ABSTRACT BOOK

Mediterranean Federation
Health and Production of Ruminants



Zanhotel Europa, Via Boldrini 11, Bologna, Italy

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1- CHALLENGES FOR RUMINANT TICKS AND TICK-BORNE DISEASES CONTROL IN THE MEDITERRANEAN REGION

I. LAFRI

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Vector-borne diseases (VBDs) are widely distributed across the globe. The majority of these diseases, classified as metazoonoses, 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 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

F. ROSSO

European Commission for the control of foot-an-mouth disease

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

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 buffalos 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 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.

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, antinflammatory) and the problem of residues in food of animal origin that is highly desirable from a one-health point of view.

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 contest 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, particullary 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 Zooprophylactic 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

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 (*f*R), systolic, mean and diastolic blood pressure (SAP, MAP and DAP) in a non-invasive manner (NIBP), and oxygen saturation (SpO_2). 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 5ºC and 25ºC, 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 (ºC) 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 (PierceTM 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 27.76ºC, compared with 15.23ºC without heat stress, while the mean humidity was 77.69% and 61.91%, respectively. So high T with high HR 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 μM/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 corelation was observed.

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 Methos

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 THI ≥ 72 showed a lower pregnancy rate (29%) compared to cows exposed to 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≤THI≤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

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-arachidonylethanolamide (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±9,82) than in summer (16,77±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 od 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 statisticaly relevant correlations between presence od *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 %).

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 analized in Dessie University by Mini Flotac Technique using flotation solution at 1350 S G.
6. This tool allows to work quikly 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 from100 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 tha Flotac thecnique.
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.

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* (Dessì 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 (χ^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.

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.thysaniesia* and *Fasciola Hepatica*. On the other hand, serum induced by band 27 KDa antigen reacted with antigens of *T. thysaniesia* 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 ≤ 25% or when their CL area was < 2 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 (ΔBCS) 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 neuroparalytic 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 (toxinfection 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.

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 (T_1, T_2, T_3, T_4, T_5, T_6 respectively) post-treatment, followed by reassessments of 15 days and 5 months to evaluate recovery and productivity.

Results

On arrival to the farm (T_0), 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, panhyperbilirrubinemia (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 T_0, 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 T_0 to 16% at T_1 (3 hours post-treatment) and eventually reached 0% at T_6 (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.

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.

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 haemorrhage's 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.

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.

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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 Dairylac 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 claves 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.

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 2^{nd} and 3^{rd} seem differed slightly, although they do differ significantly from the control group while the group 4^{th} 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 =number of days with diarrhea × 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 tpercentage deviation (PD%), calculated as PD% = ((tk - t0)/t0) × 100, representing the relative change from day 0; and maximum permissible instability (MPI), calculated as MPI = 0.5 × (CVa + CVi), where CVa is the analytical coefficient of variation and CVi 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).

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 helped to know the epidemiology of helminth infection in small ruminants thorough 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 seansonal trend of gastrointestinal nematodes in extensive and semi-extensive sheep farming systems. Understanding how climate and management strategies influence GIN infections and their tren 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 × 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 acidic 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.

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 $\sim 2\times10^{-14}$ per random portion for adults. In contrast, the risk of STEC infection has been estimated at an average of $\sim 4\times10^{-4}$ 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

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.

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 CO_2 eq. (0.91-5.84); terrestrial acidification 9.76E-03 kg SO_2 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 m^2a crop eq. (0.42-3.91); fossil resource scarcity 0.14 kg oil eq. (0.03-0.94); water consumption 0.09 m^3 (0.01-0.25). The main contributors to GWP (kg CO_2 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.

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-CO_2 sources, with methane (CH_4) representing approximately 80% of emissions from livestock. The CH_4 is the major non-CO_2 greenhouse gas produced in the rumen. Metabolomics analyses have shown that ketotic cows possess higher levels of CH_4 precursors, indicating a potential connection between metabolic disorders and CH_4 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 ± 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 ≥ 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 CH_4, CO_2, and H_2 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.

Regarding GHG emissions, KET cows emitted significantly more CH_4 (392 ± 27.2 g/day) than CTR (216 ± 16.3 g/day; $p < 0.001$). CO_2 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 H_2 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 CH_4 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. 20223HSYPJ) 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 (72m^2). Two boxes housed 7 animals per box and represented the low stocking density group (LowD group, 10.3m^2/animal), while one box housed 13 animals and represented the high stocking density group (HighD group, 5.5m^2/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 cm^2 in LowD vs. 94 cm^2 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.

