

**25th International Congress of the
Mediterranean Federation for Health and
Production of Ruminants**

Fe. Me. S. P. Rum.

PROCEEDINGS

Session 1: Ruminant welfare and production

Keynote speakers

Starič Jože - Veterinary Faculty, University of Ljubljana
Metabolic Stress in Dairy Cows: Implications for Dairy Cow Welfare

Paola Nicolussi - Istituto Zooprofilattico Sperimentale della Sardegna - and **Alessandra Gaffuri** - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, CRN per il Benessere Animale
Welfare in dairy sheep and goat farming: methodologies and assessment of critical points

Oral communications

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Investigating the copper-zinc ratio in calf pneumonia: insights from vitamin D and trace element analysis

Alonso-Diez AJ, Martínez-Pastor F, Hefnawy Abd Elghany AE, Blanco-Fernández MA, Sanz-Jerónimo I, Cal-Pereyra LG, González-Montaña JR
Use of a portable electronic device to monitor metabolic status in sheep under field conditions

Cantou I, Neimaur K, Abreu C, Scaglione F, Rodríguez P, Cal-Pereyra LG, González-Montaña JR
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González-Montaña JR, Fernández-Olivera A, Martín-Alonso MJ, Domínguez JC, Alonso-de la Varga ME, Alonso Alonso P, Alegre-Gutiérrez B, Alonso-Diez AJ
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Maedi-Visna in dairy sheep in Sardinia: from animal welfare and legislative view-point

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Boubaker Bensmida – Service Santé Animale, Commisariat Regional au Développement Agricole (CRDA) de Tataouine, Tunisia

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Antonello Carta - AGRIS Sardegna

Advances in understanding the genetic architecture of resistance to main diseases in sheep

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Enhancement of Sardinian breed goats in marginal areas: considerations relating to genetic heritage, resistance to pathologies, adaptability to the local environment and longevity

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Pablo Díaz Fernández - Faculty of Veterinary Sciences, Universidade de Santiago de Compostela

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Giovanni Savini - Istituto Zooprofilattico dell'Abruzzo e Molise
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Alessandra Martucciello – National Reference Centre on Water Buffalo Farming and Productions Hygiene and Technologies - Istituto Zooprofilattico Sperimentale del Mezzogiorno
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Antonio Bosco - Department of Veterinary Medicine and Animal Production, University of Naples Federico II
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Simone Dore - Istituto Zooprofilattico della Sardegna, Centro di Referenza Nazionale per le Mastopatie degli Ovini e dei Caprini
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POSTER SESSION

Preliminary results on the diffusion of *Dicrocoelium* spp. in roe deer from Central Italy

Manuela Diaferia^{1*}, Valentina Calgaro², Iolanda Moretta¹, Marco Gobbi³, Leonardo Brustenga¹

¹University of Perugia, Perugia, Italy

²Freelance Veterinary Professional, Italy

³Istituto Zooprofilattico dell'Umbria e delle Marche "Togo Rosati", Italy

*Correspondence: manuela.diaferia@unipg.it

Keywords: *Capreolus capreolus*, *Dicrocoelium*, Italy

INTRODUCTION

The European roe deer (*Capreolus capreolus*) is one of the most widely distributed ungulates in Europe and in Italy. In the last decades, the Italian population has grown exponentially, resulting on roe deer sharing the same pastures and territories with domestic species (cattle, sheep, goats and equids). In particular, because of the promiscuity of pastures with sheep, the roe deer is predisposed to be infected by *Dicrocoelium* spp., a liver fluke whose adult form parasites the small bile ducts and gall bladder of domestic and wild ruminants.

MATERIALS AND METHODS

In the present study, the prevalence of *Dicrocoelium* spp. in roe deer from Central Italy, Umbria and Marche regions, was investigated directly searching for adult parasites in the livers of 67 individuals rescued by Wildlife Rescue Centers that died spontaneously or as a result of euthanasia from November 2021 to June 2023.

RESULTS AND CONCLUSIONS

Only 3 livers (4.5%) were positive for the presence of *Dicrocoelium* spp., all from the Marche region and with low parasite loads. The DNA of the isolated parasites was then extracted and sequenced, confirming the taxonomical identification of *Dicrocoelium dendriticum*. Data from our study showed circulation of the parasite in Central Italy, with lower prevalences than expected, basing on available works in the literature. The low parasite loads found in roe deer suggest its secondary role in environmental contamination, in contrast to sheep, which remains the main reservoir of the parasitosis in Central Italy.

Taken from the 25th International Congress of the Mediterranean Federation for Health and Production of Ruminants (Fe.Me.S.P.Rum.), Centre for Monitoring Parasitic Diseases (CREMOPAR), Eboli (Salerno, Italy), 19th – 20th October 2023.

Effect of MTNR1A gene polymorphism on the reproductive performance of sheep subjected to oestrus synchronization in different months of the year

Sebastiano Luridiana¹, Maria Consuelo Mura¹, Mortadha Ouadday¹, Giovanni Cosso¹, Ben Smida Boubaker¹, Vincenzo Carcangiu^{1*}

¹Department of Veterinary Medicine, University of Sassari, Via Vienna 2, 07100, Sassari, Italy

²National School of Veterinary Medicine, Sidi Thabet, University of Manouba, La Manouba 2010, Tunisia

*Correspondence: vcarcangiu@uniss.it

Keywords: Sarda ewes; MTNR1A gene,

INTRODUCTION

The MT1 receptor belongs to the G protein-coupled receptor family and its gene has been cloned and mapped in sheep and in other several species. The melatonin receptor 1A (MTNR1A) gene has several polymorphic sites, which are associated with seasonal reproductive activity in sheep. In Sarda sheep, two polymorphisms have shown association with reproductive seasonality, precisely those known as rs430181568 and rs407388227. The aim of this study was to highlight the effect of the polymorphisms rs430181568 and rs407388227 of the MTNR1A gene on reproductive performance in Sarda sheep after oestrus synchronization treatment in the months of March, April, May, and June.

MATERIALS AND METHODS

The research was conducted on two farms that raised approximately 1000 animals; a total of 800 sheep (400 for each farm) were genotyped. The two SNPs considered were totally linked, so that they were considered as a single SNP. Therefore, 240 sheep were chosen and divided into four homogeneous groups (A, B, C and D) of 60 subjects, each group based on age (range 3-6 years), body condition score (BCS) (range 2.5–3.5) and genotype (20 sheep carrying the CC/CC, 20 CT/CT and 20 TT/TT genotype). The oestrus synchronization dates of each group were March 10, April 10, May 10, and June 10, respectively. Each sheep was synchronized using a vaginal sponge containing 40 mg of Cronolone (Intervet, Italy). After 14 days the sponges were removed, and 400IU of PMSG was administered (Intervet, Italy). At removal of sponges, two fertile rams were introduced into the groups. All the rams were provided with marker harness, so that matings could be registered daily; colour marker harnesses were changed every 7 days. In all groups, the lambing date and the number of lambs born from 150 to 220 days after the ram introduction were recorded. The distance in hours between sponge removal and oestrus was the same between different genotypes in different treatment periods.

RESULTS AND CONCLUSIONS

In all groups, the CC/CC and CT/CT genotypes of the two polymorphisms, rs430181568 and rs407388227, showed the highest fertility rate ($P < 0.01$) and the distance shorter between the ram introduction to the lambing ($P < 0.01$), compared to the TT/TT genotype. Treatment and genotype did not influence litter size. In conclusion, we determined that the rs430181568 and rs407388227 polymorphisms influence the reproductive response after oestrous and this effect is not modified by the treatment period.

Taken from the 25th International Congress of the Mediterranean Federation for Health and Production of Ruminants (Fe.Me.S.P.Rum.), Centre for Monitoring Parasitic Diseases (CREMOPAR), Eboli (Salerno, Italy), 19th – 20th October 2023.

Mandatory permanent stabulation of ruminants in densely populated areas of Africa: practical and sustainable solutions implemented in Burundi

Joana Goncalves Pontes Jacinto ^{1,2*}, Valentina Morini ³, Gérard Habonimana ³, Sonia Santucci ⁴, Luca Farina ^{1,4} and Arcangelo Gentile ^{1,4}

¹ Vet for Africa, Bologna, Italy

² University of Bologna, Bologna, Italy

³ LVIA, Bujumbura, Burundi

⁴ IBO Italia, Ferrara-Padova, Italy

*Correspondence : arcangelo.gentile@unibo.it

Keywords: Permanent stabling, Sustainable farming system, stable design

INTRODUCTION

Burundi has a predominantly rural population, mostly dedicated to subsistence farming. The population density of more than 460 inhabitants per km² (third highest on the continent and almost doubling over the past two decades) is constantly increasing and requires a nutritional sustenance that is only possible by cultivating every available agricultural space. The importance of arable land in relation to extensive livestock farming or the grazing of animals, especially ruminants, led the Burundian authorities to issue a law that prevent free grazing (Law N 1/21 of 4 October 2018, in force as of 4 October 2021 *portant stabulation permanente et l'interdiction de la divagation des animaux domestiques et de la basse-cour au Burundi*). This law, in fact, heavily punishes those who do not comply with the rules of keeping animals in permanent housing by preventing them from grazing. In most cases, owners have applied this legislation by housing animals in shelters that had been previously used for confinement limited to the night, being therefore not suitable for a permanent housing and relative adequate animal management and welfare.

The aim of two projects co-funded by the Emilia-Romagna Region, respectively in 2022 and 2023, was to propose and develop an organic and sustainable animal housing compliant with the legislation but also with acceptable animal welfare.

MATERIALS AND METHODS

Improved stables were designed, conceived and built, using local materials including poles and wooden planks. They were proposed in the provinces of Kirundo, Ruyigi and Rutana. While adhering to the standard depth of 230 cm required to use the metal tiles available on the local market (clay tiles were also used in some cases and depending on availability), the following width dimensions were proposed: 360 cm for a single adult cow and 180 cm for the two to three goats that are usually in the availability of a family. The possibility of including chickens and rabbits was also considered.

At the same time, training and practical actions on feed supplementation techniques (in particular, the manufacturing of multinutrient blocks) as well as the enhancement of fodder crops such as *Trypsacum laxum*, *Pennisetum purpureum*, and forage leguminous shrubs such as *Calliandra* spp. and *Leucaena leucocephala* were proposed.

RESULTS AND CONCLUSIONS

Thirteen improved stables were built for cattle, sheep and goats as well as rabbits and poultry, guaranteeing compliance with the principles of animal welfare in terms of the size of the animals reared.

The proposed building model was well received by the local population, who began to request that it be erected on their property, and serve as a template for the construction of similar buildings, taking into account certain principles such as protection from rainwater and winds, and respect for animal welfare such as drinking troughs, feeding troughs, and benches on which the goats like to rest.

Acknowledgements

Projects co-funded by the Emilia-Romagna Region: a) “*DONNE E MESTIERI: empowerment di giovani donne della provincia di Kirundo (Burundi) attraverso programmi di formazione professionale e autoimprenditorialità*” (CUP E49J21016570009);

b) *TWITEZIMBERE: “Rafforzamento delle capacità di resilienza della popolazione dell’Est del Burundi”* (CUP E41J22000590009)

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Taken from the 25th International Congress of the Mediterranean Federation for Health and Production of Ruminants (Fe.Me.S.P.Rum.), Centre for Monitoring Parasitic Diseases (CREMOPAR), Eboli (Salerno, Italy), 19th – 20th October 2023.

***Twitezimbere* project (Burundi): education of farmers on feed integration for ruminants in permanent stabling and manufacturing of multinutrient blocks**

Luca Farina ^{1,2}, Valentina Morini ³, Joana G. P. Jacinto ^{2,4}, Gérard Habonimana ³, Daniel Nyandwi ⁵, Siméon Habonimana ⁵ and Arcangelo Gentile^{2,4}

¹ IBO Italia, Ferrara-Padova, Italy

² Vet for Africa, Bologna, Italy

³ LVIA, Bujumbura, Burundi

⁴ University of Bologna, Bologna, Italy

⁵ University of Burundi, Bujumbura, Burundi

*Correspondence: arcangelo.gentile@unibo.it

Keywords: nutrient block, Sustainable farming system, feed supplement, animal housing

INTRODUCTION

The national law *NI/21_2018* that came into force in Burundi on 04/10/2021 entails the obligation to keep animals permanently housed to better manage agricultural and livestock resources. It has simultaneously resulted in the necessity to improve livestock rearing conditions both in terms of animal housing and feeding.

The *Twitezimbere* project, co-funded by the Emilia-Romagna Region, has subsequently developed an organic and sustainable proposal that considers the above-mentioned requirements together with the need to gradually improve skills and educate farmers over time.

The aim of this study was to educate farmers about the importance of ruminant feed supplementation and to support the production of multinutrient blocks.

MATERIALS AND METHODS

Eight improved buildings for goats and cattle were built in the provinces of Ruyigi and Rutana; this was followed by eight meetings with interested farmers affiliated to the Rural Service Centres (RSCs) to raise awareness for the need to improve animal conditions, including housing and nutrition.

The farmers' training covered the main points of feed supplementation for permanently housed ruminants. Many of the participants had fodder crops of *Tripsacum laxum*, *Pennisetum purpureum*, *Calliandra* spp. and *Leucaena leucocephala*, although not in the quantities and proportions required. Multinutrient blocks are available but, often imported from abroad, so they are too expensive for the local rearing system characterized by subsistence livestock.

The ingredients used for manufacturing of feed blocks in this project were all readily available locally and known to the farmers who showed particular interest in the practical formulation of the product provided by the training sessions. During the training they made the multinutrient blocks themselves. The following ingredients were used: table salt (47.5%), cement (19.7%), water (9.8%), rice bran (8.2%), bone meal (8.2%), urea (2.5%), premix (2.4%), molasses (1.7%).

RESULTS AND CONCLUSIONS

The production of multinutrient blocks for the dietary supplementation of ruminants - which if modified by removing urea and molasses can also be used for pigs - was an important part of the *Twitezimbere* project, as evidenced by the active participation of 357 farmers in the eight training meetings held in June-July 2023 in the eight selected CSRs.

Two sizes of blocks (approximately 1.5 kg and 7 kg) have been produced and several farmers have started to produce them both for their own use and for sale. This confirms the validity of the project that provided an effective and practical feed supplement for their livestock, which is crucial given the legislation implemented.

Educational campaigns for farmers on the nutritional needs of permanently housed livestock and consistent supplementation are of major importance to improve animal health and productivity in such system.

Acknowledgements: TWITEZIMBERE: Rafforzamento delle capacità di resilienza della popolazione dell'Est del Burundi. Project co-funded by the Emilia-Romagna Region, CUP E41J22000590009

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***Neospora caninum* antibodies in tank bulk milk from dairy cows in northern Italy: spatial analysis and effects on herd performances.**

Carolina Allievi^{1*}, Luca Villa¹, Anna Rita Di Cerbo¹, Flavio Sommariva², Lucio Zanini², Alessia Libera Gazzonis¹, Sergio Aurelio Zanzani¹, Michele Mortarino¹, Maria Teresa Manfredi¹

¹ Università degli Studi di Milano, Department of Veterinary Medicine and Animal Sciences, Lodi, Italy

² Regional Breeders Association of Lombardy (ARAL), Crema, Italy

Correspondence: carolina.allievi@unimi.it

Keywords: neosporosis, dairy cows, bulk tank milk

INTRODUCTION

Neospora caninum is a major cause of infectious abortion in cattle which is the only clinical sign of neosporosis in adult animals [1]. Among the available diagnostic techniques, antibody detection in bulk tank milk (BTM) represents a useful tool to estimate and monitor *Neospora caninum* herd prevalence [2]. Thus, a serological study was planned in order to evaluate both the prevalence and spatial distribution of *N. caninum* infection in dairy cattle in northern Italy and the relation with herd performances.

MATERIALS AND METHODS

BTM samples collected from 586 dairy herds located in Lombardy (northern Italy) were analyzed by an indirect ELISA (Innovative Diagnostics, France). All farms were geo-referenced and reproductive and productive data were collected. A purely spatial analysis scanning for clusters with high or low rates for *N. caninum* serology using the Bernoulli model (SaTScan vers.10.1) was performed. The probability of distribution of the pathogen according to 19 environmental variables (BIO01- BIO19) was estimated by a maximum entropy approach (MaxEnt vers.3.4.4). Univariate generalized linear procedures (GLMs) were also developed.

RESULTS AND CONCLUSIONS

Overall, 180 herds resulted positive to *N. caninum* antibodies on tank bulk milk (P=30.7%). Higher and lower prevalences were evidenced in four (P=32-40%) and three provinces, respectively (P=13-24%). The geographical distribution of *N. caninum* positive farms with the highest level of infection probability were in central and the western sectors of the Po valley. One high-risk cluster with a prevalence of 50% and relative risk of 2.1 and one low risk cluster with no infection cases and expected value of 20.6% were evidenced. For positive farms the climate variables with the higher risk, when used in isolation, were related to both temperature and precipitation. A higher risk of parasite infection was revealed in small-medium farms (101-300 animals) and in older animals with more than 4 years. Further, the number of inseminations for conception were higher and the period from calving to conception were longer in positive farms. Besides, *N. caninum* positive herds presented lower head daily milk production and somatic cell counts higher than 300.000 cells/ml.

Neosporosis is widely distributed in dairy herds in northern Italy and an impact of the parasite on herd performances could be hypothesized.

Acknowledgements

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A new report on the epidemiology of *Oestrus ovis* infection in sheep in Sardinia, Italy

Carlo Carta¹, Alberto Coghetto¹, Luisa Meloni¹, Claudia Tamponi¹, Lia Cavallo¹, Pamela Zeinoun¹, Maria Francesca Sini¹, Francesca Nonnis¹, Plamena Pentcheva¹, Antonio Varcasia¹ and Antonio Scala^{1*}

1. Department of Veterinary Medicine, University of Sassari (Italy);

*Correspondence: scala@uniss.it

Keywords: *Oestrus ovis*, sheep, epidemiology

INTRODUCTION

The larvae of *Oestrus ovis* L. 1758 (Diptera, Oestridae) are obligatory parasites of the nasal cavities and sinuses of sheep and goats. The infestation is prevalent in hot and dry regions, such as Mediterranean countries.

Different surveys carried out indicate its presence in all areas where sheep breeding is practiced with a prevalence of 38% in Puglia, 51% in Emilia Romagna, 56% in Sicily, highlighting the widespread of the disease throughout Italy (Caracappa et al., 2000; Scala et al. 2001).

The aim of this study was to update the data on the prevalence of Oestrosis in the Sardinia region, which is the Italian region with the highest number of sheep reared (2,873,647 data: BDN Vetinfo.it).

MATERIALS AND METHODS

From March 2022 to February 2023 192 heads of adult Sarda sheep, regularly slaughtered, were collected from 35 farms in Sardinia. The number of animals included in the study was determined using the Epi Info software, taking into account the size of the regional population, with expecting prevalence of 50% and a confidence interval of 80%. The heads were dissected transversely at the level of the eye sockets and longitudinally from the muzzle, along the nasal cavities until the intersection point with the first cut. Then the two halves were washed and filtered using 150µm filters. The contents were analyzed by stereomicroscope and *O. ovis* larvae were counted and classified into first, second and third stage larvae.

RESULTS AND CONCLUSIONS

The 87.5% of the samples examined (168/192) were positive, with a higher prevalence in the province of Sassari (89.7%) compared to Nuoro (85%) ($\chi^2 = 1.758$; $P = 0.185$).

The data pertaining the harvesting season yielded a prevalence of 98.7% in autumn, 90% in spring and 81.9% in summer, while the lowest prevalence, at 72.7%, was observed in the winter period (χ^2 trend = 7.087; $P = 0.008$).

The mean number of larvae found per positive subject was 7.3 L1 larvae; 8.1 L2 larvae and 1.8 L3 larvae. These data varies according to the season, with an increase in the average number of L1 in the winter and spring period, 12.9 and 13.8 L1 respectively, followed by a decrease in the summer (5.4) and autumn season (5.7). Conversely, the majority of L2 and L3 larvae were found in the summer-autumn period, with respective values of 5.5 and 11.2 for the L2, and 1.9, and 1.8 for the L3. In the winter-spring period the values ranged from 3 during winter to 2.8 during the spring season for the L2, and from 0 to 1 for the L3.

The survey has further confirmed that Oestrosis is strongly present in the Sardinia region with a prevalence of over 87%, with peaks of 98% in the autumn season. The prevalence reported in this study is one of the highest in Mediterranean countries along with Tunisia (93.6%) (Kilani et al. 1986).

Continuous larval infections occurred year-round, likely due to increasing temperatures, including in winter. To address this, regular antiparasitic treatments may be necessary to reduce infection rates.

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Broncho-pulmonary nematodes of sheep in Sardinia (Italy): direct parasitological surveys

Lia Cavallo¹, Syrine Rekik², Carlo Carta¹, Claudia Tamponi¹, Plamena Pentcheva¹, Maria Francesca Sini¹, Francesca Nonnis¹, Ennio Bandino³, Antonio Varcasia¹ and Antonio Scala^{1*}

¹ Department of Veterinary Medicine, University of Sassari (Italy);

² Ecole Nationale de Médecine Vétérinaire, Sidi Thabet, Tunis (Tunisia);

³ Zooprophyllactic Institute of Sardinia "G. Pegreffi," Nuoro branch (Italy).

*Correspondence: scala@uniss.it

Keywords: Broncho-pulmonary nematodes, sheep, epidemiology

INTRODUCTION

Ovine broncho-pulmonary nematodes (BPN) are widespread parasites that can have significant adverse effects on the production and well-being of sheep. The diagnosis of these parasites in live animals primarily relies on clinical suspicion, with respiratory symptoms such as coughing and difficulty breathing. Confirmation typically involves a copromicroscopic investigation, which aims to isolate and identify the larvae expelled in the feces.

MATERIAL AND METHODS

From July 2021 to June 2022, 432 regularly slaughtered adult Sarda sheep (from 4 to 8 years old) from 36 farms in Sardinia were inspected. The study involved the examination of both the lungs and the entire bronchotracheal tree of each animal for broncho-pulmonary nematodes (BPNs). This examination involved pulmonary lesions associated with protostrongilids, or in cases where such lesions were absent, samples were taken from the dorsal margin of each lung. At least 4 pieces, with a diameter of 1x1 cm, were collected with a scalpel and tweezers from all patients and processed through a Baermann techniques. Isolated larvae were classified according to the morphometric keys given by Boev (1975) and by Euzeby (1982) and then counted.

RESULTS AND CONCLUSIONS

Larvae or adult and preadult species of BPN were detected in 44.7 % of the examined organs (n.193) and in 86.1 % of the farms (n.31).

The BPN species found were as follows: *Dictyocaulus filaria* 2.1% (n.9); *Muellerius capillaris* 36.4% (n.157); *Neostrongylus linearis* 22.7% (n.98); *Cystocaulus ocreatus* 6.7% (n.29). The differences in prevalence of the detected species were highly significant ($\chi^2=226.2568$; $P<0.00001$).

The data processing of the prevalence rates in relation to the season, using the χ^2 trend test, revealed no significant difference for any of detected BPNs ($P>0.05$). However higher rates were observed mainly in the summer for all detected species (72.8% - 110/151), with Odds Ratio (OR) values of 8.68 compared to the reference season with OR of 1.00. Only prevalence rates for *D. filaria* were higher in the spring (5.5% - 59/145). Among the BPN species detected, the highest number of larvae found was recorded for *M. capillaris*.

The survey found that *Protostrongylus rufescens*, a nematode detected in both goats and mouflons on the island, is never found among Sardinian sheep, despite the fact that these small ruminant species share the same habitat or are even bred together (e.g., goats and sheep). Nevertheless, it is evident that BPNs can be an important health problem for Sardinian sheep farming, being present in 86.1% of the herds, with prevalence rates of 44.7%, represented mainly by *M. capillaris*. However, these parasitosis are often underestimated by veterinarians and breeders who do not request the appropriate precise copromicroscopic investigation for them.

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Eimeriosis in dairy cattle in Sardinia (Italy): first epidemiological findings

Antonio Scala ^{1*}, Lorenzo Stevanato ², Alberto Coghetto ¹, Lia Cavallo ¹, Carlo Carta ¹, Maria Francesca Sini ¹, Francesca Nonnis ¹, Pamela Zeinoun ¹, Plamena Pentcheva ¹, Angelo Ruii ³, Antonio Varcasia ¹ and Claudia Tamponi ¹

¹ Department of veterinary medicine, University of Sassari (Italy);

² Freelance Veterinarian, Arborea (OR) (Italy);

³ Zoophylactic Institute of Sardinia “G. Pegreffi”, Oristano branch (Italy).

*Correspondence: scala@uniss.it

Keywords: Eimeriosis, cattle, epidemiology

INTRODUCTION

In Sardinia, there has been present a dairy cattle sector of primary national importance concentrated in the Arborea-Terralba (OR) district (approximately 38,000 heads). However, there is currently a lack of data regarding the spread of Eimeriosis and the specific species involved in this region. Hence, it has been considered useful to conduct this investigation within this district.

MATERIAL AND METHODS

In total, 342 faecal samples were collected from 16 farms: 70 from suckling calves aged up to 30 days; 67 weaned calves aged up to 6 months; 70 heifers; 63 dry cows; and 72 from lactating cows. The research and quantification of the oocysts of *Eimeria* spp. was performed using McMaster slides according to the method indicated by Raynaud (1970) and a super-saturated solution of zinc sulfate (specific gravity 1200). Coprocultures were established for positive stool samples from calves and heifers with the purpose of identifying the *Eimeria* species present in according to the morphometric keys indicated by Eckert et al. (1995).

RESULTS AND CONCLUSIONS

Oocysts of *Eimeria* spp. were found in all monitored farms (100% - 16/16) and in 30.4% of the samples analyzed (104/342). Prevalence rates and average Oocysts Per Gram of feces values (OPG) differed significantly in the different categories of cattle examined (χ^2 trend=18.835; $P < 0.000$; Kruskal-Wallis test: $H = 30.26$; $P < 0.000$): Prevalence rates were higher in weaned calves (53.7%) and lower in dairy cows (5.6%), while the highest OPG averages were recorded in suckling calves (average OPG = $3,155.2 \pm 6,434.9$). In the various farms monitored the prevalence rates ranged from 16% up to 52.9% (χ^2 trend = 2.794; $P = 0.094$). In adult cattle, it was observed that dry cows had higher oocyst shedding compared to lactating cows; in fact, the latter only eliminated a maximum of 500 OPG in an extremely limited percentage of animals (1.4%). In total, 10 species of *Eimeria* were highlighted, among which the most common findings were *E. bovis* (39.8%), followed by *E. canadensis* (17.5%), *E. zuernii* (15.9%), *E. ellipsoidalis* (6.3%), *E. auburnensis* (5.8%), *E. subspherica* (4.4%), *E. bukidnonensis* (4%), *E. cylindrica* (2.8%), *E. pellita* (2%) and *E. alabamensis* (1.5%).

The substantial prevalence rates, especially among weaned calves and heifers, along with the high percentage of *E. bovis* and *E. zuernii* species both of which are known to be particularly pathogenic for cattle brings out that this parasitosis is largely underestimated in the monitored district. This is further exacerbated by the limited implementation of therapeutic and prophylactic measures in the area.

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Cytokine responses after Bubaline alphaherpesvirus 1 (BuAHV-1) infection in calves

Giulia Franzoni^{*1}, Cecilia Righi^{*2}, Silvia Dei Giudici¹, Giulia Costantino², Susanna Zinellu¹, Annalisa Oggiano¹, Alessandra Martucciello³, Francesco Feliziani², Stefano Petrini².

¹ Department of Animal Health, Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy

² National Reference Centre for Infectious Bovine Rhinotracheitis (IBR), Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy

³ National Reference Centre for Hygiene and Technologies of Water Buffalo Farming and Productions, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Salerno, Italy

*These authors equally contributed to this work

Correspondence: giulia.franzoni@izs-sardegna.it

Keywords: BuAHV-1, cytokines, calves

INTRODUCTION

Cytokines are small proteins that play an essential role in controlling the homoeostasis of the immune system [1]. Their levels are often monitored as indicators of infection or vaccine-induced immune responses [2]. *Bubaline alphaherpesvirus 1* (BuAHV-1) is an important pathogen of water buffaloes and is able to infect calves [3], causing significant economic losses to the dairy industry worldwide [4]. To date, little is known about the impact of BuAHV-1 on the bovine immune system. Therefore, this research aimed to characterize the humoral immune response after challenge infection with BuAHV-1 in calves. Five calves were used in the experiments.

MATERIALS AND METHODS

Four animals were challenged infected with a high dose of virulent BuAHV-1 administrated by the intranasal route, whereas one calf was used as a negative control. Clinical signs were monitored during all experiments, and immunological parameters were monitored up to 29 days postinfection (dpi). In particular, nine key immune cytokines were investigated by *multiplex* ELISA tests (IFN- γ , IL-1 α , IL-1 β , IL-4, IL-6, IL-10, MIP1 β , IL-36Ra, TNF). The serum samples used to investigate the cytokines were collected at 2, 4, 7, 10, 14, 21, and 29 dpi and were compared with those pre-infection (0 dpi).

RESULTS AND CONCLUSIONS

The results showed respiratory symptoms up to 10 dpi and a transient increase in circulating IFN- γ values early post-infection (2 dpi) and a subsequent second wave (10 dpi). Furthermore, a transient increase in serum levels of four cytokines was observed: IL-1 α (4, 7 dpi), IL-4 (4, 7 dpi), IL-10 (4, 7 dpi), and MIP1 β (10 dpi), which returned to baseline values at later time points. Small fluctuations in serum IL-36Ra values were observed: infection with this herpesvirus resulted in a transient decrease in circulating levels of this receptor antagonist (14 dpi). Finally, BuAHV-1 infection did not trigger sustained fluctuations in serum IL-1 β , IL-6, and TNF values.

Our results highlighted that the increase in circulating IFN- γ values was transitory and is associated with the pro-Th1 response and the ability of the animals to overcome the infection. In parallel, the ability of animals to clear the infection was also associated with a transient raise of IL-10 and pro-inflammatory cytokines (IL-1 α , MIP1 β). A slight decrease in the IL-36Ra receptor antagonist was also observed, like that described for BoHV-1 (unpublished results).

Overall, we provided a better representation of the impact of BuAHV-1 on several key cytokines in calves. A better understanding of these immunopathological mechanisms would help to design suitable vaccines against this disease.

Acknowledgements

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Genetic variation of ORF virus: a perspective based on the VIR gene with new data from the island of Cuba.

Coradduzza E^{1*}, Scarpa F², Cacciotto C⁵, Fiori M.S¹, Rodriguez-Valera Y⁴, De Pascale A.M³, Azzena I^{2,5}, Casu M⁵, Pintus D¹, Ligios C¹, Scagliarini A³, Sanna D², Puggioni G¹

1 Istituto Zooprofilattico Sperimentale della Sardegna, 07100 Sassari, Italy

2 Dipartimento di Scienze Biomediche, Università degli studi di Sassari, 07100 Sassari, Italy

3 Dipartimento di Scienze Mediche e Chirurgiche, 40138 Bologna, Italy

4 Faculty of Agricultural Sciences, University of Granma, Bayamo 95100, Cuba

5 Dipartimento di Medicina Veterinaria, Università degli studi di Sassari, 07100 Sassari, Italy

*Corrispondence: elisabetta.coradduzza@izs-sardegna.it

Keywords: ORFV, contagious ecthyma, phylogenetic analysis

INTRODUCTION

Orf virus (ORFV), the causative agent of contagious ecthyma (CE), is an epitheliotropic DNA virus belonging to the *Parapoxvirus* genus of the *Poxviridae* family (1, 2). It is clinically characterized by mild papular and pustular to severe proliferative lesions, mainly occurring in sheep and goats. Similar lesions have also been reported in other animal species and humans (3, 4), thus resulting in a zoonotic disease spread worldwide. In this study we analyzed the genetic variation of the ORFV VIR gene in sheep and goat samples collected both in the Sardinia and Cuba islands.

MATERIALS AND METHODS

The Sanger method was used for sequencing and the sequence alignments were performed using the Clustal Omega software package after editing by the Unipro UGENE software.

RESULTS AND CONCLUSIONS

Relative to ORFV phylodynamic worldwide, Phylogenetic Bayesian tree and Principal Coordinates analyses were consistent in evidencing the occurrence of two large highly supported sequences groups. In particular, the most common genetic group included a reduced number of sequences from Cuba and Sardinia together with sequences isolated mainly in Europe and North America. Conversely, the less frequent group included the largest number of Sardinian and Cuban sequences together with samples mainly from South-East Asia. Analyses on ORFV phylodynamics performed on a high number of ORFV strains from the isolated populations of Sardinia and Cuba evidenced the occurrence of a common evolutionary trend. Indeed, each island shows the presence of private lineages that are exclusive to the area.

However, the genetic affinity of the Sardinian and Cuban ORFV lineages with strains isolated in the neighboring areas, may suggest a viral evolution specifically restricted to each island as a possible consequence of the effect of the geographic isolation and economic embargo in the case of Cuba. The phylodynamic patterns retrieved for ORFV in Sardinia and Cuba may represent a typical evolutionary model of this virus for isolated areas.

Acknowledgements

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Diet Supplementation with Hemp (*Cannabis sativa* L.) Inflorescences: Effects on Quanti-Qualitative Milk Yield and Fatty Acid Profile in Grazing Goats

Ruggero Amato¹, Marianna Oteri², Biagina Chiofalo², Fabio Zicarelli¹, Nadia Musco¹, Fiorella Sarubbi³, Severina Pacifico⁴, Marialuisa Formato⁴, Pietro Lombardi¹, Federica Di Bennardo¹, Piera Iommelli¹, Federico Infascelli¹ and Raffaella Tudisco¹

¹ Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, 80100, Napoli, Italy

² Department of Veterinary Sciences, University of Messina, 98122 Messina, Italy

³ Institute for the Animal Production System in the Mediterranean Environment, National Research Council, Italy

⁴ Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Italy

Correspondence: piera.iommelli@unina.it

Keywords: hemp; dairy goats; fatty acid profile

INTRODUCTION

Hemp (*Cannabis sativa* L.) is an annual plant belonging to the family of Cannabaceae with several varieties characterised by different fatty acid profiles, content in flavonoids, polyphenols and cannabinoid compounds. Hemp is mostly used in livestock nutrition as oil or as protein cake, not as inflorescences. The aim of this study was to evaluate the effect of dietary hemp inflorescences on milk yield and composition in grazing goats.

MATERIALS AND METHODS

Twenty goats (Camosciata delle Alpi), after kidding, were equally divided into two groups (G: Grazing and GH: grazing and hemp), homogeneous for milk yield in the previous lactation, parity and live weight. For three months, all goats were fed on a permanent pasture and received 700/head/day of concentrate (barley, oats and faba bean); diet of group GH was supplemented with 20 g/head/day of hemp inflorescences. Pasture DM intake was estimated according to previous research performed in the same area. In addition, goats' body weight did not change along the trial meaning that their energy requirements were guaranteed. Individual milk yield was daily registered and samples collected every 20 days for chemical composition (Milkoscan) and fatty acid profile (Gas Chromatography).

RESULTS AND CONCLUSIONS

The data were analysed by ANOVA (GLM procedure of SAS, 2000), for repeated measure. No significant differences were found for milk yield and chemical composition. Lauric acid (C12:0) was significantly higher in milk of group GH (4.83% vs 4.32%; $P < 0.01$) as well as total conjugated linoleic acids (CLAs) (0.435% vs 0.417%; $P < 0.01$). Additionally cortisol level was evaluated and the data are now object of evaluation with ANOVA techniques.

Our results showed that hemp supplementation affects milk fatty acid composition, increasing the total content of CLAs and of other beneficial FA, therefore suggesting that this plant could be a potential integration in animal diet useful to modify milk production, with advantages in terms of milk quality. Further studies are needed to evaluate different level of inflorescences supplementation and to investigate on aromatic characteristics of the product obtained. Moreover, the great attention that hemp had gained in the last years offers the possibility to use the different products that the cultivation of this plant offers in animal field.

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Snail borne infections in cattle in southern Italy -new insights into the biology and diagnosis of liver flukes

Lavinia Ciuca^{1*}, Ines Hammami², Maria Paola Maurelli¹, Antonio Bosco¹, Paola Vitiello¹, Laura Rinaldi¹

¹Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Via Delpino, 1, 80137 Napoli, Italy

²École Nationale de Médecine Vétérinaire de Sidi Thabet, Univ Manouba, 2020 Sidi Thabet, Tunisia

*Correspondence: lavinia.ciuca@unina.it

Keywords: snail-borne infections, cattle, liver flukes

INTRODUCTION

Fasciolosis is a worldwide parasitic infection caused by the main trematodes *Fasciola hepatica* and *Fasciola gigantica* (known as liver flukes) (Itagaki et al., 2022. Genet Evol. 99 105248). It affects a wide variety of hosts including ruminants, horses, pigs, rabbits and humans (Beesley et al., et al., 2018. Transbound Emerg Dis.; Evack et al., 2020. J Parasitol. 106(2), 316-322). In Europe, the main intermediate host of *F. hepatica* is represented by the lymnaeid snail populations (i.e. *Galba truncatula*) (Vázquez et al., 2018. CAB Rev.: Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 13, 10.1079). In Italy the prevalence of *F. hepatica* in ruminants appears to be low (0.7–6.0% in sheep and 0.9–7.8% in cattle); however *Calicophoron daubneyi* (known as rumen flukes) has been reported at high prevalence (4.5–51.1% in sheep and 9.6–60.9% in cattle) (Bosco et al., 2021. Area. Pathogens. 2021;10:1122; Maurizio et al., 2023. Parasitol. 2023; 1–14). However, there is large gap regarding the morphological and molecular data on the liver fluke species in cattle and the intermediate host (i.e. *Lymnaeidae* species) responsible for the transmission of *F. hepatica* in cattle in southern Italy. Furthermore, the only morphological study that has been carried out on the Italian liver flukes was described in wild boars hosts (Sgroi et al., 2021. Comp. Immunol. Microbiol. Infect. Dis. 77) and so far no studies have been reported on existing populations of lymnaeid snails from southern Italy. Therefore, the study aimed to assess the following data regarding: (i) the identity of the adults of *Fasciola* spp. in cattle through morphological and molecular analysis in southern Italy; ii) the snail species found on pastures grazed by cattle infected with both *F. hepatica* (FH) and *C. daubneyi* (CD) using morphological and molecular analysis in southern Italy.

MATERIALS AND METHODS

The study was carried out from May 2022 to September 2023 in cattle farms and slaughterhouses located in three regions of southern Italy, specifically Campania, Basilicata and Molise. Briefly, a total number of 84 adult liver flukes were collected from 8 animals and stored in 70% alcohol until used for morphological and molecular analyses. Snails (N= 212) were collected from 10 water-sources close to the 13 FH and CD-positive selected cattle farms. In order to characterize the morphology of Italian *Fasciola* flukes isolated from bovine livers, five morphometric parameters were performed, as follows: the total body length (BL), the abdomen diameter (AD), the distance between oral sucker and ventral sucker (OS-VS), the distance between ventral sucker and the tail (VS-T) and the tail diameter (TD). Moreover, for the morphology of the collected snails, the height of both mouth and shell, were considered as the main morphometric parameters performed in the study. The molecular analyses were performed on the Italian *Fasciola* specimens isolated from bovine livers, using ITS2 gene coding for 5.8S and 28S rRNAs of approximately 500 bp in length (primers: ITS2F and ITS2R) according to the protocol described by Itagaki et al. (2005. Parasitol. 131 679-

685). The snail specimens were tested by amplifying the ITS2 gene coding for 5.8S and 28S rRNAs of approximately 400 bp in length (primers: LT1-F and ITS2-Rixo-R) according to Bargues et al. (2001. Inf Gen Ev. 1 85-107). The multiple sequences alignment was performed using MEGA XI software (version 11.0.11) and the phylogenetic tree of Italian *Fasciola* spp. was created following 1000 bootstrap replicates using the neighbor joining method (Saitou and Nei 1987) applied in MEGA XI software.

RESULTS AND CONCLUSIONS

The morphometric data of *Fasciola* specimens isolated from cattle in southern Italy revealed that the mean of the total body fluke length (BL) was 19.17 ± 7.38 mm with a minimum (min) and maximum (max) of 11.68 and 35 mm respectively and that the mean of fluke's abdomen diameter (AD) was 4.53 ± 0.93 mm (ranged between 2.94 and 6.92). While, the ratio length/width of the fluke body was 4.29 ± 1.03 mm (min and max: 2.72 - 7.21, respectively). The morphometric measurements carried out on snails collected from southern Italy showed that the mean of the shell height was 6.019 ± 1.98 , with min and max values of 2.986 and 14 mm respectively, and that the mean of mouth height was 3.53 ± 1.39 mm (ranged between 1.50 and 9 mm). Sequencing of all *Fasciola* spp. ITS2-PCR amplicons revealed the presence of a single *Fasciola* species, as *Fasciola hepatica* (99.79 – 100% identity) in cattle in southern Italy. Similarly, all the snail ITS2-PCR amplicons indicated the occurrence of a single *Galba* species, as *Galba truncatula* (99.58 – 100% identity). Alignment of all the sequences obtained for *F. hepatica* and *G. truncatula* revealed a single variant, therefore, only one sequence, one for each species. In conclusion, the morphometric results were in agreement with those of the molecular and phylogenetic analyses revealing the existence of only *F. hepatica* species in cattle and only *G. truncatula* species of snails analyzed so far in southern Italy. However, this study is ongoing, and further results will allow deeper knowledge on the occurrence of freshwater snail species found on pastures grazed by cattle infected with both *F. hepatica* and *C. daubneyi* and also assess the presence of infection with these parasites in potential intermediate host snails in southern Italy.

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Baiting stray dogs in an extra-urban area as innovative tool for an integrated control of cystic echinococcosis

Elena Ciccone ^{1,2*}, Antonio Bosco ^{1,2}, Martina Nocerino ¹, Paola Pepe ¹, Nicola Lattero ^{1,2}, Mirella Santaniello ¹, Gerald Umhang ³, Laatamna AbdElkarim ⁴, Samia Lahmar ⁵, Yousra Said ⁵, Giorgio Saralli ⁶, Rania Baka ⁷, Smaro Sotiraki ⁷, Marshall W. Lightowlers ⁸, Peter Deplazes ⁹ Franck Boue ³, Laura Rinaldi ^{1,2}

¹ Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, 80137, Naples, Italy.

² Regional Reference Centre for Animal Health (CReSan), Campania Region, Italy.

³ French Agency for Food, Environmental and Occupational Health Safety (ANSES), Nancy Laboratory for Rabies and Wildlife Diseases, Technopôle agricole et vétérinaire, BP 40009, 54220 Malzéville, France

⁴ Laboratory of Exploration and Valorization of Steppic Ecosystems, Faculty of Nature and Life Sciences, University of Djelfa, Moudjbara Road, BP 3117, Djelfa, Algeria

⁵ Parasitology Laboratory, National School of Veterinary Medicine, University of Manouba, 2020 Sidi Thabet, Tunisia

⁶ Istituto Zooprofilattico Sperimentale of Lazio and Tuscany M. Aleandri, Via Appia Nuova, 00178 Rome, Italy

⁷ Veterinary Research Institute, Hellenic Agricultural Organisation-Demeter, 57001 Thessaloniki, Greece

⁸ Department of Biosciences, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia

⁹ Institute of Parasitology, Vetsuisse, and Medical Faculty, University of Zürich, Zürich, Switzerland

*Correspondence: elenaciccone@unina.it

Keywords: baits; stray dogs; *Echinococcus granulosus*.

INTRODUCTION

Cystic echinococcosis (CE) caused by the larval stage of the cestode *Echinococcus granulosus*, is one of the major zoonosis, affecting humans as well as domestic and wild animals. This parasitic disease represents one of the most widespread problems in Mediterranean countries, including Italy, causing economic losses both in the public health sector and in the livestock industry.

The CE control in a highly endemic area of southern Italy (Campania region) is based on a control program focused on the development of new procedures and tools that includes among the activities the treatment of shepherd dogs. Therefore, the aim of this study was to implement the anthelmintic treatment by extending it also to stray dogs in the proximity of CE positive sheep farms, by dropping baits with a drone in peri-pasture areas.

MATERIALS AND METHODS

Assessments were focused on the resistance of three different types of the baits over the time (at least ten days) to different climatic conditions while preserving the palatability and attractiveness for dogs, and on integrity resistance after release by drone from different heights. Subsequently, grazing areas were located using mobile global positioning system (GPS) devices applied to sheep and shepherd dogs of a CE positive sheep farm, and a pilot baiting site for bait delivery was selected based on the movements of the sheep and dog in the farm. Baits were placed in several arrangements and at different locations and the uptake by stray dogs was investigated using camera traps.

RESULTS AND CONCLUSIONS

The double praziquantel-laced baits showed the greatest resistance in the environment while also preserving the attractiveness for ten days, withstanding different climatic conditions. In addition, the double layer coverage withstood heights of 25 meters dropped by drones on hard surfaces remained perfectly intact after the impact, thus, they resulted also the mainly suitable for delivery by drone. The results on field showed that 80% of the baits were eaten after 1 day, 15% after 2 days, 5% after 3 days. Most of the baits (93%) were consumed by stray dogs and the remaining (7%) were consumed by foxes, badger and wild boar.

In conclusion, the double praziquantel-laced baits tested have shown better reliability resulted optimal for the treatment of stray dogs presents in the peri-pasture areas of CE positive sheep farms and seem to be well suited in a control plan for CE.

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Environment enrichment possibilities for beef cattle

Branka Kramberger¹ and Jože Starič^{2*}

¹ Veterinary faculty, University of Ljubljana, Ljubljana, Slovenia

² Section for ruminants, Veterinary faculty, University of Ljubljana, Ljubljana, Slovenia

*Correspondence: Joze.Staric@vf.uni-lj.si

Keywords: Welfare, housing enrichment, fattening bovine

INTRODUCTION

Understanding the behaviour and needs of production animals and designing the environment to help establish and maintain natural behaviours allows for adequate animal welfare.²

There are 5 categories of environmental enrichment: social, occupational, physical, sensory, and nutritional.¹ Optimisation of the environment can facilitate in an increase of normal behaviours and improvement in animal health.² Environmental enrichment improves animal welfare and productivity.

An insight into current published data on enrichment possibilities for beef cattle are presented.

MATERIALS AND METHODS

Scientific publications published from 1991 to 2023 and available on PubMed, ResearchGate and Cambridge University Press were reviewed.

RESULTS AND CONCLUSIONS

Nutritional enrichment can be accomplished by presenting varied or novel types of food or by changing the food delivery method.³ This form of enrichment can alleviate boredom, which is often the main reason for adopting abnormal behaviours.² Occupational enrichment includes enrichment that encourages exercise and psychological activity.¹ Animals can benefit from cognitive tasks such as opening gates to reach food rewards or exercise that can reduce lameness. Play behaviour is an indicator of positive welfare in calves, and providing larger pens is a suitable method for instigating this positive behaviour.³ Sensory enrichment stimulates animals' senses through auditory enrichment like music, visual enrichment by using mirrors or special lighting, and tactile enrichment by using cow brushes. Animals need an appropriate physical environment to remain healthy and productive. Sufficient space is needed in their environment to engage in normal behaviours such as bulling and hierarchy formation, while allowing the submissive animal to withdraw. Suitable substrates such as straw and woodchips, can also help reduce aggression. Social enrichment can benefit welfare and stress in farm animals.⁴ Positive human contact with farm animals is important and can reduce stress in animals.²

The extent to which environmental enrichment improves animal welfare, if at all, should be considered. Substrates and objects provided for environmental enrichment may have no effect on animal welfare and may even negatively impact welfare (increased competition and aggression when a limited resource is offered to beef cattle).⁵

Enrichment interventions for livestock can be cost effective and used successfully on farm. The benefits outweigh the costs of providing enrichment and should be considered when designing housing facilities for farm animals. It also reduces abnormal behaviours commonly seen in production animals, thus decreasing issues associated with poor animal health. This abstract has shown the importance of understanding farm animal behaviour and the benefits of enrichment to the welfare and productivity of livestock. The lack of research on beef cattle

represents an issue to wellbeing and production in the meat sector and should encourage further research.

Acknowledgements

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Acute stress response at disbudding in calves

Lena Veren Geč, Rok Marzel, Jožica Ježek, Jože Starič

University of Ljubljana, Veterinary faculty, Ljubljana, Slovenia

Correspondence*: lena.verengec@vf.uni-lj.si

Keywords: Cortisol, Horn bud removal, Calf

INTRODUCTION

Disbudding is a procedure in which the horn bud is removed in order to prevent the horn from developing. The reason behind this practice is safety of the herd, people interacting with the animals and ultimately the quality of the carcass.

It is usually done in the first two months after birth. Later, horns become too large and require amputation i.e., dehorning.

The methods of horn removal and use of medication vary across Europe (1).

In Slovenia, only veterinarians are allowed to perform cautery disbudding with concurrent use of a sedative, local anaesthetic, and an anti-inflammatory, which is a standard practice.

To better understand and monitor stress levels caused by the procedure, serum cortisol was measured.

MATERIALS AND METHODS

Blood was sampled at the initial contact with the animal. The calf was then sedated (xylazine) and a local anaesthetic (procaine) was applied to desensitize the cornual nerve, after which cautery disbudding was performed. The animals were given a NSAID (meloxicam) immediately after the procedure. Blood was sampled again 10 minutes after disbudding.

A control group of calves had their blood sampled twice in the space of 30 minutes without application of drugs or disbudding.

Serum cortisol was measured using ELFA immunoassay using MINI VIDAS (Biomerieux, France).

RESULTS AND CONCLUSIONS

Significant individual differences are apparent in the initial cortisol values. On average, the control group showed a lesser increase (average: 9.99 ng/mL) in cortisol compared to the experimental group (average: 19.12 ng/mL). It is difficult to draw specific conclusions on correlation between the higher rise in cortisol and stress without direct comparison to other methods. Further studies are needed to expand on these findings.

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Evidence of the circulation of EHDV-8 in sheep in Sardinia

Massimo Spedicato², Roberto Bechere¹, Barbara Bonfini², Marcella Maxia³, Daniela Manunta¹, Elisabetta Coradduzza¹, Simone Pulsoni², Ottavio Portanti², Bianca Maria Falchi⁴, Annamaria Coccollone¹, Vilma Giglio¹, Anna Ladu¹, Giulia Franzoni¹, Graziella Puggioni¹, Angelo Rui¹, Annalisa Oggiano¹, Giovanni Savini², Giontonella Puggioni¹

¹ Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy

² Istituto Zooprofilattico Sperimentale Abruzzo e Molise, Teramo, Italy

³ Azienda Sanitaria locale n. 7, Carbonia, Italy

⁴ Azienda Sanitaria locale n. 8, Cagliari, Italy

*Correspondence: giontonella.puggioni@izs-sardegna.it

Keywords: EHDV, sheep, natural infection

INTRODUCTION

Epizootic haemorrhagic disease (EHD) is a Culicoides-borne viral disease caused by epizootic haemorrhagic disease virus (EHDV). It is often associated with clinical manifestations in domestic and wild ruminants, primarily white-tailed deer (*Odocoileus virginianus*) and cattle (*Bos taurus*). EHDV is related to Bluetongue virus (BTV), the etiological agent of bluetongue (BT). Both viruses belong to the genus *Orbivirus* (family *Sedoreoviridae*) and circulate in multiple serotypes. By the end of September 2021, EHDV-8 was reported in cattle farms in west-central Tunisia, with more than 200 confirmed outbreaks (1). From October to early November 2022, BTV-3 and EHDV-8 circulation was detected in the Southern of Sardinia (SU), municipality of Arbus. EHDV-8 was reported in three cattle farms located in the municipalities of Arbus and Guspini (SU). Contemporaneously, BTV-3 and EHDV-8 were detected in one farm located in Sicily. This is the first evidence of EHDV in the European Union (EU) (2). In early November 2022, blood samples were taken for antibodies and virus detection in the EHD outbreak epidemiologically related farms. Surprisingly, in a flock of 125 healthy sheep located in the municipality of Fluminimaggiore (SU), out of 85 samples, 74 tested positive for antibodies and 73 for the RNA virus. Here, we describe the follow-up carried out on the animals of the farm from November 2022 to September 2023.

MATERIALS AND METHODS

Serum and EDTA-blood samples were collected from 85 sheep on 8 November 2022, from 82 sheep on 28 February 2023, from 43 sheep on 20 March, from 117 sheep on 14 of April, and from 106 sheep on 4 September 2023. Serum samples were tested for the presence of EHDV antibodies using a commercial EHDV c-ELISA kit (IdVet, ID Screen® EHDV) according to the manufacturer's instructions. Samples testing positive by c-ELISA were tested by seroneutralization for EHDV

serotype 8 to confirm positivity and determine serotype. A real time RT-PCR assay based on the sequence of segment 9 was used for Pan-EHDV detection (3). All positive samples were analyzed with a real-time RT-PCR serotyping assay that specifically amplifies EHD serotype 8 segment 2.

RESULTS AND CONCLUSIONS

Follow up of serological results revealed a high percentage of positivity in all samples collected up to September 2023 (86% of 85 tested, 89% of 82 tested, 72% of 43 tested, 86% of 117 tested and 81% of 106 tested). The seroneutralization test showed antibody titers against EHDV-8 ranging from 1:20 to 1:640. On the other hand, EHDV-8 RNA was detected in blood samples up to March 2023. Given the peculiarity of high positivity, which is unusual in vector-borne diseases and has never been detected for EHDV in sheep, investigations are currently underway to understand the epidemiological role of sheep and the dynamic of EHDV serotype 8 infection.

Acknowledgements

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Gastrointestinal nematodes of cattle and sheep: evaluation of anthelmintic resistance in farms of the Campania region

Rosa D'Ambrosio¹, Roberta Brunetti¹, Stefania Cavallo¹, Antonella Pesce¹, Diletta Mandato¹, Mirella Santaniello², Marialuisa Buonanno¹, Loredana Baldi¹, Laura Rinaldi², Antonio Bosco^{2*}

¹ Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Naples, Italy

² Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Naples, Italy

*Correspondence: antonio.bosco@unina.it

Keywords: Ruminants, sustainable parasite control.

INTRODUCTION

Gastrointestinal nematode (GIN) infection endangers livestock health and welfare and is commonly associated with economic losses mostly through subclinical diseases impairing weight gain and milk yields (Charlier et al., Prev Vet Med, 2020).

The worldwide increased difficulty to combat GIN infection in ruminant, due to progressing anthelmintic resistance (AR), calls for an enhanced and standardized implementation of early detection of AR. This study provides a snapshot of the current AR status against benzimidazoles and macrocyclic lactones in sheep and cattle farms of southern Italy, generated with standardized techniques.

MATERIALS AND METHODS

The trial was conducted in 10 cattle and 20 sheep farms of the Campania region. In each farm the animals were divided into 2 groups of 20 animals randomly chosen, one group treated with ML (ivermectin) and one group with BZ (albendazole), without using an untreated control group (Bosco et al., Parasit Vectors, 2020). On each farm the enrolled animals were individually weighed and the correct dose of drugs was administered using an appropriate equipment, calibrated to deliver the dose accurately. Specifically, animals were treated with an oral suspension of albendazole (ALB, Valbazen® Zoetis, Rome, Italy; 3.8 mg/kg of body weight) and an oral solution for sheep and an injectable solution for cattle of ivermectin, Boehringer Ingelheim Animal Health (IVM, Oramec® for sheep and Ivomec® for cattle; 0.2 mg/kg of body weight).

Individual faecal samples were collected rectally on the day of treatment (D0) and after 14 days (D14) and analysed using the Mini-FLOTAC Technique (Cringoli et al., Nat Protoc, 2017). The FECRT was calculated from individual samples. Efficacy was classified as 'reduced', 'suspected' and 'normal'. Coprocultures were set for D0 and D14 faecal samples of each group. From farms with FECR < 95%, an in vitro egg hatch test (EHT) and a follow-up FECRT using fenbendazole (FBZ) were conducted.

RESULTS AND CONCLUSIONS

Based on the FECR, high efficacy (from 96.7% to 100%) was observed for ALB and IVM in all cattle farms and in 18 sheep farms. On two sheep farms, the efficacy for the macrocyclic lactones was classified as 'normal', but 'reduced' efficacy was observed for ALB on Farm 1 (FECR = 86%) and 'suspected' efficacy on Farm 2 (FECR = 92.4%) with the predominant GIN genus *Trichostrongylus* followed by *Haemonchus* at D14. The in vitro EHT confirmed AR in both sheep farms (Farm 1: 87%; Farm 2: 77%).

In regions like southern Italy, where the negative impacts from AR have played a minor role, efficient monitoring of AR is important in order to evaluate potential risks and being able to promptly respond with countermeasures.

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Session 1: Ruminant welfare and production

Keynote speakers

Starič Jože - Veterinary Faculty, University of Ljubljana
Metabolic Stress in Dairy Cows: Implications for Dairy Cow Welfare

Paola Nicolussi - Istituto Zooprofilattico Sperimentale della Sardegna - and **Alessandra Gaffuri** - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, CRN per il Benessere Animale
Welfare in dairy sheep and goat farming: methodologies and assessment of critical points

Oral communications

Bilgiç B, Tarhan D, Erdoğan H, Erdoğan S, Pinar O, Ural K, Kayar A, Ercan AM, Or ME
Investigating the copper-zinc ratio in calf pneumonia: insights from vitamin D and trace element analysis

Alonso-Diez AJ, Martínez-Pastor F, Hefnawy Abd Elghany AE, Blanco-Fernández MA, Sanz-Jerónimo I, Cal-Pereyra LG, González-Montaña JR
Use of a portable electronic device to monitor metabolic status in sheep under field conditions

Cantou I, Neimaur K, Abreu C, Scaglione F, Rodríguez P, Cal-Pereyra LG, González-Montaña JR
Effect of prepartum shearing on some metabolic parameters of ewes and their lambs

González-Montaña JR, Fernández-Olivera A, Martín-Alonso MJ, Domínguez JC, Alonso-de la Varga ME, Alonso Alonso P, Alegre-Gutiérrez B, Alonso-Diez AJ
Clinical case: Mastitis in sheep flock. Diagnosis and control by somatic cells counting

Biondi V, Mereaglia F, Previti A, Pugliese M, Passantino A, Cubeddu GM
Maedi-Visna in dairy sheep in Sardinia: from animal welfare and legislative view-point

Session 2: Conservation of “ruminant” biodiversity in Mediterranean Basin

Keynote speakers

Boubaker Bensmida – Service Santé Animale, Commisariat Regional au Développement Agricole (CRDA) de Tataouine, Tunisia

State-of-the-Art of dromedaries in Tunisian zootechnics

Antonello Carta - AGRIS Sardegna

Advances in understanding the genetic architecture of resistance to main diseases in sheep

Oral communications

Cabras PA, Zidda C, Dore S, Orrù A, Deiana AM, Bandino E

Enhancement of Sardinian breed goats in marginal areas: considerations relating to genetic heritage, resistance to pathologies, adaptability to the local environment and longevity

Jacinto JGP, Gentile A, Sbarra F, Drögemüller C

Frequency of deleterious mutations causing recessive disorders in Italian Chianina, Marchigiana and Romagnola cattle

Scarcelli S, Buono F, Castaldo E, Varuzza P, Argenio F, Lepri E, Diaferia M, Pepe P, Maurelli MP, Veneziano V

Dicrocoelium dendriticum in roe deer (Capreolus capreolus) in central Italy: distribution and risk factors

Session 3: Parasitic disease in ruminants

Keynote speakers

Pablo Díaz Fernández - Faculty of Veterinary Sciences, Universidade de Santiago de Compostela

Paramphistomidosis: an emergent parasitic disease of ruminants in Europe

Oral communications

Masu G, Bonelli P, Serra E, Loi F, Buonanno M, Carvelli A, Cerioli MP, Crotti S, Danesi P, Iurescia M, Santi A, Rolesu S, Peruzzu A, Masala G, Piseddu T

Development of an active surveillance system in Italy to estimate the real prevalence of Echinococcosis in sheep

Khan MY, Barlaam A, Gazzonis AL, Ferrari N, Jiménez-Meléndez A, Robertson LJ, Giangaspero A

Toxoplasma infection in goats in Pakistan; risk factors and public health significance

Scala A, Spezzigu A, Tamponi C, Cavallo L, Carta C, Sini MF, Nonnis F, Varcasia A

Efficacy of treatments with macrocyclic lactones in different formulations against gastrointestinal nematodes of sheep in Sardinia, Italy

Mancusi A, Giordano A, Bosco A, Girardi A, Proroga YTR, Rinaldi L, Capuano F, Cringoli G, Maurelli MP

Development of highly sensitive digital droplet PCR for detection of Toxoplasma gondii in bovine meat

Azara E, Longheu CM, Foddai A, Nives MR, Cillara G, Broccia S, Puddu G, Addis MF, Tola S

Cloning, expression and purification of recombinant proteins from Staphylococcus aureus and their potential for vaccine development

Session 4: (Re-)Emerging ruminant infectious diseases

Keynote speakers

Giovanni Savini - Istituto Zooprofilattico dell'Abruzzo e Molise
EHDV-8: virus of a lesser God

Alessandra Martucciello – National Reference Centre on Water Buffalo Farming and Productions Hygiene and Technologies - Istituto Zooprofilattico Sperimentale del Mezzogiorno
Brucellosis: recent advances and future challenges

Oral communications

López-Novo C, Prieto A, García-Dios D, Díaz-Cao JM, Remesar S, López-Lorenzo G, Fernández G, Morrondo P, Díaz P
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Pintus D, Scivoli R, Herder V, Rocchigiani AM, Maestrale C, Bechere R, Oggiano A, Palmarini M, Ligios C, Puggioni G
Experimental Bluetongue virus (BTV) infections in sheep using two different inoculum sources mimic fatal natural disease

Attili AR, Gigli F, Guerrini A, Nocera FP, Corradini CM, Fiorito F, Cerracchio C, De Martino L, Cuteri V
Isolation of ESKAPE bacteria in healthy cattle, antibiotic resistance, and potential zoonotic risk

Luridiana S, Mura MC, Ouadday M, Cosso G, Boubaker BS, Carcangiu V
30 years of melatonin use in Sardinian sheep: considerations

Session 5: Sustainability of ruminant farms

Keynote speakers

Antonio Bosco - Department of Veterinary Medicine and Animal Production, University of Naples Federico II
Sustainable approaches to parasite control in ruminants

Simone Dore - Istituto Zooprofilattico della Sardegna, Centro di Referenza Nazionale per le Mastopatie degli Ovini e dei Caprini
Sustainable practices for small ruminant mastitis management

Oral communications

Vastolo A, Nocerino M, Kiatti D, Bosco A, Calabrò S, Cutrignelli MI, Rinaldi L
In vitro evaluation of grape pomace in the diet of adult sheep

Kiatti D, Vastolo A, Koura BI, Cutrignelli MI, Calabrò S
Characterization of non-conventional feedstuff for ruminants' nutrition: case of cashew and pineapple by-products

Hammami I, Ciuca L, Maurelli MP, Romdhane R, Rjeibi MR, Farhat N, Sassi L, Simo AK, Rinaldi L, Rekik M, Gharbi M
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Falzarano A, Bosco A, Scarano P, Prigioniero A, Maurelli MP, Rinaldi L, Cringoli G, Quaranta G, Claps S, Sciarrillo R, Guarino C
Use of perennial plants in the fight against gastrointestinal nematodes of sheep

Persichilli C, Biffani S, Senczuk G, Di Civita M, Bosco A, Cringoli G, Grande G, Pilla F

Identification of Genetic Regions Associated with Resistance to Gastrointestinal Nematodes in Comisana Sheep using a Genome-wide Association Study based on EBV Ranking

Amato R, Vitiello P, Bosco A, Rinaldi L

Parasitological Evaluation of Diet Supplementation with Hemp (Cannabis sativa L.) Inflorescences in goat and in vitro Usage of Different Cannabis Extract: a preliminary study

Ciccone E, Paola P, Cacciola NA, Campanile C, Bosco A, Maurelli MP, Oliva G, Guccione J, Rinaldi L

Application of RNA technologies for improved control of cystic echinococcosis (ok)

Altieri D, Gombia Y, Rossi D, Neri M, Carnovale F, Di Palo R

Could a locomotion score be useful in the type trait assessment of the Italian Mediterranean Buffalo?

Session 6: Ongoing projects on ruminant sector

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Pugliese A

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Artificial insemination in Mediterranean Buffaloes: a field research in southern Italy

Pepe P, Bosco A, Umhang G, Said Y, Baka R, Alterisio CM, Piegari G, Ciaramella P, Paciello O, D'Orilia F, Sarnelli P, Saralli G, Laatamna A, Lahmar S, Lightowlers MW, Deplazes P, Boué F, Sotiraki S, Rinaldi L

Innovative and sustainable strategies to control Cystic echinococcosis in the Mediterranean area

Di Vuolo G, Cappelli G, Serrapica M, Ambra CD, Vecchio D, De Carlo E, Lucchese L, Scali F, Lorenzi V, Bosco A, Lecchi C

Risk categorization of antimicrobial and anthelmintic consumption in Italian Mediterranean buffalo farming - IZSME15/22 RC

Calbi A

Poster Session

1. Diaferia M, Calgaro V, Moretta I, Gobbi M, Brustenga L
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2. Luridiana S, Mura MC, Ouadday M, Cosso G, Boubaker BS, Carcangiu V
Effect of MTNR1A gene polymorphism on the reproductive performance of sheep subjected to oestrus synchronization in different months of the year
3. Jacinto JGP, Morini V, Habonimana G, Santucci S, Farina L, Gentile A
Mandatory permanent stabulation of ruminants in densely populated areas of Africa: practical and sustainable solutions implemented in Burundi
4. Farina L, Morini V, Jacinto JGP, Habonimana G, Nyandwi D, Habonimana S, Gentile A
Twitezimbere project (Burundi): education of farmers on feed integration for ruminants in permanent stabulation and manufacturing of multivitamin blocks
5. Allievi C, Villa L, Di Cerbo AR, Sommariva F, Zanini L, Gazzonis AL, Zanzani SA, Mortarino M, Manfredi MT
Neospora caninum antibodies in tank bulk milk from dairy cows in northern Italy: spatial analysis and effects on herd performances
6. Carta C, Coghetto A, Meloni L, Tamponi C, Cavallo L, Zeinoun P, Sini MF, Nonnis F, Pentcheva P, Varcasia A, Scala A
A new report on the epidemiology of Oestrus ovis infection in sheep in Sardinia, Italy
7. Cavallo L, Rekik S, Carta C, Tamponi C, Pentcheva P, Sini MF, Nonnis F, Bandino E, Varcasia A, Scala A
Broncho-pulmonary nematodes of sheep in Sardinia (Italy): direct parasitological surveys
8. Scala A, Stevanato L, Coghetto A, Cavallo L, Carta C, Sini MF, Nonnis F, Zeinoun P, Pentcheva P, Rui A, Varcasia A, Tamponi C
Eimeriosis in dairy cattle in Sardinia (Italy): first epidemiological findings
9. Franzoni G, Righi C, Dei Giudici S, Costantino G, Zinellu S, Oggiano A, Martucciello A, Feliziani F, Petrini S
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Gastrointestinal nematodes of cattle and sheep: evaluation of anthelmintic resistance in farms of the Campania region

Use of a portable electronic device to monitor metabolic status in sheep under field conditions

Alonso-Diez, A J^{1,2}; Martínez-Pastor, F ^{2,3}; Hefnawy Abd Elghany, AE ⁴; Blanco-Fernández, M A⁵; Sanz-Jerónimo, I⁵; Cal-Pereyra, LG ⁶; González-Montaña, J R ^{1,2}

1. *Medicine, Surgery and Anatomy Veterinary Department, Veterinary Faculty, University of León, Spain.*

2. *Institute of Animal Health and Cattle Development (INDEGSAL), University of León, León, Spain.*

3. *Faculty of Veterinary Medicine Minoufia University- Egypt.*

4. *Consorcio de Promoción del Ovino, Zamora, Spain.*

5. *Molecular Biology Department (Cell Biology), University of León, Spain.*

6. *Patobiología Department, Faculty of Veterinary, UDELAR, Montevideo, Uruguay.*

Keywords: pregnancy toxaemia, ketonemia, ewes

INTRODUCTION

Pregnancy toxaemia is a metabolic disease, due to a negative energy balance, that affects sheep in the last third of gestation, with a marked economic impact due to its high morbidity and mortality rate. It presents with digestive and neuromuscular clinical signs and is characterized by hypoglycaemia, hyperketonaemia, and ketonuria [1–4]. Although various parameters may appear altered, glucose and β -hydroxybutyrate (BHB) allow it to be diagnosed, even without the presence of clinical symptoms [5–8]. Given that early diagnosis is key when establishing the necessary measures for its control and prevention, we have evaluated the usefulness of a portable measuring device (GlucoMen areo2K®, Menarini diagnostics) together with adapted test strips.

MATERIALS AND METHODS

We have assessed in situ glycaemia and BH and BHB in blood obtained from the jugular and auricular vein of sheep in the final on period, and we have compared them with the values obtained in a reference laboratory. Additionally, we have evaluated the suitability of a blood sample obtained from the auricular vein, as it is a simpler sampling method to be measured using a portable device.

RESULTS AND CONCLUSIONS

Although there is not always complete agreement between the different measurements made, the portable meter allows an approximation of the blood glucose and ketone body values under field conditions. Furthermore, the results show the suitability of analysing the concentration of both glucose and BHB in a drop of blood obtained from an ear vein. The measurement of these parameters will allow early detection of a negative energy balance, detecting in situ sheep with gestation toxaemia or at risk of suffering from it, and thus establish the appropriate control measures.

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Investigating the Copper-Zinc Ratio in Calf Pneumonia: Insights from Vitamin D and Trace Element Analysis

Bengü Bilgiç^{1*}, Duygu Tarhan^{2,3}, Hasan Erdoğan⁴, Songül Erdoğan⁴, Orhan Pınar⁵, Kerem Ural⁴, Abdullah Kayar¹, Alev Meltem Ercan², M.Erman Or¹

¹Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Internal Medicine Department, Avcılar, Istanbul, Turkey

²Istanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Biophysics, Fatih, Istanbul, Turkey

³Bahcesehir University, School of Medicine, Department of Biophysics, Istanbul, Turkey

⁴Adnan Menderes University, Veterinary Faculty, Department of Internal Medicine, Aydın, Turkey

⁵Istanbul University-Cerrahpaşa, Vocational School of Veterinary Medicine, Equine and Equine Training Program, Avcılar, Istanbul, Turkey

*Correspondence: bengu.bilgic@iuc.edu.tr

Keywords: elements, magnesium, cholecalciferol, respiratory disease, cattle

INTRODUCTION

The disruption of lung defense mechanisms increases susceptibility to infections, which can lead to complications ending in the calf's death. The most critical bacterial pathogens include *Pasteurella multocida*, *Mycoplasma bovis*, and *Mannheimia haemolytica* (İder and Maden, 2022). Environmental factors not only predispose calves to respiratory diseases, but they can also generate various free radicals, leading to stress in animals. Oxidative stress emerges due to an imbalance between oxidative and antioxidant substances resulting from the excessive production of oxidative compounds. Antioxidants primarily defend against oxidative stress. In case of a potential imbalance, oxidative stress could cause severe damage to the lung tissue (Özbek and Özkan, 2020). Oxidative stress plays a substantial role in the pathogenesis of lung diseases (Shoieb et al., 2016). According to recent studies, it is believed to be associated with an excessive accumulation of reactive oxygen species (ROS), generated by immune system cells accumulated in the lungs due to the inflammatory response. This mechanism, formed to kill pathogens, can lead to local tissue damage and excessive cytokine release throughout the body when it occurs in excessive quantities (Xu et al., 2020). As a result, the excessively released ROS can non-specifically and rapidly react with chemical groups in lipids, proteins, and DNA, potentially leading to the shaping of extracellular matrices, apoptosis, increased mucus secretion, and pulmonary fibrosis (Poljsak et al., 2013; Labuschagne and Brenkman, 2013).

Zinc is an immunoregulatory microelement with antiviral features that plays a role in the maturation of immune system cells, the development and activation of lymphoid cells, the regulation of inflammatory cytokines, and oxidative stress control (Shakeri et al., 2021). It has been found that natural killer (NK) cell activity is low in patients with zinc deficiency. Additionally, susceptibility to bacterial, viral, and fungal infections increases in patients with zinc malabsorption syndrome (Sandström, 1994; Kahmann et al., 2008). Copper, on the other hand, not only plays a significant role in metabolism but also has the ability to act as both an antioxidant and a pro-oxidant. In this situation, it can prevent the formation of free radicals, but it can also trigger free radical damage under certain circumstances (Osredkar and Sustar,

2011). It is known that vitamin D and its metabolites play many roles related to the immune system and host susceptibility to infection (Di Rosa et al., 2011; Ooi et al., 2012; Hewison, 2012). Vitamin D receptors are found in most immune system cells, and some immune system cells can synthesize the active form of vitamin D, suggesting that vitamin D may play a significant immunoregulatory role (Calder, 2020). In many human studies, an inversely linear relationship has been identified between vitamin D levels and respiratory tract infections (Berry et al., 2011). Moreover, another comprehensive study in the US showed an independent inverse correlation between serum 25(OH)-D vitamin and recent upper respiratory tract infections (Ginde et al., 2009). As a result, meta-analyses have indicated that vitamin D supplementation may reduce the risk of respiratory tract infections (Bergman et al., 2013; Pham et al., 2019). In light of the aforementioned information, it's worth noting that while research is available examining the role of trace elements in assessing immune status in calves with pneumonia, there appears to be a gap in the literature concerning vitamin D, a critical player in cellular immunity. The purpose of this study was to examine the association between serum levels of Mg, selected trace elements (Cr, Cu, Fe, Mn, Se, Zn), Cu:Zn ratio and vitamin D levels in calves with the acute pneumonia at weaning period.

MATERIALS AND METHODS

The study involved two groups of Holstein calves aged 45 to 70 days: a healthy group and a pneumonia-affected group. The healthy group was composed of seven calves, while the pneumonia-affected group had 14 calves. Blood samples were collected from both groups for the evaluation of vitamin D and trace element levels. The pneumonia-affected calves underwent a treatment protocol involving subcutaneous administration of tilmicosin, meloxicam, and Vitamin D3. The analysis of vitamin D and trace elements was conducted using the immunochromatographic test device (Savant-100, China) and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES Thermo iCAP 6000 series) respectively.

RESULTS AND CONCLUSIONS

Significant alterations were detected in chromium, magnesium, manganese, and vitamin D levels among calves with pneumonia compared to healthy controls. Particularly, levels of magnesium and manganese decreased, while copper levels markedly increased following initial treatment. However, by the third day, copper levels equalized between the groups. Additionally, an insignificant positive correlation ($r=0.74$) was observed between the copper-zinc ratio and vitamin D levels in calves with pneumonia.

This study is the first to evaluate the impact of supplementary vitamin D on copper-zinc ratios in calves with pneumonia. Although significant changes in some trace elements were observed, the addition of vitamin D did not result in significant changes in the copper-zinc ratios. Further research is required to deepen our understanding and potentially guide strategies to decrease incidences of pneumonia in calves during the weaning period.

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MAEDI-VISNA IN DAIRY SHEEP IN SARDINIA: FROM ANIMAL WELFARE AND LEGISLATIVE POINT OF VIEW

Vito Biondi¹, Ferdinando Mereghaglia², Annalisa Previti¹, Michela Pugliese^{1*}, Annamaria Passantino¹, Giovanni Maria Cubeddu²

¹Department of Veterinary Sciences, University of Messina (Italy)

²MELEFOVET, Gruppo di studio AIVPA - Medicina legale e Forense, Bioetica e Deontologia applicate alla Professione Veterinaria.

*Correspondence: michela.pugliese@unime.it

Keywords: dairy sheep, Visna-Maedi, legislation.

INTRODUCTION

The traditional farming system of Sardinia is linked to extensive and free-range grazing, limitations on farming premises and infrastructures availability, and low stocking rates. These features of the farming sector are also associated with specific animal diseases that are not usual in the rest of Europe such as Visna Maedi (VM).

MATERIALS AND METHODS

It is a viral disease affecting sheep transmitted through colostrum, contaminated milk, direct contact between animals in confined spaces and through the sharing of feeders and drinkers. A health emergency was recently declared in Sardinia due to the spread of VM and, in dairy sheep, the economic losses related to the disease are quite extensive. Unfortunately, the slow onset of clinical symptoms that characterizes VM (1) has important implications for their epidemiology and control at both the farm and regional levels, as well as for animal health and welfare. VM, in fact, clinically affects the quality of life of animals due to pain and disability.

From a legislative point of view, although Regulation (EU) 2016/429 (2) establishes control rules and measures to contrast and to eradicate several transmissible infectious diseases, it has repealed several diseases including VM. However, recognizing the socioeconomic impact of VM, the World Organization for Animal Health (WOAH) has included it in the list of notifiable terrestrial animal diseases, with significant impact on international trade of animals and their products (3).

RESULTS AND CONCLUSIONS

In the absence of EU legislation, the control of VM is the responsibility of national authorities of Member States who, with a compulsory or voluntary national control or monitoring program for this disease, may require additional guarantees. Therefore, Sardinia has the freedom to establish any methods of control and management of VM. In this context, it is necessary to highlight the need to review animal health legislation regarding VM in sheep.

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Effect of prepartum shearing on some metabolic parameters of ewes and their lambs

Cantou, I¹, Neimaur, K¹, Abreu, C¹, Scaglione, F¹, Rodríguez, P¹, Cal-Pereyra, LG¹, González-Montaña JR².

1. Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay.

2. Facultad de Veterinaria, Universidad de León, León, Spain.

Keywords: insulin, placenta, cotyledon, lamb weight

INTRODUCTION

Prepartum shearing (PS) has traditionally been associated with increased lamb survival under extensive grazing conditions. This could be explained by a multifactorial effect: an increase in the size of the placenta, greater flow of nutrients to the foetus, greater mobilization of the sheep's body reserves [1] and a change in maternal patterns of behaviour and in the supply and utilization of uterine nutrients [2]. Kenyon *et al.* [3,4] described those ewes sheared towards the middle of gestation had greater development of the placenta and, therefore, a greater weight of the lamb at birth. However, the mechanisms by which prepartum shearing achieves an increase in the birth weight of lambs are still unclear [5].

MATERIALS AND METHODS

To evaluate the effects of prepartum shearing at two different times of gestation on some metabolic and placental parameters of the mothers and its impact on the lambs, on day 70 of gestation, 37 ewes carrying a single foetus were randomly divided into three groups: sheep sheared on day 70 (PS70, n=12), sheep sheared on day 110 (PS110, n=12) and unshared ewes (US, control group, n=13). Energy metabolism (glycemia, insulin, NEFA, and BHB) and several placental parameters (placental weight, number and characteristics of cotyledons, and placental efficiency) were evaluated in the ewes. Live weight, various morphometric measurements, and body composition were determined in their lambs.

RESULTS AND CONCLUSIONS

Early and late prepartum shearing caused changes in energy metabolism, resulting in a decrease in serum insulin and an increase in blood values of NEFA and BHB. Shearing on day 70 (PS70) increased placental weight and cotyledon number and weight by 2 to 3 cm, while PS110 increased placental weight and only changed cotyledon weight by 2 to 3 cm. Both shearing, on days 70 and 110 prepartum, caused an increase in the birth weight of the lambs. Therefore, we can affirm that prepartum shearing, under extensive livestock farming conditions, is capable of inducing changes in the metabolic and placental profile of pregnant ewes, which resulted in an increase in weight and development of their lambs at birth, and even in later life.

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Clinical case: Mastitis in sheep flock. Diagnosis and control by somatic cells counting

González-Montaña, JR^{1,2}, Fernández-Olivera, A¹; Martín-Alonso, MJ³, Domínguez, JC^{1,2}; Alonso-de la Varga, ME⁴; Alonso Alonso P¹; Alegre-Gutiérrez B¹, Alonso-Diez, AJ^{1,2}

1. Medicine, Surgery and Anatomy Veterinary Department, Veterinary Faculty, University of León, Spain.

2. Institute of Animal Health and Cattle Development (INDEGSAL), University of León, Spain.

3. Facultad de Veterinaria. Universidad de Lleida, Spain.

4. Animal Production Department. Veterinary Faculty, University of León, Spain.

INTRODUCTION

On a dairy sheep farm, somatic cell count (SCC) is an essential and common procedure to ensure adequate milk quality. This count is used as an indicator of possible infection in the mammary gland, that is, animals with a high SCC in milk possibly suffer from mastitis, either clinical or subclinical [1,2]. A high SCC is associated with the loss of milk production and, therefore, with important economic losses, while a low number of somatic cells in milk translates into a correct health status of the animals and a greater economic profitability of livestock [3,4].

MATERIALS AND METHODS

A farmer requested our collaboration due to a high SCC in the milk produced, with a consequent decrease in the price of milk by the collection company.

For this reason, it was necessary to propose a series of control and prevention methods aimed at reducing the number of sheep affected by mastitis. These measures will depend on several factors, such as the animal itself, the microorganism involved, the time of lactation, the degree of udder involvement, the ewe's production, its age, etc. [5]. These factors will be fundamental in choosing how to proceed with each ewe, which traditionally ranges from the elimination of severely affected animals, to the use of antibiotic therapy during lactation, to drying therapy using intramammary cannulas or the so-called "selective management and/or selective drying" [6,7].

RESULTS AND CONCLUSIONS

For all these reasons, we established an action protocol aimed at: 1. Identifying sick animals with clinical mastitis; 2. Identify animals with high somatic cell counts in milk, 3. Establish a treatment and control strategy for sick animals; and 4. Apply selective treatment in different batches of animals.

The implementation of the indicated measures has had important economic and health consequences, significantly reducing the SCC in milk, while maintaining the quantity of milk produced unchanged. Furthermore, it also made it possible to reduce the cost of treatment, to avoid unnecessary work for the operators and, above all, to maintain an adequate health status of the farm.

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Welfare in dairy sheep and goat farming: methodologies and assessment of critical points

Paola Nicolussi¹ and Alessandra Gaffuri²

1 Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy

2 Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Bergamo, Italy

*Correspondence: paola.nicolussi@izs-sardegna.it

Keywords: small ruminants, welfare protocol, scoring

The Italian National Centre for Animal Welfare (CRENBA) has developed a protocol for the assessment of welfare in farmed animals (ruminants, pigs, poultry, rabbits), following the results of the European Welfare Quality Project and scientific opinions provided by EFSA (Check lists based on risk assessment). In European Union and National level there are currently no specific rules for sheep and particularly for goat's welfare; for these specie welfare indicators were based on the available scientific literature, on the European legislation (Council Directive 98/58/EC) and its Italian transposition (Legislative Decree 26 March 2001, n. 146), on the AWIN welfare assessment protocols for goats and for sheep and on an expert knowledge elicitation (Anzuino et al., 2010; Bertocchi et al., 2018; Mattiello et al., 2015; Muri et al., 2013). Another strong points of Classyfarm protocols are the items for the detection of observations on the animals, direct consequences of management and structures and equipment.

Classyfarm system (<https://www.classyfarm.it/>) for welfare assessment in sheep and goats is characterized by two different check lists: only one protocol for official control in sheep and goat farms, for all types of farming; the check list includes 38 total items, nine items regard ABMs (animal-based measures) and one biosafety. The judgement for each item is expressed considering all risk factors and can produce sanctions and prescription as corrective action.

Two different check lists for goats and sheep are used for self-control assessment and are applicable on dairy sheep and goat farms. There are 64/63 total items respectively for the two species; in addition there are 15 items regarding biosafety.

The check lists also include parameters divided into the following areas “Area A: farm management and staff training” “Area B: housing” “Area C: animal-based measures”, “Area D: major risks and alarm system”. For each indicator, the evaluator assigns a score based on a 2 or 3-point scale scoring system, where 1 indicated an insufficient status or high level of risk, 2 and 3 indicated respectively an acceptable and excellent status of the indicator, i.e., a low level of risk. Most of the items are the same for the two species, but there are some differences due to different ethological characteristics. For example, for goats the presence of environmental enrichments (such as stones, branches, trunk) is considered, because this makes the environment less monotonous, and responds to the ethological needs of a curious, active, and hierarchical animal like the goat.

The system can be applied to different types of farming, from intensive to extensive, and allows to carry out a risk assessment applied to animal welfare on farms.

However, only a good preparation and knowledge of each species allow to apply correctly the checklists respecting the purpose of the evaluation system. Studying the manual and attending theoretical and practical training courses are essential tools for correct application of the check list.

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Metabolic Stress in Dairy Cows: Implications for Dairy Cow Welfare

Jože Starič

Veterinary faculty, University of Ljubljana, Ljubljana, Slovenia

*Correspondence: joze.staric@vf.uni-lj.si

Keywords: negative energy balance, milk production, health

Metabolic stress is a critical concern in modern dairy farming practices due to its profound impact on the welfare of dairy cows. The multifaceted relationship between metabolic stress and dairy cow welfare is introduced to provide insights into the physiological, behavioral, and environmental factors contributing to this issue.

Dairy cows are continuously subjected to metabolic challenges as they are bred and managed for high milk production. These challenges manifest in various forms, including energy imbalances, nutrient deficiencies, and metabolic disorders such as ketosis and acidosis. These conditions not only compromise the overall health of dairy cows but also elicit a series of welfare-related concerns.

It is known that metabolic stress leads to several adverse welfare outcomes for the animal. However, it does not have consequences only for the animal, but also many for the breeder as it can result in decreased milk production, reproductive efficiency and too early culling, which not only affects farm profitability but places emotional and economic stress on the farmer.

Metabolic stress often induces painful conditions, such as lameness and mastitis, compromising the well-being of dairy cows. These physical discomforts negatively impact their mobility, behavior, and overall quality of life. Additionally, the behavioral responses of cows under metabolic stress, such as reduced feed intake and altered social interactions, can provide valuable insights into their emotional experiences and welfare state.

Mitigating metabolic stress in dairy cows involves comprehensive management practices, including appropriate nutrition, housing, and health monitoring. Proper feeding strategies that match cows' nutritional needs to their production demands can reduce the risk of energy imbalances and associated disorders. Adequate housing conditions, such as providing comfortable resting areas and minimizing social stressors, contribute to better overall welfare. Routine health assessments and early disease detection are crucial for preventing and managing metabolic disorders effectively.

In conclusion, metabolic stress in dairy cows is intricately linked to their welfare, affecting not only their physical health but also their emotional well-being. Addressing this issue requires a

holistic approach that encompasses nutritional, housing, health management practices and selection. Further research and the integration of innovative technologies are needed to develop sustainable solutions that prioritize the welfare of dairy cows while maintaining the demands of modern dairy production.

Acknowledgements

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Progress in understanding the genetic architecture of resistance to major diseases in sheep

Antonello Carta¹, Mario Graziano Usai,¹ Sara Casu¹, Sotero Salaris¹

¹Research Unit Genetics and Biotechnologies, Agris Sardinia, Loc. Bonassai, 07100 Sassari

Correspondence: acarta@agrisricerca.it

Keywords: Genetic resistance, Disease, Sheep

Selective breeding through genomic tools is crucial to reduce the impact of diseases on the economic sustainability of local breeds of sheep. A female population in a nucleus flock (FRP) has been set up to study the genetic architecture of resistance to main diseases in the Sarda breed. The introduction of resistance to Gastrointestinal Nematodes infestation (GIN), Paratuberculosis (PTB) and Maedi Visna (MV) infections as selection objectives needs to estimate their heritabilities and genetic correlations as well as the existence of genomic regions with major effects. The FRP ewes were genotyped (Illumina 50Kchip) and pedigree recorded from 2000 to 2022 (4,940 ewes). Moreover, 17,679 individual fecal eggs counts *i.e.* the number of GIN eggs per g of faeces (FEC), of 4,681 ewes were available. FEC was then log-transformed (lnFEC) for genetic analyses. The infection status for PTB of 3,032 ewes (1 for infected and 0 for not infected) was determined by 14,349 ELISA screening tests from 2001 to 2012. A ewe with at least one positive test along the recording period was considered affected by PTB. From 2016 to 2019, 770 ewes were first tested at one year age to determine the infection status for MV (1 for infected and 0 for not infected). ELISA tests were repeated every six months until 36 months of age just for ewes with a negative previous test. Finally 1,963 ewes had a MV status and were genotyped for the E35K polymorphism on TMEM154 gene. Means and standard deviations were 4.82 ± 1.43 for lnFEC. The ewes considered affected by PTB and MV were 906 and 740 respectively. The infection status along the lifetime for PTB and MV and repeated measures for GIN were used to estimate h^2 with single trait analyses considering the genomic relationship matrix. Genetic correlations were then estimated with bi-trait animal models using pseudo-phenotypes derived from the single trait models: the sum of genetic and residual predictions for MV and PTB and the sum of the genetic, permanent environment predictions and the average of residuals for GIN. The pseudo-phenotypes were also used for the detection of significant genomic regions with a Linkage Disequilibrium Linkage Analysis. Heritabilities were 0.22 for GIN, 0.22 for PTB and 0.20 for MV. Genetic correlations of GIN were weakly favorable with PTB (0.17) and negligible with MV (-0.04). The genetic correlation between PTB and VM was moderately favorable (0.33). For MV, it was confirmed the strong effect of the E35K polymorphism of TMEM154 gene: KK genotype much more resistant than KE and EE. Ten significant regions were found on 9 chromosomes for GIN. One high significant region was found for PTB on chromosome 20 in the MHC region. Selective breeding for resistance to GIN, PTB and MV is a realistic option since relevant polygenic additive variations and one major gene for MV were detected. Further research is needed to discover causal mutations in the significant regions for PTB and GIN.

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Breeding camels in Tunisia: reality and prospects

Aboubaker Ben Smida¹ and Monia Lachtar²

¹ UNISS-PhD student Department of Veterinary medicine-SASSARI Italy

² National Center of Zoosanitary Vigilance, Tunisia

Correspondence:

boubaker.bensmida@gmail.com

lachtarmonia@gmail.com

Keywords: rearing, dromedary, Maghreb, disease, camel production

In Tunisia, Camel breeding is crucial at the socioeconomic level. Ninety-nine percent of camel herds are mainly concentrated in southern Tunisia. The Maghrebi camel is the only breed raised in this region. There are three distinct camel breeding systems. The extensive system is the principal method of dromedary raising, in which breeders permit camels to roam without restriction. In most cases, herd management is under the supervision of the camel herders. During the period from March to September, herders moved their animals, then they grouped, and delivered feed supplementation to their herds between October to March, which coincides with heat and birth season. At the same time, the office of livestock and pasture and the veterinary service organizes annual campaigns to control trypanosomiasis and scabies, vaccinated against camelpox, and identified animals using modern (ear tags) and traditional methods (branding).

The common diseases that infect camels in Tunisia are scabies, camelpox, ringworm, and trypanosomiasis. However, the control of those diseases remains difficult due to herd mobility and farm dispersion.

Concerning production, meat is the main product of the camel, while milk is mostly for household consumption. In addition to meat and milk, camel fiber and leather are also secondary products achieved through camel farming.

Finally, camel breeding in Tunisia exhibits inherent strengths, such as the adaptability of camels to arid environments, the foundation of mutual agricultural services, and the establishment of a national program for development and research on camel production.

Diversification through camel products like milk, meat, and hides and low production cost provides multiple revenue streams. However, weaknesses include the development of reproductive biotechnologies and genetic improvement, challenges in market access, inadequate infrastructure, and the decrease in qualified breeder numbers. Opportunities lie in the growing demand for camel products, tourism and cultural promotion, investment in research and development, and government support. Meanwhile, threats include the impact of climate change on grazing land, the absence of marketing camel products, and the increase in feed prices. Regular monitoring and adaptation of strategies are crucial to navigate these factors and ensure the sustainable development of the camel breeding industry in Tunisia.

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Enhancement of Sardinian breed goats in marginal areas: considerations relating to genetic heritage, resistance to pathologies, adaptability to the local environment and longevity

Piera Angela Cabras ¹; Cosimo Zidda ²; Simone Dore ³; Andrea Orrù ²; Anna Maria Deiana ¹; Ennio Bandino ⁴

¹ Istituto Zooprofilattico Sperimentale della Sardegna - Centro Territoriale Tortolì (Nu)

² IZS Sardegna - Centro di Referenza Nazionale per le Produzioni Biologiche (CRNPB)

³ IZS Sardegna - Centro di Referenza Nazionale per le Mastopatie degli Ovini e dei Caprini (CReNMOC)

⁴ IZS Sardegna - SC Diagnostico Territoriale Nuoro (Nu)

Correspondence: pierangela.cabras@izs-sardegna.it

Keywords: Ancient Goat, Sardinia, biodiversity

INTRODUCTION

The aim of this work is to deepen the health, genetic, production, adaptability and resistance knowledge of the Sardinian breed goat in marginal environments, for the purposes of protection and enhancement of local biodiversity. The study was carried out in the territories of Ogliastra (Sardinia, Italy), located in a *Blue Zone*, where there is a high concentration of centenarians, also with the aim of identifying the possible existence of factors linked to longevity.

MATERIALS AND METHODS

Nineteen goat farms were selected and included in the study. Clinical tests were carried out with the compilation of herd and individual records, with blood sampling for serological and genetic tests, milk sampling for the research of milk characteristics and mastitis agents, stool samples for parasitological examination and nasal swabs for Enzootic Nasal Tumor (ENT).

RESULTS AND CONCLUSIONS

From the examination of 103 hemoserum, the following results are highlighted: Brucellosis and Toxoplasmosis, all negative; Chlamydia, 13 positive (12,6%); Q fever, 16 positive (15,5%); Paratuberculosis, 13 positive (12,6%); Caprine Arthritis Encephalitis (CAE), 75 positive (72,8%). mass fecal tests carried out on 30 samples showed the presence of gastrointestinal strongyles, coccidia, tapeworms and bronchopulmonary strongyles while flukes were found in no farm. Enzootic Nasal Tumor was found in 3 cases out of 96 animals examined (3,1%).

The genetic examination highlighted genetic diversity parameters of the population of Ancient Sardinian Goat comparable with those of the comparison populations. These results are congruent with a high level of genetic variability of the Sarda breed and high adaptive potential, due to high levels of ancestral variability not reduced by selection practices.

A statistically significant higher content of SFA (Saturated Fatty Acids), UFA (Unsaturated Fatty Acids), total protein, casein, Solid Non Fat (SnF) and a significant reduction in chloride content were observed in the milk of Sarda goats compared to other breeds. In 90 out of 102 milk samples (88.2%) the result of the bacteriological examination was negative.

This study highlighted relevant information related to the conservation of the genetic heritage of the primitive Sarda breed. In fact, noteworthy is the presence of five individuals of ancient Sardinian breed with 'pure' genomic make-up, belonging to three different farms, in which no positivity for CAE and the main zoonoses was found. They live in territories particularly impervious, in which other breeds could hardly survive. In conclusion, on the basis of what emerged from the study, worthy of further investigation, it certainly appears desirable to undertake a complex path of protection and enhancement of the Sardinian breed species, symbol of local biodiversity and resilience linked to the

characteristics of the harsh territories where they live, which are part of the ancestral cultural heritage of the centenarians.

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Frequency of deleterious mutations causing recessive disorders in Italian Chianina, Marchigiana and Romagnola cattle

Joana G. P. Jacinto^{1,2,*}, Arcangelo Gentile¹, Fiorella Sbarra³, Cord Drögemüller¹

¹ Department of Veterinary Medical Sciences, University of Bologna, Italy

² Institute of Genetics, Vetsuisse Faculty, University of Bern, Switzerland

³ National Association of Italian Beef-Cattle Breeders, Italy

Correspondence: joana.goncalves2@studio.unibo.it

Keywords: Disease allele frequency, Inbreeding, Systematic genotyping

INTRODUCTION

In the last two decades, the molecular cause of six monogenic autosomal recessive disorders has been identified in native Italian beef cattle: two different ATP2A1 variants for the pseudomyotonia congenita, the first in Chianina and Romagnola (PMT1) and the second in Romagnola (PMT2); a KDM2B variant for the paunch calf syndrome (PCS) in Marchigiana and Romagnola; a NID1 variant for the congenital cataract (CC) in Romagnola; a LAMB1 variant for the hemifacial microsomia (HFM) in Romagnola; an ABCA12 variant for the ichthyosis fetalis (IF) in Chianina and a FA2H variant for the ichthyosis congenita (IC) in Chianina. The aim of this study was to evaluate the potential impact of these disorders in the affected Italian populations.

MATERIALS AND METHODS

In total, 3331 Chianina, 2812 Marchigiana and 1680 Romagnola male animals were included in the study. The study involved semen samples from artificial insemination (AI) sires top listed in the annual catalogue, as well as blood samples from young males shortlisted for admission to performance testing at the testing station. Genomic DNA was isolated from these samples using standard methods. Three variants (ATP2A1:p.Arg164His; KDM2B:p. Asp835Asn; ABCA12:p.His1935Arg) were genotyped either by Sanger sequencing of PCR products as described before or by extraction of available SNP array genotyping data from routine genomic selection. Four further variants (ATP2A1:p.Glu211Val; NID1 deletion, LAMB1:p.Arg668Cys; FA2H insertion) were genotyped via PCR-based methods.

RESULTS AND CONCLUSIONS

The allelic frequency (AF) of the variant for PMT1 was 1.0% in Romagnola, 4.6% in Marchigiana and 5.9% in Chianina. The AF of the variant for PMT2 was 3.3% in Romagnola and 0% in the other two breeds. The AF of the variant for PCS was 11.7% in Romagnola, 2.0% in Marchigiana and 0% in Chianina. The AF of the variants for CC, HFM, IF and IC resulted below 3%, being the variants detected only in the breed populations in which they were previously reported. Considering a selected male population in the single breed, Chianina showed carrier prevalence of 11.9% for PMT1, 7.7% for IC and 6.4% for IF. Romagnola showed carrier prevalence of 23.4% for PCS, 6.7% for PMT2, 4.1% for HFM, 3.2% for CC and 2.0% for PMT1. Marchigiana showed carrier prevalence of 9.1% for PMT1 and 4.0% for PCS. With respect to the Romagnola cattle, the concerning presence of a total of five defect alleles in the population hampers a general approach based on the prevention of carriers from artificial insemination. However, identification of carriers may allow conscious mating to prevent the risk of homozygous descendants as well as the spread of heterozygous offspring.

Therefore, systematic genotyping for all seven known harmful alleles is recommended to prevent risk mating between carriers, particularly to avoid affected offspring.

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***Dicrocoelium dendriticum* in roe deer (*Capreolus capreolus*) in central Italy: distribution and risk factors**

Stefano Scarcelli¹, Francesco Buono¹, Elisa Castaldo¹, Paolo Varuzza¹, Felice Argenio¹, Elvio Lepri², Manuela Diaferia², Paola Pepe¹, Maria Paola Maurelli¹ and Vincenzo Veneziano¹

¹ Department of Veterinary Medicine and Animal Productions, University of Naples 'Federico II', Naples, Italy.

² Department of Veterinary Medicine, University of Perugia, Perugia, Italy.

*Correspondence: yinvene@unina.it

Keywords

Hunting, Liver flukes, Trematodes

INTRODUCTION

Dicrocoelium dendriticum (lancet fluke or small liver fluke) is a trematode that infects both domestic and wild animals (Cringoli et al., 2002. Vet Parasitol, 108: 137-43). It is characterized by an indirect life cycle involving two invertebrate intermediate hosts: terrestrial snails and ants. In Italy *D. dendriticum* is the most widespread fluke in sheep farms (Bosco et al., 2015. Small Rumin Res, 126: 2-5). The aim of the present study was to investigate the liver flukes' infection in roe deer (*Capreolus capreolus*) in central Italy and the risk factors associated with infection.

MATERIAL AND METHODS

The study was conducted from June 2021 to March 2022 in central Italy (Tuscany, Marche and Umbria regions) on 265 roe deer's liver. Age class (juvenile, <1 year; yearlings, 1-2 years; adults, >2 years), sex, hunting area, and hunting period were recorded for each animal and considering infection predictor factors. Each liver was macroscopically evaluated for the presence of lesions using a five-point liver lesion score (Jithendran and Bhat, 1996) and cut into slices, washed with tap water, and palpated to dislodge flukes within the bile and hepatic ducts to detect the adult stage (Sgroi et al., 2021. Comp Immunol Microbiol Infect Dis, 77: 101672). The water was filtered with a sieve (0.5 mm) and the remaining contents were examined under stereomicroscope. Adult parasites were collected, counted, and fixed in 70% ethanol for morphological examination using dichotomous keys (Otranto et al., 2007. Acta Trop, 104: 91-8). Statistical analysis was performed using a chi-squared test (χ^2). *P* values <0.05 were considered statistically significant.

RESULTS AND CONCLUSIONS

Of 265 roe deer, 54 were juvenile, 60 yearlings, and 151 adults; 97 were males and 168 females. Regarding hunting area 198, 60, and 7 came from Tuscany, Umbria, and Marche regions, respectively. Moreover, 111 livers were collected during summer and 154 during winter. An overall prevalence of 19.6% (52/265 - mean intensity (MI) of 17.1; min 1 – max 354) was reported. The prevalence of infection was 24.1% in juveniles (MI 43.0, min 2 – max 354), 20.0% in yearlings (MI 8.6, min 1 – max 43), and 17.9% in adults (MI 8.5, min 1 – max 41) ($\chi^2 = 0.97$, *p* = 0.61); 14.4% in male (MI 10.6, min 1 – max 42) and 22.6% in female (MI 19.6, min 1 – max 354) ($\chi^2 = 2.61$; *p* = 0.11). In Tuscany and Marche regions a prevalence of 24.7% (MI 17.7, min 1 – max 354) and 42.9% (MI 8.3, min 1 – max 16) was reported whereas no positive samples were recorded in Umbria region ($\chi^2 = 20.34$; *p* = <0.001). The prevalence in summer and in winter was 24.3% (MI 8.3, min 1 – max 43) and 16.2% (MI 26.7, min 1 – max 354), respectively ($\chi^2 = 2.68$; *p* = <0.10). No macroscopic lesions were observed. Roe deer is characterized by a low intensity of infection of *D. dendriticum*

and it should be considering as animal *in refugia* reducing the pressure for the development of anthelmintic resistance in sheep.

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Cloning, expression and purification of recombinant proteins from *Staphylococcus aureus* and their potential for vaccine development

Elisa Azara¹, Carla Maria Longheu¹, Antonio Foddai², Nives Maria Rosa¹, Grazia Cillara¹, Simona Broccias¹, Gonaria Puddu¹, Maria Filippa Addis^{3,4}, Sebastiana Tola^{1*}

¹Istituto Zooprofilattico Sperimentale della Sardegna, Italy

²Teesside University, School of Health and Life Sciences, Middlesbrough, Tees Valley, TS1 3BX, UK

³Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, 26900 Lodi, Italy

⁴Laboratorio di Malattie Infettive degli Animali (MiLab), Università degli Studi di Milano, 26900 Lodi, Italy

*Correspondence: sebastiana.tola@izs-sardegna.it

Keywords : *Staphylococcus aureus*, sheep, recombinant protein

INTRODUCTION

Staphylococcus aureus is the leading cause of clinical mastitis and is associated with persistent subclinical infections in ewes, significantly compromising the quality and quantity of milk productions. To date, vaccines intended for use in sheep have focused mainly on biofilm production traits, but many pathogenic *S. aureus* isolates do not produce biofilm, including those circulating in Sardinia, a major producer of sheep's milk in Europe. The aim of this work was to identify suitable alternative immunodominant candidates to biofilm components for vaccine and diagnostic development (1,2). An immunoproteomics study was carried out by analyzing sera from naturally infected sheep with a prevalent *S. aureus* lineage against cellular and secreted antigens, followed by tandem mass spectrometry identification of the most prominent immunogens. Seven *S. aureus* antigens: pyruvate kinase, elongation Factor Tu, dihydrolipoyl dehydrogenase, alpha-keto acid dehydrogenase, the bifunctional autolysin (Atl) and the two components of the Panton-Valentine leukocidin, lukF-PV/lukM, elicited strong host humoral immune response (3).

MATERIALS AND METHODS

In the present study, a 3D structural prediction analysis was carried out with the identification of the best B cell epitopes of *S. aureus* AtlA and LukF-PV/LukM. Fragments of *atlA* and *lukF-PV/lukM*, containing the predicted main epitopes, were amplified and cloned for recombinant protein production. Oligonucleotide sets were designed for directional and ORF cloning of the gene fragments by inserting engineered restriction sites into the forward and reverse primers. Both the PCR products and the expression vector pQE-30 (Qiagen, Chatsworth, CA, USA) were digested with the restriction enzymes *Bam*HI and *Kpn*I. Each construct was used to transform *Escherichia coli* DH5α cells containing pREP-4 repressor plasmid (*lacIq*). Cloning into pQE-30 allows the production of recombinant proteins linked to a polyhistidine (His) stretch that binds strongly to nickel-chelated columns (Qiagen).

RESULTS AND CONCLUSIONS

To prevent mastitis and its spread, and consequently reduce the use of antibiotics, it is essential to guarantee adequate breeding conditions and animal health, placing the emphasis on the adoption of good farm management practices and the application of adequate biosafety measures. Vaccination can also play a strategic role in the control of mastitis. Vaccines are essential for stimulating the immune response and protecting against disease (4). In the present study, computation analysis, one of the most important branches of bioinformatics, was used to analyze both *S. aureus* AtlA and LukF-PV/LukM, providing information on the main predicted epitopes and enabling the production of recombinant proteins including these epitopes. The selected clones produced recombinant proteins showing strong reactivity with hyperimmune sera against the native AtlA (5) and LukF-PV/LukM and with blood sera collected from sheep with clinical *S. aureus* mastitis. These could represent

potential protein-based vaccines candidate able of eliciting a protective immune response to be evaluated by vaccination and subsequent challenge of the vaccinated sheep.

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Paramphistomidosis: an emergent parasitic disease of ruminants in Europe

Pablo Díaz

Universidade de Santiago de Compostela, Lugo, Spain

*Correspondence: pablo.diaz@usc.es

Keywords: Paramphistomid, ruminants, Europe

Paramphistomids are trematodes affecting the digestive tract of domestic and wild ruminants; they can cause severe lesions leading to significant productive losses. Ruminants, the definitive hosts, pass eggs in their faeces, contaminating the environment. Under suitable conditions, a miracidium emerges from eggs, searching for freshwater snails (*Galba truncatula*) which act as intermediate hosts (IH). Within the snail, miracidia develop to sporocysts, then rediae and finally cercariae, which encyst in the nearby vegetation. Ruminants become infected by ingesting the metacercariae. Immature stages feed on the duodenal mucosa and later show a backward migration reaching the rumen and reticulum, where they develop into adults and begin to eliminate eggs. Although adult rumen flukes are generally well tolerated, heavy infections with immature paramphistomids can result in clinical disease, characterized by watery diarrhoea, dehydration, anaemia and oedema; severe cases can be fatal.

Although paramphistomids infections are particularly prevalent in tropical and subtropical regions, recent studies in cattle indicate a significant increase in their incidence in Europe in recent decades, especially in Western countries. For example, in cattle from central France, Mage et al. (2002) reported an increase in the prevalence from 5.2% (1990) to 44.7% (1999). In Ireland, Zintl et al. (2014) observed prevalences up to 8.8% in the period 2004-2008, whereas the percentage of positive cows ranged from 17.3% to 31.6% between 2009 and 2013. In addition, a significant increase was also observed in cattle from northern Spain; thus, prevalences rose from 12% in 2006 to 52% in 2021. In small ruminants, prevalences are usually lower than in cattle (Table 1). Nevertheless, analysis of available data reveals that the percentage of sheep and goats positive to this trematode is also increasing in the continent. Therefore, paramphistomidosis is currently considered an emerging disease in Europe.

Some investigations have suggested that rumen fluke prevalences are progressively increasing due to several causes such climatic change, lack of effective treatments, livestock movements from endemic regions, better laboratorial diagnosis or the increase of grazing farms. Moreover, a competition of *Fasciola hepatica* and paramphistomids within the IH has been suggested; measures applied for controlling liver fluke infections, including effective treatments, favour the completion of the life cycle of paramphistomids.

In conclusion, paramphistomids are more common in cattle than in small ruminants; available data reveals that they are widespread in Europe but especially prevalent in Western countries. The increase in the prevalence is related to increased environmental contamination, which may lead to an increase in clinical cases in a rather short period. Therefore, paramphistomidosis should be included in the differential diagnosis of digestive problems of ruminants in this continent.

Acknowledgements

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Table 1. Percentage of animals infected with paramphistomids in European studies including at least two ruminant species

Country	Cattle	Sheep	Goat	Reference
Ireland	52%	14%		Toolan et al. (2015)
UK	59% farm	42% (farm)		Jones et al. (2017)
UK (Scotland)	43,3%	13,5%		Busin et al (2023)
Italy	55% (farm)	7,9% (farm)	2,7% (farm)	Bosco et al. (2021)
Italy (Sardinia)	10,9% (55,9% farm)	2% (13,9% farm)		Sanna et al (2016)
Germany		5,1% (farm)	0% (farm)	Alstedt et al (2022)
Germany	12,7%	3,8%		May et al. (2022)
Netherlands	15,8% (farm)	8% (farms)		Ploeger et al. (2017)
Greece	0,3%	0%	0%	Theodoropoulos et al. (2002)
Spain	51,2%	14%		García Dios (2023)

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***Toxoplasma* infection in goats in Pakistan; risk factors and public health significance**

Muhammad Y. Khan^{1,2*}, Alessandra Barlaam¹, Alessia L. Gazzonis³, Nicola Ferrari³, Alejandro Jiménez-Meléndez⁴, Lucy J. Robertson⁴ and Annunziata Giangaspero¹

¹University of Foggia, Foggia, Italy

²University of Veterinary & Animal Sciences, Lahore, Pakistan

³University of Milan, Lodi, Italy.

⁴Norwegian University of Life Sciences (NMBU), Ås, Norway

*Correspondence: muhammadyaser.khan@unifg.it

Keywords: *Toxoplasma gondii*, goats, epidemiology

INTRODUCTION

Toxoplasma gondii is a parasitic protozoan infecting warm-blooded animals and humans associated with fetal mortality as well as a spectrum of other symptoms. The infection mainly occurs through ingesting undercooked or raw animal products (meat, but also milk) from infected animals or water or raw vegetables contaminated with the oocyst transmission stage shed in the feces of the feline definitive hosts (Dubey, 1991. Southeast Asian J. Trop. Med. Public Health. 22: 89–92). Our study in Layyah District (Pakistan) investigated: the prevalence of *T. gondii* in goats via the analysis of blood for anti-*T. gondii* antibodies, the risk factors associated with infection, the presence of *T. gondii* DNA in milk, and its potential public health risk.

MATERIALS AND METHODS

All goat farms in the study area were stratified by size, and 110 were randomly selected. From 12 goats from each farm (>1 year old), 1320 sera samples were collected and tested by ELISA. A questionnaire on conditions and management practices of each farm was administered to farmers for risk factor analysis. From three highly seropositive farms, 360 milk samples were collected from 40 goats in each farm at three different lactation intervals. Furthermore, brain and placenta tissue samples from three aborted goats were collected. Milk and tissue samples were tested by qPCR (B1 gene) for *T. gondii* DNA (Temesgen et al., 2019. Food Res. Int. 125: 108636).

RESULTS AND CONCLUSIONS

Overall, 31.5% (95% CI: 28.3–34.8) sera samples were positive for anti-*T. gondii* antibodies and 89.1% of the flocks had at least one seropositive goat. Based on questionnaire responses, all farms had semi-intensive systems with 79% of dirt floors. Cats were present in 61.8% of farms with up to 52.7% having access to stables; 82.7% of farms reported goat abortions. Moreover, 68.2% of farmers practiced domestic slaughter and the majority (80%) had from no to primary-level education; 60% had no access to drinking water, while consumption of raw milk, raw meat, and raw vegetables was reported by 54.5%, 55.5%, and 57.3% of respondents, respectively. In addition, 20% of farmers reported a family abortion history. Significant effects on *T. gondii* seroprevalence were associated with goat farms having dirt floor and kitten presence ($P < 0.001$). *T. gondii* seroprevalence was also proportional to adult cat presence, age class, abortion history and water source supply modulated by owner education levels. Among three highly seropositive farms, all had at least three goats in which *T. gondii* DNA was detected in the milk samples. All aborted tissues, except one fetal brain tissue, were found positive for *T. gondii*.

This is the largest epidemiological study on *T. gondii* in goat farms in Pakistan. The presence of *T. gondii* amongst goats in areas where goat farming has a crucial economic role may pose a

production threat to the small-stock industry and public health. Identification of high-risk goat populations could facilitate the implementation of targeted local control strategies.

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Development of highly sensitive digital droplet PCR for detection of *Toxoplasma gondii* in bovine meat

Andrea Mancusi¹, Angela Giordano¹, Antonio Bosco², Santa Girardi¹, Yolande T.R. Proroga^{1*}, Laura Rinaldi², Federico Capuano¹, Giuseppe Cringoli², Maria Paola Maurelli²

¹Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy

²Università degli Studi di Napoli Federico II, CREMOPAR, Italy

Correspondence*: yolande.proroga@izsmportici.it

Keywords *Toxoplasma gondii*, Toxoplasmosis, Droplet digital polymerase chain reaction (ddPCR)

INTRODUCTION

Toxoplasmosis is a zoonotic infection with a worldwide distribution caused by the intracellular protozoan *Toxoplasma gondii*. *T. gondii* infection has also important veterinary implications especially in small ruminants where toxoplasmosis mainly affects the reproductive organs resulting in abortions. The molecular biology methods are the most used direct techniques. Different protocols of PCR end-point, nested PCR and real-time PCR (qPCR) to amplify the B1 gene or the 529 bp repeat element that are the most used target have been described. However, the development of new diagnostic tools more sensitive and specific is always in progress. The droplet digital polymerase chain reaction (ddPCR), novel PCR that provides absolute and direct quantification of target DNA, has been successfully used for detection of different parasites, e.g., *Cryptosporidium* in different animal hosts and humans. The aim of this paper is to develop and validate a new ddPCR assay for detection and quantification of *T. gondii* DNA in meat of intermediate hosts.

MATERIALS AND METHODS

To optimize the digital droplet PCR (ddPCR), specific reference strains were obtained from the American Type Culture Collection (ATCC). The stock solution of *Toxoplasma gondii* ATCC 50174D contained $\sim 2 \times 10^5$ cg/ μ l genome equivalent. Twenty-five grams of 80 minced negative cattle meat samples were homogenized in a stomacher, then total DNA was extracted, using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany), according to the manufacturers' instructions. To evaluate the limit of detection at 95% of probability (LOD₉₅) of ddPCR five concentration levels: 8000 cg/ μ l, 800 cg/ μ l, 80 cg/ μ l, 8 cg/ μ l were used. The ddPCR reaction was performed using the protocol, primers and probe to amplify the region Toxo-529bp repeat element of the parasite described by Mancusi et al. (2023) [1]. The ddPCR was performed in a QX200 system. The LOD₉₅ obtained for ddPCR was 8 gc/ μ l. The sample was considered positive if showed \geq two droplets.

RESULTS AND CONCLUSIONS

The LoD₉₅ obtained for ddPCR was 8 gc/ μ l in ddPCR. The number of droplets generated for reaction ranged from 8985 to 13,940, with an average of 11,384 droplets. Reactions with more than 8000 accepted droplets for well were used for analysis. The sensitivity of ddPCR was 97.5% (95% CI=85.3–99.9) and specificity 100%. Of the 171 samples examined, the qPCR reference method detected *T. gondii* in only two samples (1.2%; 95% CI=0.2–4.6) while ddPCR detected 13 positive samples (7.6%; 95% CI=4.3–12.9). The positive samples not detected by qPCR showed

concentrations ranging from 0.3 to 17.1 gc/μl. However, this innovative approach could be very useful for a rapid detection of small amounts of *T. gondii* in meat, as well as in other food matrices, e.g. milk, cheese, vegetables and molluscs, so to perform valid control strategies, aimed to reduce the risks of toxoplasmosis infection in animals and humans, according to the One Health approach.

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Development of an active surveillance system in Italy to estimate the real prevalence of Echinococcosis in sheep.

Gabriella Masu¹, Piero Bonelli¹, Elisa Serra¹, Federica Loi², Marialuisa Buonanno³, Andrea Carvelli⁷, Monica Pierangela Cerioli⁴, Silvia Crotti⁵, Patrizia Danesi⁶, Manuela Iurescia⁷, Annalisa Santi⁸, Sandro Rolesu², Angela Peruzzu¹, Giovanna Masala¹ and Toni Piseddu^{1*}

¹Istituto Zooprofilattico Sperimentale della Sardegna, WOA Reference Laboratory, National Reference Laboratory of Echinococcosis (CeNRE), Sassari, Italy

² Osservatorio Epidemiologico Veterinario Regionale della Sardegna, Cagliari, Italy

³Istituto Zooprofilattico Sperimentale del Mezzogiorno, Napoli, Italy

⁴ Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Brescia, Italy

⁵Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy

⁶Istituto Zooprofilattico Sperimentale delle Venezie, Legnago,

⁷Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Roma.

⁸Istituto Zooprofilattico della Lombardia e dell'Emilia-Romagna.

*Correspondence: toni.piseddu@izs-sardegna.it

Keywords: Parasitic disease in ruminants, Zoonosis, Animal Health

INTRODUCTION

Cystic Echinococcosis (CE) is a chronic and disabling neglected zoonotic infection caused by the larval stage of taeniid tapeworm *Echinococcus granulosus* s.l., a complex species that includes several genotypes and cryptic species [Romig, T. et al. (2017) 'Ecology and Life Cycle Patterns of Echinococcus Species', Adv. in Par., 95, pp. 213–314]. Environmental and anthropogenic factors influence the CE distribution in different parts of the world [Deplazes, P. et al. (2017) 'Global Distribution of Alveolar and Cystic Echinococcosis', Adv. in Par., 95, pp. 315–493]. Previous studies conducted by CeNRE demonstrate that official data on the prevalence and distribution of EC in sheep and humans are imprecise and underestimated [Piseddu, T. et al. (2017) 'The disease burden of human cystic echinococcosis based on HDRs from 2001 to 2014 in Italy', PLoS Negl. Trop. Dis., 11(7)] [Loi, F. et al. (2019) 'Prevalence estimation of Italian ovine cystic echinococcosis in slaughterhouses: A retrospective Bayesian data analysis, 2010–2015', PloS One, 14(4)]. In Italy the monitoring of the presence of CE in sheep farms occurs by passive surveillance during post-mortem inspection in slaughterhouses.

MATERIAL AND METHODS

In this project the CeNRE proposed an active surveillance plan that assembled the activities with those of the official monitoring of the National Plan of ovine-caprine TSE (Reg. 999/2001/CE) during slaughter. In this way, after the evaluation of the possible presence of E.g.s.l. cysts, the samples were collected and subsequently sent to the territorial IIZZSS for diagnostic confirmation and molecular analysis. The objectives of the research were to verify the effectiveness and efficiency of an experimental model of active surveillance and to validate, with prevalence estimation data, the elaborations prior carried out.

RESULTS AND CONCLUSIONS

The active surveillance protocol was correctly applied in the Sardinia, Emilia-Romagna and Lombardy regions. The sample size and distribution allowed us to obtain processable results, which confirm the definition of endemic area for the Sardinia region (61%) and a low-risk geographical area for the Lombardy and Emilia-Romagna regions (<2%). The prevalence observed in Lazio was higher than expected (20.4%), for Tuscany

the data cannot be compared with previous research, but the observed prevalence stands at low levels (5%). Most of the remaining UU.OO. by establishing a fruitful collaborative relationship with the Regional Prevention Departments, while not applying the new protocol instead adopting passive surveillance, they were able to find EC-positive farms even in geographical contexts not recognized as "endemic" until now. It has been highlighted how the different genotypes of E.g.s.l. show different characteristics from an epidemiological, morphological and biological point of view which can have important implications from a diagnostic point of view and in the surveillance or control strategies of the pathology. In a relatively short fragment of the mitochondrial nad5 gene of E.g.s.s. there are six SNPs capable of effectively distinguishing the G1 genotype from the G3 genotype. In our previous work we confirmed the informative value of three SNPs (758, 1123, 1380) and their potential use in a TaqRTPCR assay capable of adequately distinguishing the two genotypes. Our results confirm the high genetic variability of E.g.s.s. highlighting the importance and need to continue the study of the genetic diversity of the parasite for a more adequate control strategy in endemic areas.

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Efficacy of treatments with macrocyclic lactones in different formulations against gastrointestinal nematodes of sheep in Sardinia, Italy

Antonio Scala^{1*}, Antonio Spezzigu², Claudia Tamponi¹, Lia Cavallo¹, Carlo Carta¹, Maria Francesca Sini¹, Francesca Nonnis¹ and Antonio Varcasia¹

¹Department of Veterinary Medicine, University of Sassari (Italy);

² