biodiversity. Among these, the Gentile di Puglia (GdP) is traditionally renowned for its aptitude for producing high-quality wool, meat, and milk. Historically, this breed has been a key element of the region's cultural and economic heritage, with millennia-old traditions that underscore its significance as a local genetic resource. Despite its resilience and adaptability to the challenging environmental conditions of marginal areas, GdP is currently threatened by genetic erosion, primarily due to the wool market crisis and extensive crossbreeding over the past 50 years. In addition to this breed, the Gentile Moretta (alias Carapellese), a genetically related population, is similarly valued for its adaptability and productive potential, although it faces a critically low population size. Another important local sheep population is the Fasanese, which is appreciated for its rusticity and productivity but also faces threats from genetic erosion and declining numbers. The conservation and sustainable management of the above-mentioned genetic resources is crucial, requiring accurate pedigree records and reliable breeding schemes. Parentage testing is a fundamental tool in this context, as it ensures the verification of genealogical data, enhances the precision of estimated breeding values, and supports informed selection decisions. In light of the challenges faced by these populations, the development of a robust and breed-specific tool for parentage assignment is crucial for safeguarding these valuable genetic resources.

Objective

To address this need, we developed a panel of SNPs for parentage assignment in small apulian sheep populations.

Materials and methods

Due to the extremely small numbers of Fasanese and Gentile Moretta, biological samples from 110 GdP were used as a representative proxy for these endangered Apulian sheep populations and were genotyped using Illumina OvineSNP50 BeadChip. After the quality control of the dataset, we adopted a multi-step approach for SNP selection. Specifically, only SNPs that (i) exhibited the highest Minor Allele Frequency (MAF) across the dataset, (ii) were in Hardy-Weinberg equilibrium, (iii) had the highest Probability of Identity (PI), and (iv) ensured an even distribution along the genome, were retained for the inclusion in the panel.

Results

The final panel included SNPs that met all the aforementioned selection criteria, providing a reliable tool for parentage testing in Apulian native sheep populations. Key population genetics and probability parameters were employed to assess the accuracy of the panel, thus offering valuable insights into its reliability and potential applications.

Conclusion

The newly developed tool represents an essential resource for improving breeding programs in Apulian sheep populations. It enhances pedigree accuracy and supports the conservation and sustainable management of these local genetic resources.

95- FROM THE PAST TO THE FUTURE: HISTORY AND NEXT PERSPECTIVES OF THE “LECCESE” SHEEP

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Origin and Distribution:

The “Moscia Leccese” sheep breed is believed to derive directly from *Ovis aries asiatica* or Syrian sheep (Sanson classification), and more specifically from the Zackel lineage. The term “moscia” (meaning “limp” or “soft”) refers to the slightly crimped and drooping wool fibers of the fleece. The breeding area is located in the Apulia Region (South Italy) and includes the provinces of Lecce, Brindisi, and Taranto, particularly covering the Salento peninsula and parts of the hilly terrain of the Ionian arc.

Population:

As the purebred population is limited to just over 500 animals across only 6 farms, the “Moscia leccese” is considered at risk of genetic erosion.

Breed Description:

The “Moscia leccese” is a medium-sized sheep. The fleece is white in all individuals, with a black fleece variant found in a limited number of animals. The skin is pink in white-fleeced individuals and has black spots in those with black fleece. The head is black, light, elongated, and lean, with a convex (roman-nosed) profile. Males have spiral-shaped horns, while females have a straight profile and are hornless. The ears are small and horizontal. The neck is slender and of medium length. The trunk is long, with the withers slightly lower than the rump. The flanks and ribcage are flat; the tail is long and thin. The udder is well-developed, globular in shape, well-attached, with fine skin and firm teats. The legs are black, long, straight, with correct conformation and posture, and the hooves are dark and compact.

Future Goals:

Recovery of the breed through both in situ and ex situ conservation strategies.

96- GENETIC POLYMORPHISMS OF GDF9 AND BMP15 FECUNDITY GENES IN SYRIAN AWASSI SHEEP

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This study was conducted to identify the genetic polymorphisms in growth differentiation factor 9 GDF9 and bone morphogenetic protein 15 BMP15 fecundity genes in Syrian Awassi sheep, using sequencing analysis for exons 1 and 2 in both genes. Ewes blood samples were collected from ACSAD research station in Ezraa, in addition to Merj-Kreem and Hamah research stations (GCSAR). Alignment with the reference sequences was carried out using sequencer version 5.4.6 and BioEdit version 7.2.1., which revealed seven mutations (SNPs) in GDF9 gene in the flanking region, exon 1 and exon 2. Three out of them are functional mutations led to amino acids substitution such as G1 (Arg87His), G4 (Glu241Lys) and G6 (Val332Ile). Regarding the BMP15, the alignment analysis identified two mutations, the first is a frameshift in exon 1 due to deletion of 3 nucleotides (CTT at positions 58 - 60) of the coding sequence and led to removal of the amino acid No. 20 leucine. While the second mutation was in exon 2 caused amino acid No. 261 from leucine to Proline.

The identified polymorphisms of these fecundity genes can be used to speed up the selection process for fertility traits such as twinning rate by integrating modern biotechnologies into genetic improvement plans.

Key words: Syrian Awassi sheep, genetic polymorphism, fecundity genes, GDF9, BMP15.

97- MOLECULAR DETECTION AND CHARACTERIZATION OF PIROPLASMS (*BABESIA* AND *THEILERIA*) AND OTHER TICK-BORNE PATHOGENS IN FALLOW DEER OF SICILY, SOUTHERN ITALY

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Introduction

In recent years, multiple factors have intensified interactions among wildlife, domestic animals and humans, enhancing pathogen transmission and threatening public health, livestock, and biodiversity. Tick-borne pathogens (TBPs), in particular, exhibit a complex transmission cycle, influenced by vector abundance, host density, climate and landscape. Wild ruminants play a key role in maintaining TBPs, many of which are zoonotic, by hosting ticks and serving as natural reservoirs for certain pathogens.

In Sicily (Southern Italy), a recent and uncontrolled increase in fallow deer populations may have implications for the circulation of TBPs.

Objective

This study aimed at investigating the presence of ticks and TBPs in fallow deer (*Dama dama*) carcasses collected in Sicily through regional culling programs.

Materials and methods

A total of 56 fallow deer carcasses were analyzed. The study involved the health inspection of fallow deer carcasses, with clinical assessments documenting pathological lesions and ecto-/endoparasite presence. External examinations evaluated nutritional status, external injuries, and ectoparasite burden. Necropsy focused on internal organ topography and cavity effusions, with detailed characterization of any pathological lesions and associated parasitic findings. Ticks, when present, were collected and identified using morphological keys. Piroplasm (*Babesia* and *Theileria*) DNA was screened by PCR targeting 18S rDNA in 390 organ samples, including heart, spleen, liver, lung, intestine, kidney, testicle, mammary gland, tongue, and lymph nodes. Additionally, *Anaplasma* spp. and *Rickettsia* spp. DNA was analyzed in spleen samples from the 56 carcasses by nested PCRs targeting, respectively, 16S rDNA and *OmpB* gene fragments. PCR products from positive samples were sequenced and the obtained sequences were analysed to identify the closest matching species based on sequence homology.

Results

No pathognomonic lesions attributable to the targeted pathogens were observed in any of the analyzed cases. A total of 189 ticks were collected from the carcasses, of which 140 (74.1%) were identified as *Ixodes ricinus*, 46 (24.3%) as *Haemaphysalis punctata*, and 3 (1.6%) as *Dermacentor marginatus*.

Piroplasm DNA was detected in 8 fallow deer (14.3%). *Anaplasma* spp. DNA was identified in 43 animals (76.8%), *Rickettsia* spp. was detected in 2 fallow deer (3.6%). Molecular characterization revealed a high sequence identity with *Theileria* spp., *Theileria cervi*, *Theileria ovis*, and *Theileria capreoli* among the Piroplasm-positive samples. Among the samples positive for *Anaplasma* spp., sequence analysis further identified *Anaplasma phagocytophilum*. Sample positive for *Rickettsia* spp. were confirmed as *Rickettsia slovaca*. Two cases of coinfection were reported: one involving *Theileria* sp. and *R. slovaca*, and another involving *Theileria* sp. and *A. phagocytophilum*.

Conclusion

The detection of multiple pathogens in the analysed wild ruminants indicates their potential involvement in the transmission dynamics and epidemiology of TBPs.

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The coexistence of TBPs, competent tick vectors and a high density of fallow deer in Sicily may enhance the transmission and dissemination of these infectious agents, the majority of which have zoonotic significance. Monitoring the presence and spread of pathogens in deer populations is essential for the implementation of control measures aimed at minimizing the risk of interspecies transmission. This research has been funded by Ministero della Salute GR-2021-12373930.