Conclusion

Preliminary results of this field study revealed that a difference of 5 m^2/animal could negatively affect the carcass yield (-1.6%), ribeye area (-13 cm^2), 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 storaged.

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 humidity78±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.

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.

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.

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

(14.4%), Macrococcus (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

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 WOAH 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*.

Conclusion

Our results show the presence of zoonotic agents in the air, demonstrating that the use of an air sampler can be a successfuland 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

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 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 esttamente 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 no 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, vanto 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 conce 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 bresaole, 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 famigliare: 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 (cha 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 pasci 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 applicated 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 matter 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 WOAH (WAHIS) reports.

Results

The first outbreak was confirmed on 8 July 2024. To date, 86 outbreaks have been reported to WOAH, 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°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 beadministered 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.

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 × *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

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 primary 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 WOAH 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 Malaguena 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 symptoms). Seropositivity does 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 0registered (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.

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 sufering 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 framework. The Milan model

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.

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 ANTIBIOMULTIRESITANTE 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 secand 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 ZOONOTIC 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 CH_4 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 Ym (%) as follows: EF = [GE x (Ym/100) x 365 days]/55.65 MJ/kg CH_4.

Results

When assessing statistical differences, the *t-statistic* value indicates a significant difference in CH_4 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.

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 $bla_{\text{CTX-M1}}$, $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-3}}$, 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 ≠0 was 0.7±0.07. The AW score was 76.9±9.8% and the Biosecurity score was 68.9±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-Bablock 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®.

Passing Bablock's comparison between iCa2+ 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 mmo/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 ispection 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. Reasearchers are currently looking for new diagnostic biomarkers of mastitis – acute phase proteins produced by the liver, unique genetic sequences of mRNA, antimicrobical 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-Bablock 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).

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, K_2EDTA, K_3EDTA, 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: K_2EDTA, K_3EDTA, 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

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% CO_2 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 Tempus™ 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.

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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 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*.

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 most commonly raised together

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 behave 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 Nº. 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 nmol/l ± 0.02 vs 1.10 nmol/l ± 0.02; $P = 0.0065$) and was also higher in sheep in the Ta group than in those in the Te group (1.18 nmol/l ± 0.02 vs 1 .10 nmol/L ± 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 nmol/l ± 0.04 vs. 1.03 nmol/l ± 0.05; $P = 0.0042$) and at 72 hours (1.24 nmol/l ± 0.04 vs. 1.02 nmol/l ± 0. 05; $P = 0.0015$) compared to unshorn sheep. The T4 concentration was higher in the group of shorn sheep (95.56 nmol/l ± 1.56 vs. 80.88 nmol/l ± 2.14; $P<0.0001$) and was also higher in the Ta group than in the Te group (91.00 nmol/l ± 1.90 vs. 85.44 nmol/ l ± 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.

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 MilkoScan ™ 7 RM (Foss Electric, Hillerød, Denmark), while the somatic cell count (**SCC**) was performed through flow-cytometry method using the Fossomatic ™ 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

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 (CH_4) and nitrous oxide (N_2O) 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 CH_4 (45.1%) and N_2O (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.

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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 ins 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 meltonin-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 x 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, unsheared 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 f ROS and BAP. These findings suggest that exogenous melatonin could be utilized to mitigate stress and improve animal welfare.

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.

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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Arab Center for Studies of Arid Zones and Dry Lands (ACSAD)

This study was conducted to identify the genetic polymorphisms in growth differentiation factor 9 GDF9 and bone morphogenetic prptein 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 version7.2.1., which revealed seven mutations (SNPs) in GDF9 gene in the flanking region, exon1 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 twining 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.

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

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 (especilally 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.

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 µmol/L) exhibited significantly lower cobalt concentrations compared to bulls of the same breed (0.217 ± 0.052 µmol/L) and Charolais steers (0.146 ± 0.099 µmol/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 bout the epidemiology of beisnoitiosis 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 conduced.

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 asymptomatically 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.

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 Ogliastra, 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 Ogliastra, 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.

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

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 thre 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 (PrecR *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 =contour x 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.

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 CO_2, ECG, heart rate (HR), oxygen saturation (SpO_2), 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 positioneted 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

of ≥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 combatthis 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, isinnocuous 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). Form 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.perfrigens* 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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