98- SEPTICEMIA CAUSED BY *E. COLI* ST23 IN A HEIFER HERD IN APULIA, ITALY

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Introduction

Escherichia coli is the most common bacterial cause of calf diarrhea and, occasionally, pathogenic strains can evolve into more severe forms, such as septicemia. The pathogenic strains of *E. coli*, are mostly responsible for diarrheal forms affecting neonatal calves, usually in the first 4 days of life, but colibacillosis can occur in older calves. This report demonstrates the involvement of *E. coli* O78:H9, ST 23, in a heifers herd raised in southern Italy with sudden increase in mortality rate, related to multiple organ dysfunction syndromes and septic shock. *E. coli* ST23 is reported as an agent of infections in cattle farming, poultry farming, sheep, and humans, where it is responsible for many infections, including urinary tract infections.

Objective

In this paper is described a case report in a heifer herd located in Apulia (Italy), in which three heifers were struck simultaneously by a severe form of septicemia caused by *E. coli* ST23. Field isolates of *E. coli* were characterized using Whole Genome Sequencing (WGS), investigating virulence genes responsible for pathogenicity and multi-drug resistance factors.

Materials and methods

Necropsy examination was performed. Organs were taken for the histopathological, microbiological, and molecular tests. Tissue samples were fixed in 10% formalin, microtome sectioned (3–5 µm), and stained with hematoxylin and eosin for histopathologic evaluation. Microbiological procedures were performed by streaking pathological material with a swab onto Columbia Blood Agar plates (CBA); one strain was isolated, identified by using MALDI-TOF, sequenced and submitted to bioinformatic analysis on Illumina MiSeq® platform. The antimicrobial susceptibility was evaluated by the Minimum Inhibitory Concentration (MIC) method according to the CLSI.

Results

At necropsy, a severe pattern of diffuse sierofibrinous inflammation was evident, including peritonitis, pleuritis, polyarthritis, severe meningitis, hemorrhagic petechiae on the aortic arch and myocardium. Cerebrospinal fluid (CSF) was turbid, bloody, and contained a fibrinous strand. Histologically, cytological picture was attributable to suppurative inflammation of bacterial origin. Purulent meningoencephalitis with parenchymal microabscesses, thrombotic vasculitis and perivasculitis was observable, with bacterial aggregates present in colonies and in the granulocytic cytoplasm. Bacteria phenotypically referred to *Enterobacteriaceae* were isolated on CBA in pure culture from the brain, CSF, lungs, liver, spleen, carpal joint, lymph node and kidney. Strains were identified as *E. coli* by using MALDI-TOF. Genetic relationships were investigated and revealed the same sequence type (ST23) and cgMLST allele profile. Isolate serotype was O78:H9, while the main predicted virulence factor was *Hlye*. In addition, other virulence factors that are responsible for the bacterium's adhesion and pathogenicity mechanisms have also been predicted. MIC test showed resistance against ampicillin, tetracyclines and intermediate susceptibility for florfenicol and cefazolin. WGS confirmed the multidrug resistant character of the isolate: multidrug efflux pumps genes and antimicrobial resistance genes acting against aminoglycosides, beta-lactam antibiotics and tetracyclines were found.

Conclusion

In this study, a multidisciplinary diagnostic approach was adopted, to investigate about the virulence factors and antimicrobial resistance of the responsible agent. Thanks to this diagnostic strategy, it was possible to define an appropriate therapeutic approach. *E. coli* septicemias, although frequent in cattle herds, usually occur in very young animals. Therefore, the occurrence of such severe disease in five-month-old animals is rare and requires expeditious intervention. Finally, the environment and management practices on the farm should be

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investigated, in order to identify useful elements for herd infection risk analysis and Animal Welfare assessments.

99- FROM COW TO CURE: BOVINE MODELS OF RARE DISEASES AND TRANSLATIONAL RESEARCH FOR HUMAN HEALTH

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Introduction

Recent paradigm shifts in global health have expanded its scope beyond human-centric approaches. The 'One Health' concept now integrates human, animal, and plant health, recognizing their interdependence within ecosystems. The intricate interrelationship between human and animal health has become increasingly evident in recent years. Numerous human pathologies, including rare diseases, have been found to have analogous counterparts in animal species, particularly in large mammals such as bovine species. This phenomenon, known as translational research, has significantly enhanced our understanding of disease mechanisms and potential therapeutic approaches.

Objective

The study of naturally occurring diseases in cattle, has provided valuable insights into human disorders, particularly in areas such as metabolic syndromes, reproductive disorders, and especially in certain rare genetic conditions. This cross-species relevance underscores the importance of a 'One Health' approach in biomedical research and public health strategies.

The aims of this project are to investigate rare inherited neuromuscular diseases in cattle in order to: i) elucidate the pathogenetic mechanisms underlying specific human diseases and ii) subsequently propose pharmacological interventions. The research methodology incorporates alternative models to enhance our understanding of disease processes and facilitate the development of targeted therapeutic strategies

Primary focus was given to Congenital Pseudomyotonia (PMT) and to Bovine Spastic Syndrome (BSS). PMT represents the real mammalian counterpart of human Brody myopathy (BM) and BSS shares clinical similarities to human hereditary spastic paraplegias (HSPs).

Materials and methods

We have recently proposed a new pharmacological approach to treat BM, based on the repositioning strategy of Cystic Fibrosis Transmembrane Regulator (CFTR) correctors (especially C17 molecule) (EU 2925317 and US 9987256 B2 patents). These correctors have been extensively studied for the treatment of human Cystic Fibrosis (CF). The first stage in evaluating the efficacy of a drug candidate is provided by preclinical investigations. The main limit in employing bovine models for *in vivo* studies is the complexities inherent in administering and evaluating pharmacological treatments in large animal subjects. To address these limitations:

- 1) we generated and characterized a new zebrafish mutant line introducing mutation causing bovine PMT in SERCA1 protein, by CRISPR/Cas9-mediated genome editing (authorization 319/2022-PR);
- 2) we developed cheap and easy-to-obtain 3D muscle models based on two different scaffolds, (de-cellularized extracellular matrix from bovine diaphragm and collagen-based scaffold derived from non-processed sea urchin food waste).

Results

We have generated and characterized the first SERCA1 mutant zebrafish models carrying the mutations R164H found in bovine PMT. Western blot analysis with antibodies to SERCA1 on sarcoplasmic reticulum (SR) microsomal fractions from adult zebrafish, evidenced a decrease of immunoreactivity in fraction from R164H mutant zebrafish, in close agreement with results found in bovine BM model.

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Collagen-based scaffold obtained from non-processed sea urchin food waste, have been cellularized with C2C12 cells, seeded inside the scaffold using a Hamilton syringe injector. The extracellular matrix from bovine diaphragm has been successfully decellularized. The refined detergent-enzymatic methodology yielded an optimized muscle-derived scaffold that maintained the structural integrity of the extracellular matrix while ensuring efficient removal of myofibers to facilitate subsequent cellular repopulation.

Conclusion

Overall, our findings have yielded two significant outcomes:

- 1) a newly generated R164H zebrafish mutant line, which currently represents the sole available zebrafish model to investigate BM. This mutant line is maintained at the Zebrafish Facility of the University of Padova and constitutes a valuable resource for future studies on this disease.
- 2) the development and validation of muscle organoids based on two different scaffolds which will be used as platform for the development of innovative therapeutic strategies for neuromuscular disorders in parallel with conventional *in vitro* studies or as alternative of *in vivo* animal models.

These studies were funded within PRIN PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2022, research project titled “Bovine neuromuscular diseases as a model for translational researches”, Project Code 2022XLJXCZ, and PRIN PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2022 PNRR (National Recovery and Resilience Plan) research project titled “MiMiC Model Muscle Construct: a 3D organoid mimicking native skeletal muscle as a tool for testing the efficacy of new pharmacological therapeutic molecules”, Project Code P2022W35KY.

100- PLASMA COBALT LEVELS IN CATTLE SUBMITTED TO DIFFERENT FEEDING MANAGEMENT SYSTEMS

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Introduction

Cobalt is an essential trace element for the synthesis of vitamin B12 by ruminal microorganisms, a fundamental process for the metabolism of propionic acid, the main energy source in ruminants. Cobalt deficiency negatively impacts growth, weight gain, disease resistance, and productive efficiency in livestock, underscoring the need to study cobalt levels under different management conditions.

Objective

The aim of this research was to evaluate cobalt levels in cattle, comparing different groups of animals under intensive and extensive production systems.

Materials and methods

Blood cobalt concentrations were measured in 41 cattle: 14 adult bulls and 15 adult cows of Lidia breed under extensive systems, and 12 Charolais steers in intensive finishing systems, using inductively coupled plasma mass spectrometry (ICP/MS).

Results

The results indicated that adult Lidia cows (0.089 ± 0.034 $\mu\text{mol/L}$) exhibited significantly lower cobalt concentrations compared to bulls of the same breed (0.217 ± 0.052 $\mu\text{mol/L}$) and Charolais steers (0.146 ± 0.099 $\mu\text{mol/L}$). Furthermore, all sampled animals exhibited blood cobalt levels below those reported for healthy dairy cows.

Conclusion

It was concluded that feeding practices in both extensive and intensive systems do not provide an adequate cobalt supply, leading to significant deficiencies in this trace element. Monitoring cobalt levels in cattle diets is suggested to ensure proper health and productivity.

101- PREVALENCE AND RISK FACTORS OF *TOXOPLASMA GONDII* INFECTION AND DETECTION IN RAW MILK FROM DAIRY BUFFALOES IN SOUTHERN ITALY

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Introduction

The aim of the present study was to assess the **prevalence of *Toxoplasma gondii* on buffalo farms in the Campania Region** through serological screening. Toxoplasmosis is a parasitic disease considered the fourth leading cause of illness within foodborne diseases and the third leading cause of DALYs (Disability-Adjusted Life Years) in Europe. *Toxoplasma* DNA and anti-*T. gondii* antibodies have been detected in the milk of various animals, including goats, sheep, cattle, and buffaloes, suggesting that transmission can occur through raw milk consumption. Although buffalo milk is consumed less fresh compared to cow's milk, it may still represent a potential source of *T. gondii* infection in humans. This study aimed to identify risk factors associated with *T. gondii* infection on buffalo farms in southern Italy and to evaluate the potential for vertical transmission of the parasite through milk.

Objectives

The primary objectives of this study were to: a) determine the seroprevalence of *T. gondii* in adult buffaloes from the Campania Region (southern Italy); b) identify risk factors associated with *T. gondii* positivity on buffalo farms through a tailored questionnaire; c) investigate the presence of *T. gondii* DNA in buffalo milk from serologically positive farms and assess the potential for foodborne transmission.

Materials and Methods

Serological analysis and risk factors - Blood samples were collected from adult buffaloes during state prophylaxis and analyzed using an indirect ELISA kit (ID Screen®, Indirect Toxoplasmosis Multi-Species, Innovative Diagnostics, Garbles, France). In total, 13,649 adult buffaloes from 184 farms in the provinces of Caserta and Salerno were tested for *T. gondii*. A specially designed questionnaire was administered to assess potential risk factors for infection. This questionnaire included several factors, such as the herd size, the presence of cats, and rodent control measures, and the occurrence of clinical symptoms in adult buffaloes.

Milk Sampling and DNA Analysis - Bulk milk samples were collected from farms that volunteered to participate and had previously tested serologically positive for *T. gondii*. A total of 43 milk samples were taken from two buffalo farms, one in the province of Salerno and one in the province of Caserta. Total DNA was extracted using a QIAamp DNA Mini kit, following the manufacturer's instructions. The ddPCR reaction was performed in a QX200 system to amplify the 529 bp repeat element of *T. gondii*.

Results

Of the 13,649 adult buffaloes serologically tested, 2,693 (19.7%) were positive for *T. gondii*. Among the 184 farms included in the study, 179 (97.3%) had at least one seropositive animal. Analysis of the questionnaire data revealed that 97% of the *T. gondii*-positive farms reported the presence of cats, while 85% did not implement any rodent control measures. Out of 43 milk samples collected from seropositive farms, 27 (63%) tested positive for *T. gondii* DNA, indicating a potential risk for foodborne transmission through raw milk consumption.

Conclusions

The results obtained from our study highlight that **significant risk factors exist on buffalo farms that do not implement proper monitoring through appropriate biosecurity plans**. The high seroprevalence in both individual animals and farms indicates widespread exposure to *T. gondii* in the Campania Region. Furthermore, the detection of *T. gondii* DNA in buffalo milk samples confirms that **vertical transmission of the parasite through milk can occur and presents a potential form of foodborne transmission if consumed raw**. These findings underscore the importance of implementing effective control measures to mitigate the risk of toxoplasmosis in buffalo populations and, consequently, reduce the potential for human exposure through dairy products.

Acknowledgements

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102- SEROPREVALENCE AND RISK FACTORS FOR INFECTION OF *BESNOITIA BESNOITI* IN BEEF CATTLE OF EMILIA ROMAGNA REGION IN NORTHERN ITALY

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Introduction

Bovine besnoitiosis is a chronic parasitic disease caused by the cyst-forming protozoa *Besnoitia besnoiti* (Apicomplexa, Sarcocystidae). It can appear as a severe, debilitating, cutaneous, or systemic form, compromising the animal's welfare and leading to economic losses caused by decreased production, bull sterility, and leather depreciation. In 2010, EFSA defined bovine besnoitiosis as an emerging disease in European countries and recommended epidemiological investigations.

Objective

The objective of this study was to acquire information about the epidemiology of besnoitiosis in beef cattle population in Emilia Romagna Region, Northern Italy, given the lack of knowledge about the prevalence and distribution of the disease in this geographical area. At the meantime a study of risk factors for conducted.

Materials and methods

A cross-sectional study was performed on the beef cattle population of the Emilia-Romagna region, Northern Italy, in order to assess the endemicity of the infection in this territory and to evaluate associated risk factors. A total of 105 farms were investigated by serological analysis twice, in 2015 and 2018. A total of 4195 animals were tested: 1892 twice and 2303 only once. A commercial ELISA test (Kit PrioCHECK®*Besnoitia* Ab 2.0-Prionics) was performed as a screening test, according to the manufacturer's instructions. All positive sera underwent IFAT for confirmation. Data related to herd management (pasture use, type of housing, type of insemination, presence of cats, use of fly treatments) and to animals (sex, breed, date and place of birth, date of purchase/entry into the farm, farm of origin in the case of purchased animals, date of death/exit from the farm) were recorded. In order to determine risk factors associated with seropositive status, statistical studies were carried out at both the farm and animal levels. A primary univariate analysis was carried out, testing the association between positive cases and factors using the Chi-Square Test by grouping continuous variables into classes. Afterwards, a multivariable logistic regression model was performed at the farm level using statistically significant factors identified in the univariate analysis, and a multilevel-modelling analysis [generalized linear mixed modelling fit by maximum likelihood (Laplace approximation)] was performed at the animal level. For farm level, the analysis was performed using the data collected through the questionnaire distributed in 2015. For this reason, the analysis of risk factors at the farm level was based only on the serological results from 2015.

Results

During the study period, seroprevalence increased from 17.1% to 21.9% at farm level, and from 9.3% to 12.2% at animal level. Within-herd seroprevalence increased too, from 28.4 to 32.4%. At farm level, the following proved to be risk factors for bovine besnoitiosis: the number of purchased and the number of directly imported animals, the presence and the number of born abroad animals, the adoption of fly treatments, the size of the herd, the presence and the number of Limousine breed cattle. The animal-level risk variables linked with seropositivity comprised age, male sex (limited to 2015), and the Limousine breed.

Conclusion

This study provided information about bovine besnoitiosis epidemiology in beef herds of the Emilia-Romagna region. The survey, conducted on 105 farms over three years, gave evidence of the presence of the disease in the area and showed that seroprevalence was increasing during the period under analysis. We identified risk factors for *B. besnoiti* both at herd and animal level. Our observations highlighted the importance of clinical

and serological examinations of the animals, especially when they are imported from abroad, to avoid the introduction and spread of this infection, which often runs asymptotically and does not have an effective therapy. We reported a high prevalence of besnoitiosis in Limousine cattle, a breed commonly imported from France, a country considered endemic for the disease. According to our analysis, herd size, fly infestation, increasing age, and male sex are characteristics to be considered.

103- ORGANIC MANAGEMENT OF TICK CONTROL IN SARDINIAN SHEEP FARMING

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Introduction

Among the health problems in sheep farms, the heart of Sardinian livestock farming, tick ectoparasitosis stands out, increasingly resistant to repeated and scheduled chemical treatments. Integrated systems are therefore evaluated (environmental management, farm management, monitoring, targeted treatments and use of organic products based on natural antagonists that therefore reduce the phenomenon of resistance to chemicals, information plans for prevention and protection of animal and human health, according to the *One Health* approach).

Objective

The project aims to improve knowledge on the spread of ticks and related diseases, promoting health education interventions and suggesting integrated control systems with organic treatments.

Materials and methods

An Integrated Tick Control System is proposed for sheep farms, inspired by the principles of Integrated Pest Management (IPM), based on the following interventions:

- Environmental/Farm management through rotation of grazing land and mulching in wet or shaded areas to interrupt the tick life cycle
- Planting of repellent aromatic plants (rosemary, mint, lemongrass, lavender)
- Treatments with essential oils (neem oil, lavender, tea tree, lemongrass).
- Adding brewer's yeast or garlic extract to the feed ration to alter the body odor of sheep and make them less attractive to ticks.
- Organic treatments: seasonal application of entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae*) and pathogenic nematodes (*Steinernema* spp., *Heterorhabditis* spp.) on soil and sheepsheds to reduce immature tick populations
- Releasing poultry into pastures to prey on tick larvae and adults.
- Systematic cleaning of environments, pruning under the canopy of hedges, application of diatomaceous earth in infested areas, bi-weekly change of litter.

Treatments were carried out with Azadirachtin-based formulations, permitted in organic farming, using neem oil (*Azadirachta indica*).

Azadirachtin has insecticidal and acaricidal action, inhibiting metamorphosis, fertility and reproduction, modulating appetite with a sub-lethal phagorepellent function, not rapid, but progressive and stable, low ecological impact and good compatibility with useful insects. Its purpose is more to prevent/repress than to eliminate. It is one of the most studied botanical products in organic livestock farming.

A solution of 25 ml of neem oil, 12 drops of lavender essential oil diluted in 1 liter of warm water, with the addition of 5 ml of liquid Marseille soap as an emulsifier, was used. The solution was applied to 16 sheep using soaked sponges rubbed in the affected areas, avoiding the eyes, nose and mouth.

Results

The techniques provided by the Integrated Tick Control System in Sheep Farming, based on ecologically sustainable methods, have proven useful for the containment of ticks, but are demanding and expensive to apply. Awareness and responsibility are necessary on the part of the breeder as well as the institutions that should facilitate this systemic approach since the benefits would fall not only on the individual but on the community and the results, as usual with the organic method, will manifest themselves in the long term, as soon as the livestock agro-ecosystem has reached a homeostasis that allows a stable control of ticks, avoiding resistance phenomena responsible for periodic peaks of overpopulation of parasites.

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Conclusion

With Azadirachtin treatments, all sheep experienced relief from tick attacks, with a significant general reduction in parasites, and, certainly, when the application of Azadirachtin will be rationalised and optimised in terms of methods and costs, it could represent a good alternative to common chemical antiparasitic drugs.

104- PREVENTION, INFORMATION AND TRAINING ACTIVITIES FOR THE PROTECTION OF HUMAN HEALTH IN SHEEP AND GOAT LIVESTOCK ENVIRONMENTS WITH TICK INFESTATION

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Introduction

In Sardinia, the frequency of notifications of human clinical cases attributable to zoonoses transmitted by arthropods makes it necessary to implement surveillance programs, in order to define the spread of pathogens in the territory. It is urgent to investigate the real distribution of ticks and the main related pathologies, applying the *One Health* concept, especially in sheep and goat farms, which represent important reservoirs of infection.

The recent death of a woman, belonging to the livestock sector in Ogliastro, due to the bite of a tick infected by *Rickettsia* spp., has determined the need to evaluate the risk for human health and, consequently, the urgency to deepen knowledge on the real spread of ticks and related zoonoses in territories with a high vocation for sheep and goat livestock farming.

Objective

The survey aimed to deepen knowledge on the real extent of the problem related to the spread of ticks and zoonoses, and to activate an adequate targeted information and training campaign, in order to safeguard animal and human health, in a context of environmental protection, according to the *One Health* approach. Furthermore, to contribute to a prevention strategy, through information plans and training meetings aimed at both operators in the livestock sector and ordinary citizens who live in the areas affected by the problem.

Materials and methods

In the livestock context studied in Ogliastro, where there is a high concentration of sheep and goat farms, in addition to the investigation of the pathologies present in ticks and sheep and goats, questionnaires were prepared for the collection of environmental and health data and for the collection of information on the incidence of parasitic diseases in humans. In particular, 95 data collection sheets were delivered on the presence of ticks on people and on any clinical symptoms potentially related to zoonoses transmitted by these parasites.

Results

The survey was conducted by examining 95 data collection sheets on the presence of ticks in humans, aged between nine and ninety years, to verify their spread, at the same time as the ticks were found on the body, with any related symptoms.

Specifically, among the 95 people involved, 58 men aged between 16 and 90 years were interviewed, and 37 women aged between 9 and 87 years.

Among these, 56 subjects, of whom 40 men and 16 women, reported having been bitten by ticks several times during their lives.

14 men and 3 women presented symptoms related to the presence of ticks, while 2 men and 1 woman required hospitalization.

Conclusion

In light of these findings, and considering that numerous ticks collected from goats and sheep were found to be infected with zoonotic pathogens, including *Coxiella* spp., *Rickettsia* spp., *Anaplasma* spp. and *Babesia* spp., pathogens known to have a significant impact on animal and human health, the need to implement surveillance programs on tick populations and the pathogens they can transmit is evident. Integrated studies on the interactions between ticks, hosts and the environment could provide useful data to develop broad *One Health* strategies.

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It is essential to raise awareness among farmers and livestock sector professionals on the management and prevention of tick infestations. The adoption of integrated control measures, including targeted pesticide treatments and good business management practices (including Organic Farming techniques to reduce the problem of resistance to chemicals that is dangerously spreading also among arthropods), could help limit the spread of ticks and the health problems associated with them and improve health safety for livestock and for people living in close contact with animals, as well as for citizens living in affected areas.

105- MIMICKING NATURAL CONDITIONS TO RESOLVE IN VITRO PRODUCTION (IVP) SIDE EFFECTS

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Introduction

The oviduct is far more than a passive conduit for gametes and plays an active and dynamic role in sperm selection, capacitation, fertilization, and embryonic development. Despite major improvements in *in vitro* production (IVP) systems, they still fail to recapitulate this environment, resulting in reduced embryo quality and developmental disorders contributing to aberrant outcomes such as large offspring syndrome and epigenetic drift. These lacks underscore the critical importance of mimicking the physiological conditions that embryos experience *in vivo*. This work explores the role of the oviduct in fertilization and early embryo development and highlights emerging techniques aimed at reproducing its intricate architecture and dynamic interactions.

Revisiting Oviductal Physiology

The oviduct comprises four distinct parts: the uterine-tubal junction (UTJ), isthmus, ampulla, and infundibulum; each with a specific role in gamete movement, fertilization timing, and embryonic development. Secretory and ciliated epithelial cells, under the influence of estrogen and progesterone, contribute to the formation and circulation of oviductal fluid (OF)—a complex mixture of nutrients, hormones, signaling molecules, and extracellular vesicles (EVs) [1–3,8]. Ciliary action and peristaltic movements synchronize gamete transport and provide mechanical and biochemical cues essential for fertilization.

Limitations of Current IVP Systems

Conventional IVP systems use static media and flat culture plates that lack spatial structure and dynamic regulation. *In vitro* sperm selection does not reflect the UTJ filtering, where only capacitated sperm expressing specific surface markers like ADAM3 can proceed [4–7]. Fertilization and early development take place in a chemically defined, hormone-free environment, devoid of peristaltic stimulation, directional fluid flow, or gamete-driven signaling [1,8]. These deficiencies contribute to a mismatch between embryonic needs and environmental support, impairing development and increasing the risk of abnormal gene expression.

Emerging Biomimetic Approaches

To resembles *in vivo* conditions, several biomimetic strategies have been tested. Reproductive fluids like OF and uterine fluid (UF), when added in low concentrations to culture media, can enhance embryo development by offering biochemical and epigenetic support [9,10]. These fluids contain EVs that act as carriers of proteins, mRNA, and microRNA, facilitating intercellular communication between the maternal environment and the embryo [11]. Three-dimensional (3D) culture systems provide a better mimic of the oviductal structure and function: they preserve epithelial polarity, ciliary function, and secretory capacity [8,13,14]. Mechanical stimulation (tilting platforms, vibration, and microfluidic flow) more closely imitates the physical forces embryos experience during their passage through the oviduct [12]. Microfluidic devices allow for continuous media renewal and gradient formation, simulating hormone fluctuations and gamete chemotaxis. Moreover, emerging platforms allow the co-culture of embryos with EV-producing oviductal epithelial cells, enabling real-time communication and feedback.

Conclusion

Replicating the oviductal microenvironment in IVP systems is a necessity for improving embryo quality and minimizing the developmental side effects. Despite significant advances, we are still far from the complete replication of the oviductal environment. Future strategies should combine 3D architecture, reproductive fluid supplementation, real-time dynamic modulation and dynamic mechanical stimulation in bringing IVP closer to nature. In parallel, advances in artificial intelligence, genomic selection, and microengineering are poised

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to further refine embryo assessment and optimize culture conditions. Continued interdisciplinary innovation will be key to translating biomimicry into scalable and clinically relevant tools.

106- EVALUATION OF SCROTAL CIRCUMFERENCE, BODY WEIGHT AND METABOLIC MARKERS AS PREDICTORS OF SEXUAL PRECOCITY IN LACAUNE RAM LAMBS

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Introduction

The combination of scrotal circumference (SC) with biochemical and hormonal parameters has proven effective in predicting early sexual development in ram lambs. SC correlates with body weight (BW) and puberty onset across other breeds, serving as a reliable indicator of reproductive potential (1–3). Key reproductive hormones (testosterone, FSH, and LH) increase during puberty, reflecting testicular activation (4). Additionally, IGF-I is linked to testicular growth and sexual precocity (5). Thus, integrating SC with endocrine and metabolic biomarkers provides a non-invasive and cost-effective method for predicting puberty onset, enhancing early sire selection and reproductive efficiency.

Objective

This study aimed to evaluate the SC, BW and selected blood biochemical parameters in Lacaune ram lambs as biomarkers of sexual precocity, early in life, contributing to more efficient sire selection strategies in sheep production systems.

Materials and methods

Twelve Lacaune ram lambs were housed at the experimental farm of the INIA (CSIC, Madrid) at the age of 3 mo. At 6 mo. of age, the animals underwent biweekly training sessions to acclimatize to semen collection with artificial vagina. Procedures followed animal welfare guidelines (PROEX 330.1/24). Rams were classified in two groups based on their precocity in ejaculating: Precocious Rams (PreR; n=3) and Delayed Rams (DelR; n=9). Measurements were taken at three time points: Time1 at the beginning of the training at days 174±8d of age, Time2 at 244±8d old and Time3 at 279±8d old. SC (in) was measured with a scrotometer, BW (kg) was recorded using a scale and blood samples were taken from the jugular vein, plasma separated by centrifugation and stored at -80 °C until analysis. Total cholesterol (TC, mg/dL), triglycerides (TG, mg/dL), high- (HDL, mg/dL) and low-density lipoprotein cholesterol (LDL, mg/dL), glucose (GLU, mg/dL), fructosamine (FRU, mg/dL), lactate (LAC, mg/dL), β-hydroxybutyrate (BHB, mg/dL), non-esterified-fatty acids (NEFA mmol/L), proteins (g/dL), lactate dehydrogenase (LDH-LQ, U/L), alanine transaminase (ALT, U/L), aspartate aminotransferase (AST, U/L), creatine kinase (CK-NAC; U/L), albumin (g/dL) and urea (UR, mg/dL) were assessed with clinical chemistry bioanalyzer (Konelab 20; Thermo Fisher Scientific, Waltham, MA, USA). Statistical comparisons between groups (PreR vs. DelR) were made using Student's t-test, with SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA).

Results

The earlier-maturing lambs (PreR) weighted numerically more at all three time points, although differences between groups were not significant ($P > 0.05$), as was also observed for SC up to Time2 (244d old). At Time1 (174d old), HDL levels were lower in PreR rams than in those ejaculating later (PreR: 20.33 ± 6.59 vs. DelR: 37.77 ± 3.80 mg/dL; $P = 0.0449$). At Time2, TG tended to be lower in PreR (18.66 ± 4.51 vs. 28.37 ± 2.76 mg/dL; $P = 0.0999$). At Time2, TC was also significantly lower in the precocious group (PreR: 55.0 ± 6.5 vs. DelR: 82.11 ± 3.75 mg/dL; $P = 0.0048$) and at Time3 (279d old) showed a trend toward significance (PreR: 59.00 ± 6.48 vs. DelR: 73.77 ± 3.74 mg/dL; $P = 0.0767$).

Conclusion

BW and SC were higher in the more precocious males; however, these parameters alone may not be sufficient for reliable selection based on sexual precocity. The observed differences in HDL early in life and in TC and TG later on, highlight the important role of lipid metabolism in sexual maturation. These findings support the integration of biochemical biomarkers with traditional morphometric measurements to enhance the accuracy of early puberty prediction in rams.

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107- VITAMIN D RECEPTOR AND VITAMIN D-METABOLIZING ENZYMES IN TESTIS OF BUCK AFFECTED BY TESTICULAR LITHIASIS

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Introduction

The reproductive efficiency of the goat is frequently decreased by testicular lithiasis (McEntee, 1990) that affects 50% of the old subjects in one or both testis (Gimbo et al., 1982). The etiology is still not well defined even if it seems to be related to an excess of group D provitamins added to their food. Testicular microlithiasis has been also found in men with a negative impact on reproduction and bone metabolism (Foresta et al., 2011). The testis has an important role in bone metabolism because expresses the vitamin D receptor (VDR), CYP2R1 and CYP27A1 the metabolizing enzymes required in the first step of the vitamin D activation. In the sheep VDR and the two enzymes are expressed by testis and sperm cells and are related with age and sperm motility (Yao et al., 2018).

Objective

The objective of this research is a) to verify the expression of VDR, CYP2R1 and CYP27A1 also in the buck testis, b) to evaluate the effect of lithiasis on their testicular expression, c) to evaluate histological lesions associated to lithiasis.

Materials and methods

In the study, bucks living in the Apulia region (age 6±2yrs) were employed. Following the andrological examination and testicular ultrasound scan, 24 subjects were selected, 12 of whom had testicular lithiasis. Testis were recovered at the local abattoir after their slaughter. From each testis slices were cut and immediately frozen in liquid nitrogen for their storage, while the rest of the testis was 10% formalin fixed and routinely processed for histological examination. Proteins were extracted from frozen samples by conventional protocols and analyzed by the western blot procedure employing anti VDR, CYP2R1 and CYP27A1 as primary antibodies (Santa Cruz Biotechnology, USA) and a biotinylated secondary antibody. Positive signals were revealed by DAB substrate kit (Vector-Laboratories, USA). The expression of each protein was related to β -actin level. Obtained signals were scanned and quantified by density contrast ($OD = \text{contour} \times \text{mm}^2$) using Gel-Doc and Quantity One software (BioRAD, Italy). Data were analyzed for statistical significance by Student's *T*-test.

Results

Testicular lithiasis was diagnosed by ultrasound examination evidencing small non-shadowing hyperechoic foci located in the testicular parenchyma. We found that the buck testis expresses VDR and the two vitamin D metabolizing enzymes. The expression of CYP2R1 was lower in the testis affected by lithiasis compared to controls ($P < 0,001$), while the expression level of CYP27A1 and VDR was only slightly decreased in the affected testis ($P > 0,05$). Histological examination of the testis revealed the presence of amorphous basophilic material and high degree of fibrosis in intratubular spaces.

Conclusion

This is a preliminary study to evaluate the impact of testicular lithiasis on the reproductive physiology of the buck, because in men the condition has been linked to both infertility and an increased testicular cancer and to bone metabolism. The reduced expression level of CYP2R1, the vitamin-D-metabolizing enzyme mainly involved in the activation of the vitamin let us to suppose an impact on calcium metabolism. For this reason, the evaluation of calcium, vitamin D and parathormone concentrations are under investigation.

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108- QUANTITATIVE AND QUALITATIVE EVALUATION OF URINE PROTEIN IN PERIPARTUM GOATS

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Introduction

The sustainability and adaptability of goat farming have led to increased research on goat health status using affordable, non-invasive tests, such as urinalysis. Urine analysis offers valuable insights into metabolic functions, disease presence, and nutritional status without causing stress to the animals. In this context, proteomics—the study of all proteins expressed by an organism—is increasingly applied to urine samples in animal research, providing a deeper understanding of physiological and pathological processes. However, while bovine and porcine proteomes are well-studied, proteomic research in sheep and goats remains limited and underexplored. As goat farming expands globally, especially in climate-challenged regions, such diagnostic tools are essential for improving herd management and productivity.

Objective

This preliminary study investigated changes in goat urine chemistry and protein profiles delivery, aiming to explore potential biomarkers associated with key physiological and pathological changes during the peripartum period.

Materials and Methods

This study involved 10 healthy Alpine does, aged 2–9 years, housed at the University of Bologna's Teaching Farm between January and March 2024. The animals were managed under standard farming conditions, with a controlled diet during late pregnancy and lactation.

Urine collection was performed by spontaneous voiding at three time points: about 22 days before delivery (T0), 7 days postpartum (T7), and 30 days postpartum (T30). Urine samples were visually inspected, centrifuged, and analysed for specific gravity using a refractometer. A semiquantitative dipstick test was used to assess chemical parameters, and sediment was examined microscopically. Total protein and creatinine concentrations were then measured using commercial kits.

Urine proteins were separated using one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (1D-SDS-PAGE).

Statistical analysis included the Shapiro-Wilk test for normality, followed by repeated measures ANOVA or Friedman test for comparing variables across time points, with significance set at $p < 0.05$.

Results

All animals stayed healthy with mostly normal urine characteristics; dipstick tests showed protein in the majority of samples but no significant changes over time for most parameters. Microscopic examination confirmed no urinary tract inflammation or infection, detecting only occasional red blood cells in some samples.

Statistical tests showed significant changes: urine pH decreased at T30, urine protein-to-creatinine ratio (UPC) increased at T7 and T30, and urinary creatinine was highest at T0.

UPC values were higher at T7 and T30 compared to pregnancy (T0), with median values exceeding healthy ruminant reference intervals, but only T30 indicated pathological proteinuria. This increase may be due to higher glomerular filtration, membrane permeability, or tubular proteinuria, potentially influenced by the high prevalence of twin pregnancies. The rise in UPC could also result from decreased urinary creatinine at T30 rather than increased protein excretion, as total urinary protein remained stable. Similar postpartum decreases in creatinine have been seen in cattle, but further research is needed to confirm this in peripartum goats.

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In the absence of a reference goat urinary proteome, some protein bands were tentatively identified by molecular weight, including transferrin (70 kDa), albumin (62 kDa), and immunoglobulin chains (55 and 25 kDa). Pregnancy-associated glycoproteins (PAGs), known to fluctuate during pregnancy and postpartum, may explain variations in band intensity but have not yet been confirmed in urine. Although not significant, higher protein bands between 23 and 42 kDa at T0 might correspond to proteins like haptoglobin (37 kDa) and cathelicidin (14–25 kDa), suggesting changes in urinary protein patterns related to pregnancy stages.

Conclusion

This study examined changes in goat urinary protein excretion during late pregnancy and postpartum, revealing important adaptive processes that may parallel those in humans. Although further research with larger samples and detailed proteomic analysis is needed, goats' resilience in challenging environments and their role in sustainable farming highlight their value both as a model for human health and a key species in low-impact agriculture.

109- THE USE OF QUADRATUS LUMBORUM (QL) BLOCK FOR ANALGESIA MANAGEMENT IN TIBETAN GOAT UNDERGOING EMERGENCY CESAREAN SECTION: A CASE REPORT

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Introduction

Pain management remains a significant challenge in both human and veterinary medicine. The benefits of loco-regional anaesthesia include reduced perioperative use of systemic drugs and fewer associated side effects. In livestock, loco-regional anaesthesia has traditionally been performed using blind techniques, such as neuraxial blocks, without the aid of advanced tools, such as nerve stimulators or ultrasound (US) guidance. In contrast, US-guided loco-regional techniques are becoming increasingly commonplace in the treatment of small animals thanks to their precision and safety. Over the past decade, there has been a growing interest in interfascial (IF) regional anaesthesia techniques. One such technique is the quadratus lumborum (QL) block, which involves injecting a local anaesthetic between the quadratus lumborum and psoas minor muscles. While the QL block is well documented in dogs and cats, particularly during ovariectomies or ovariohysterectomies, there are currently no published reports on its use in caesarean sections in goats, as far as the authors are aware. This case report describes the use of a QL block at the L1 level to manage perioperative analgesia in a Tibetan goat undergoing an emergency caesarean section.

Objective

To evaluate the feasibility and clinical analgesic effects of a unilateral (left flank/hemiabdomen) QL block in a Tibetan goat presenting with dystocia and undergoing emergency cesarean section.

Materials and Methods

A nine-month-old, 29.7 kg female Tibetan goat was referred to the Veterinary Teaching Hospital at the University of Teramo for dystocia following over ten hours of labour. On presentation, the goat exhibited signs of agitation and hyperexcitability. Other observations included a heart rate of 160 bpm, a respiratory rate of 52 bpm, pink mucous membranes with a delayed capillary refill time, a rectal temperature of 37.5 °C, normal hydration and lymph nodes, and signs of abdominal pain. The cardiopulmonary examination revealed no abnormalities for a pregnant animal. Digital vaginal examination revealed no fetal presence in the birth canal. Radiography and fetal abdominal ultrasound were performed to determine the number of fetuses, their presentation, their viability, and their circulating volume. The radiography identified a single fetus in the breech position, which was incompatible with natural delivery. This prompted immediate preparation for an emergency caesarean section. The fetal heart rate was 180 bpm on ultrasound. Preoperative blood analysis showed only mild metabolic acidosis and normo-glycaemia. Following informed consent, the goat was premedicated intramuscularly with midazolam (0.5 mg/kg). Twenty minutes later, a jugular catheter was placed and anaesthesia was induced with alfaxalone (total amount 3.5 mg/kg) (10 mg/kg, Baxter, Jurox) intravenously (IV) to achieve an appropriate plane for orotracheal intubation. Anaesthesia was then maintained using isoflurane in an air-oxygen mixture, delivered via a circular rebreathing system. A gastric tube was inserted to decompress the rumen and prevent tympany. Amoxicillin 15 mg/kg was administered IM (Zoobiotic, 100 mg/ml, Carlier S.p.A., Milan). Invasive monitoring of CO₂, ECG, heart rate (HR), oxygen saturation (SpO₂), respiratory rate (fR) and systolic, mean and diastolic arterial pressures (SAP, MAP and DAP) was performed via an aseptically placed arterial catheter in the temporal artery. The goat was positioned in sternal recumbency and the area caudal to the last rib at L1–L2 was clipped and scrubbed surgically. A high-frequency (7–12 MHz) linear ultrasound probe (LA523, Esaote) was positioned parallel to the last rib at the L2 transverse process using a latero-ventral in-plane approach. A 50 mm echogenic needle (Stimuplex, B. Braun) was then advanced in-plane dorsocranially into the interfascial plane between the QL and the Pm. After negative aspiration and a 0.3 mL test injection to confirm hydrodissection, 0.3 mL/kg of 0.5% ropivacaine and 0.1 mL/kg of 2% lidocaine were injected (a total of 12 mL, 0.4 mL/kg). Five minutes later, the goat was rotated 30° to the right to expose the surgical flank. After clipping and scrubbing the area, towel clamps were applied and an incision was made in the left paralumbar fossa. Analgesic efficacy was assessed by monitoring heart rate (HR), respiratory rate (fR) and systolic arterial pressure (SAP). An increase

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of $\geq 30\%$ from baseline in any of these parameters triggered the administration of 1 mg/kg of IV ketamine as rescue analgesia. No rescue boluses were required during surgery. Following fetal delivery, increases in HR and fR of over 30% occurred during skin closure; however, the anaesthetist deemed these transient and did not administer rescue analgesia. The goat was extubated seven minutes after surgery, remained in the sternal position with the gastric tube in place until it was tolerated, and stood on all fours after 70 minutes. Once her body temperature returned to 37 °C, meloxicam (0.4 mg/kg) was administered intravenously. The kid was resuscitated in accordance with the guidelines, received sublingual flumazenil and began breathing spontaneously within three minutes. The neonate presented with a left flexural limb deformity, likely caused by a carpal fracture, which was the probable cause of dystocia. However, the neonate had a high Apgar score, was alert and vital, and nursed within 25–30 minutes. Both the goat and the kid were discharged the following day without complications.

Conclusion

A QL block performed at L1 in a pregnant Tibetan goat undergoing caesarean section was found to be both feasible and effective. Using the latero-ventral in-plane approach with a linear ultrasound probe described for dogs and cats, this technique was successfully adapted for use on a goat in sternal recumbency for the first time, as far as the authors are aware. This position avoids lateral recumbency and potential haemodynamic instability. The technique may mitigate hypotensive events commonly associated with epidural anaesthesia. Further investigation and clinical trials are warranted.

110- ASSESSMENT OF THE INNOCUITY AND EFFICACY OF TWO COMMERCIAL IBR MARKER VACCINES AGAINST BUBALINE ALPHAHERPESVIRUS 1 (BUAHV-1) IN WATER BUFFALO (*BUBALUS BUBALIS*)

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Introduction

Bubaline Alphaherpesvirus 1 (BuAHV-1) is a pathogen that can cause respiratory infections and other complications in buffaloes and calves, leading to significant economic impacts. To combat this infection, the vaccination strategy is a fundamental measure for disease control. However, to date no commercial BuAHV-1 vaccines are available. In this study, we hypothesised that different IBR marker vaccines registered for cattle and against BoAHV-1 could protect buffaloes against BuAHV-1. To test this hypothesis, we immunised several groups of buffaloes with different IBR marker vaccines and subsequently experimentally infected them with BuAHV-1 to assess the safety and efficacy of the vaccines tested. Objective

The objective of this study is to evaluate the innocuity and efficacy of two commercial IBR marker vaccines in water buffaloes.

Materials and Methods

Animal Selection and Grouping

Thirteen buffaloes seronegative for both BuAHV-1 and BoAHV-1 were selected and divided into three groups: **VAX-1** (live gE-/tk- vaccine), **VAX-2** (inactivated gE- vaccine), and **CNT** (control). Each animal received two 2 mL injections 21 days apart. Thirty-four days after the second vaccination (PVD 34), all animals were challenged with the wild-type (wt) BuAHV-1 virus. Nasal mucus and serum samples were collected at different intervals for virological, serological, and immunological analyses.

Results

Only the control group exhibited nasal secretions and an increase in rectal temperature, both clinical signs of infection, as observed in PCD 7. PCR detected BoAHV-1 gB between PCD 2 and 7 in the vaccinated groups, while in the controls, positivity persisted until PCD 15. Neutralizing antibody titers progressively increased in the vaccinated groups starting from PVD 34, and reached significant levels by the end of the study. In contrast, in the controls, they appeared later, at PCD 10.

Conclusions

In conclusion, the results of this study support the continuation of further in-depth research into the buffalo immune system. The data demonstrate that vaccination with IBR marker vaccines (gE-/tk-; gE-), authorized for cattle, is innocuous and efficacious in water buffaloes. These findings represent an important preliminary step toward the development of an IBR control program in Italy. However, additional large-scale field studies are required to confirm these results before the vaccines can be officially implemented in this species.

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111- SCIENTIFIC AND TECHNICAL OFFICE OF REMESA (STOR) (HISTORY AND TASKS)

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Introduction

At the 18th meeting of the Joint Permanent Committee (JPC) of REMESA (REseau MEditerranéen de Santé Animale) (June 2018) a resolution was adopted to establish the Scientific and Technical Office of REMESA (STOR), containing details on its organization and objectives. The STOR is an international initiative created to facilitate and strengthen communication among REMESA member countries, with the aim of implementing a strong network to contrast emerging animal health threats in the Mediterranean area. The STOR is an extension of the REMESA Co-Secretariat (WOAH/FAO) and works under its supervision and coordination. The STOR is located and hosted at the Istituto Zooprofilattico Sperimentale (IZS) of Sicily in Palermo (Italy).

Aims and tasks

STOR was created to pursue the following objectives:

- To conduct various technical initiatives to develop and strengthen the role of REMESA;
- To facilitate cooperation between Research Centers, Institutes, Laboratories and Universities and to build partnerships under the coordination of the co-secretariat;
- To strengthen and coordinate scientific programs and measures to effectively prevent and control Transboundary Animal Diseases (TAD) and Zoonoses (One Health) through the establishment of scientific consortia, knowledge and capacity transfer;
- To map needs in order to prioritize activities;
- To share expertise and best practices of control strategies and emergency response plans;
- To provide multi-annual strategic work plans/roadmaps;
- To ensure the management of the STOR website for all REMESA-related activities;
- To develop the capacity to design and manage competitive research projects and implement fundraising strategies to ensure the necessary financial resources;
- To support REMESA's activities through the Scientific and Technical Committee (STC) of REMESA, composed of experts from the member countries;
- To cooperate with the regional thematic sub-networks: REPIVET, RELABSA, RECOMSA and RESEPSA.

Conclusion

Major tasks of STOR concern Veterinary strategies in the Mediterranean region promoting "One Health" approach. Through its experts STORemesa boosts scientific activities, promotes cooperation and initiatives between member countries, with the aim of improving Public Veterinary Health and safe animal husbandry and trades in the Region.

112- HEALTH EDUCATION: A SMALL EXPERIENCE IN MOZAMBIQUE

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Introduction

Italian Veterinary Public Health has a recognized efficiency in the inspection sector, in particular at the slaughter level. In the context of a cooperation activity in Mozambique that brought us into contact with small cattle slaughtering activities we were able to note the poor attention observed by local technician during inspection procedures. With the aim of improving the level of healthiness of the final product for consumers and reporting the main bovine diseases we have prepared a very simple poster to be posted in slaughterhouses, schools and public offices in the villages from where the animals sent to slaughter came. The poster was written in Portuguese, the official language of Mozambique, and enriched with images that could help inspectors and, at the same time, spark debate among farmers and the population. The poster received praise from European Community inspectors.



113- VIRTUAL SLAUGHTERHOUSE SIMULATOR: INNOVATING MEAT PRODUCTION AND FOOD SAFETY TRAINING

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Introduction

Slaughterhouses are critical for early detection of food safety, animal health, and welfare issues, making veterinarians and slaughterhouse professionals essential for the protection of public and animal health.

A multidisciplinary approach must be taken by veterinarians to conduct high-quality inspections and audits, which requires them to have knowledge about live animals, production processes, infectious diseases, food safety, and welfare. Therefore, it is important for veterinary medicine students to be adequately trained with theoretical and practical skills.

It is essential to provide practical training in slaughterhouses, enabling students to understand the slaughtering process, identify and manage risks related to food safety, animal health, and welfare, and develop skills to perform official controls.

Due to closures, biosecurity restrictions, and further disruptions caused by the COVID-19 pandemic, the access to the facilities for students has been limited. To address these challenges, a bovine Virtual Slaughterhouse Simulator (VSS) was developed as an interactive tool to support student training in slaughterhouse procedures and official controls.

Objective

The simulator aims to give veterinary students a realistic understanding of slaughterhouse operations and prepare them for practical official control tasks. While it cannot replace hands-on experience, it complements onsite training by familiarising students with procedures and optimising their learning time in real slaughterhouses.

Materials and methods

The bovine VSS is a three-dimensional, real-time training tool designed to enhance veterinary education in meat hygiene and food safety. Developed using dynamic building modelling software, the simulator provides students with access to a realistic beef abattoir environment where they can safely perform ante-mortem, post-mortem, and auditing procedures.

The VSS incorporates 34 authentic case-based scenarios, enabling learners to apply critical thinking, solve problems, and integrate knowledge of food safety, animal health, and animal welfare legislation.

The platform also provides links to electronic resources, including pathology images, disease examples, process documentation, and relevant legislation, supporting self-directed learning. Accessible via PC, tablet, or mobile VR, the bovine VSS represents an innovative educational tool that complements practical slaughterhouse training and better prepares veterinary students for professional practice.

Results

The bovine VSS enhanced veterinary education by providing a realistic and interactive training environment for slaughterhouse procedures and official controls (Seguino et al., 2014). The VSS has been expanded through a multidisciplinary approach under the Una Europa Seed Funding umbrella, improving content quality, raising awareness, and attracting additional resources, and has been adopted by many veterinary schools in Europe and beyond. It has proven effective in supplementing practical training, especially during COVID-19 restrictions as VSS was recognised as an alternative to real abattoir visits (Garcia-Ara et al., 2023).

Conclusion

There is a growing need for high-quality, interactive tools to train veterinary students and professionals in slaughterhouse procedures. The bovine VSS demonstrates processes, equipment, and legal requirements related to hygiene, animal health, and welfare, helping students visualise and integrate complex operations with legislation. By complementing hands-on experience, the VSS enhances teaching quality, supports skill development in food safety and welfare, and promotes a harmonized approach to official controls, facilitating professional mobility across Europe. Ongoing developments aim to expand the simulator to other animal species and adapt it to different national regulations, making it a versatile and widely accessible educational resource.

114- A RETROSPECTIVE POST-MORTEM STUDY OF GROSS PATHOLOGICAL AND MICROBIOLOGICAL FINDINGS IN THE ABOMASUM OF SHEEP AND GOAT

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Introduction

Abomasal diseases in sheep and goats are an important but often overlooked issue. The knowledge of the diffusion of certain diseases and etiological agents in a specific territory (Umbria region, central Italy) can help practitioner to address diagnostic investigations and diagnosis, underlying the role of the necropsy room as an epidemiologic observatory.

Objective

The aim of this study is to describe the anatomopathological and microbiological findings from the abomasum of sheep and goats in the period 2019 and 2024. This study ultimately aims to increase the knowledge about abomasal pathology and its microbiological counterpart in Umbria (central Italy) region.

Materials and methods

Necroscopic reports of ovi-caprine necropsies from two institutions were reviewed with a search of the key words “abomasum” or “abomasal” or “gastric” or “abomasitis”; the records have been checked for the presence of microbiological investigations (routine bacteriology followed in some cases by MALDI/TOF) and the results recorded. Furtherly, the pathological diagnoses have been clustered in categories and compared with microbiological results.

Results

A total of 1374 ovine and 456 caprine necropsies, coming from Umbrian farms, have been reviewed, excluding all the cases of abortion or stillbirth. Among them, 394 animals were reported to have abomasal lesions at post mortem (291 ovine and 103 caprine); finally, the enrollment criteria (abomasal lesion + bacteriology) was fulfilled in 162 cases (120 ovine and 42 caprine cases). In sheep, the most common pathological finding was abomasitis with different pattern: hemorrhagic (characterized by edema with either petechial or more diffuse intraparietal hemorrhage: 41 cases), acute (hyperemia and edema: 22 cases), catarrhal (hyperemia with yellowish exudate and eventually parietal hyperplasia: 17 cases); ulcerative (with distinct ulcers: 11 cases); abomasal meteorism, parasitism and pure hemorrhages without inflammation (cases) and were less common (14, 10 and 3 cases respectively); rarely found were constipation and nodular hyperplasia (1 case each). From a bacteriological perspective, most common isolate was *E. coli*, that was the only isolated bacteria in 50 cases, in mixed infections in 34 cases, mostly associated with clostridia (31 cases). *Clostridium* sp. was quite common, both as the sole species (15 cases) or in co-infection with other bacteria (33 cases); at a species level *C.perfringens* and *P.sordellii* were found. Association of these bacteria to a pathological pattern of lesion is not evident; clostridia were isolated in 14/41 (34%) cases of hemorrhagic and 5/10 (50%) ulcerative abomasitis, while 33 of the remaining 79 cases (41%), including 5/11 cases of abomasal parasitism, tested positive. Two non-inflammatory cases are noteworthy, specifically a case of intraparietal hematoma associated to intraluminal fatal hemorrhage with unknown cause, and a case of severe diffuse abomasal impaction. In caprine species catarrhal (10 cases), acute (9 cases), ulcerative (5 cases) and hemorrhagic (3 cases) abomasitis were observed. Abomasal meteorism (5 cases) is as common than in ovine, and rare cases of laceration (1 case) and impaction associated with foreign body (trichobezoar: 3 cases) were diagnosed; parasitism occurred in 4/42 cases. Microbiology results mirror those detailed for ovine: *E. coli* was the most common isolated bacterium, as the sole species in 12 cases, in mixed infections in 8 cases. Notably, in 3 cases *E.coli* was associated with *Streptococcus gallolyticus*, a bacterium that has been associated to the occurrence of human colonic carcinoma.

Conclusion

Abomasal lesions are quite common both in sheep (291/1374: 21%) and goat (103/456: 23%), even if caprine lesions appears to be less severe compared to ovine. The association between specific gross pattern and bacterial species remain unclear, and the causative role of isolates must be interpreted with caution.

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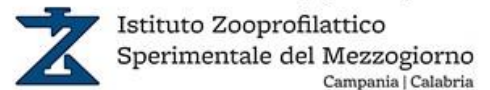
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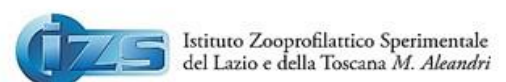
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FORMACIÓN ACADÉMICA

PhD – Environmental Science. Università di Camerino, Italia (2013).

Magíster en Estadística Aplicada. Universidad Nacional de Córdoba (2010).

Ingeniera Agrónoma. Universidad Católica de Córdoba (1990).

CARGOS DOCENTES

Profesor Adjunto (DSE)– Mejoramiento Animal. Facultad de Ciencias Agropecuarias – UNC (por concurso).

Profesor Adjunto (DS)– Mejoramiento Animal / Metodología de la Investigación. UNVM. (por concurso).

Profesor Asistente – Bioestadística I y II. UNC (2019–2023).

INVESTIGACIÓN

Categoría III – Programa de Incentivos (Res. 1854/17).

Programas UNC: Estadística Aplicada y Aprendizaje Automático (2019–2022).

Proyecto: Análisis de datos en lechería de precisión (2022–2023).

Assegno di Ricerca – Università di Bari (2023–2024; 2025–2026).

Miembro Investigador – International Dairy Research Program (UNR, 2024–presente).

LÍNEAS DE TRABAJO

- Genética molecular y diversidad genética en poblaciones animales.
- Análisis genómico (SNP, PLINK, imputación).
- Producción lechera sostenible y adaptación al cambio climático.
- Modelos estadísticos avanzados y análisis multivariado.

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PRODUCCIÓN CIENTÍFICA DESTACADA

Más de 20 publicaciones en revistas indexadas (Journal of Dairy Science, Livestock Science, Sustainability, etc.). En 2025:

Topputi R, Molina MG, Ventriglia G, Quiroz Valiente J, Ramirez Guillermo MA, Bruno S, Ciani E, Guerra L, Cesarani A, Landi V and Martinello T (2026) Environmentally independent histological markers of wool quality: a comparative study of Gentile di Puglia and Sarda breeds. Front. Anim. Sci. 6:1670137. doi: 10.3389/fanim.2025.1670137

Presentaciones en congresos internacionales (ASPA, EAAP, FEMESPRUM). En el 2025:

Molina, M. G., Bruno, S., Marino, A., Gatta, G., Albenzio, M., & Ciani, E.

Genetic characterization of local sheep and goat populations in Apulia within the frame of the regional project L.O.C.A.L.

International Congress on Biodiversity for Sustainable Development, Perugia, Italy, 2025.

Poster presentation. Book of Abstracts, p. 161. ISBN: 978-88-98139-58-7

(Caracterización del patrón folicular y densidad de fibra de lana en la raza ovina Gentile di Puglia.)

Molina, M. G., Bruno, S., Albenzio, M., Landi, V., De Palo, P., & Ciani, E.

Joint efforts for sustainable resilient farming of local Apulian sheep and goat populations: the contribution of the “LOCAL”, “Agritech”, “BioZooCare” and “WOOLLY” projects

FEMESPRUM International Congress, Bari, Italy, 2024.

Poster presentation. Abstract Book, p. 84. ISBN: 978-88-944687-4-9

(Esfuerzos conjuntos para una agricultura sostenible y resiliente de las poblaciones locales de ovejas y cabras de Apulia.)

Molina, M. G., Bruno, S., Tarricone, S., Landi, V., Ragni, M., Giannico, F., Rillo, L., Matassino, D., Crepaldi, P., Marsico, G., & Ciani, E.

Investigating the origins of the “Murciunara” goat: genetic insights from the ValBiOviCap project

FEMESPRUM International Congress, Bari, Italy, 2024.

Poster presentation. Abstract Book, p. 85. ISBN: 978-88-944687-4-9

(Investigación sobre los orígenes de la cabra “Murciunara”: información genética del proyecto ValBiOviCap.)

Molina, M. G., Bruno, S., Albenzio, M., Landi, V., De Palo, P., & Ciani, E.

Development of a SNP parentage panel for Apulian autochthonous sheep breeds within the frame of the L.O.C.A.L. project

FEMESPRUM International Congress, Bari, Italy, 2024.

Poster presentation. Abstract Book, p. 94. **ISBN: 978-88-944687-4-9**

(Desarrollo de un panel de SNPs para verificación de parentesco en razas ovinas autóctonas de Apulia, en el marco del proyecto LOCAL.)

Molina, M. G., Bruno, S., & Ciani, E.

Selection of a dromedary SNP parentage panel

FEMESPRUM International Congress, Bari, Italy, 2024.

Poster presentation. Abstract Book, p. 92. **ISBN: 978-88-944687-4-9**

(Selección de un panel de SNPs para verificación de parentesco en dromedarios.)

Molina, M. G., Bruno, S., Meghelli, I., Ciani, E., Sharaf, A., & Gaouar, S. B. S.

Unraveling genetic determinants of trypanosome infection in Algerian dromedaries

FEMESPRUM International Congress, Bari, Italy, 2024.

Poster presentation. Abstract Book, p. 93. **ISBN: 978-88-944687-4-9**

(Determinantes genéticos de la infección por tripanosomas en dromedarios argelinos.)

Molina, M. G.

Invited scientific meetings on genetic conservation programs.

Dipartimento Agricoltura, Ambiente e Alimenti, Università del Molise, Campobasso, Italy, October 29–30, 2025.

Participation in scientific discussion sessions within the framework of the PSR Puglia – LOCAL project collaboration.

ESTANCIAS INTERNACIONALES

Universidad de Lleida – España (2019, 2023, 2025).

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Università di Bari – Italia (Assegni di Ricerca 2023, 2025).

Universidad de Córdoba – España (Posdoctorado AUIP, 2021).

IDIOMAS Y COMPETENCIAS

Italiano: C2 | Inglés: B1 (en perfeccionamiento).

Software: R, SAS, Python, SPSS, BLUPF90, PLINK, Linux.



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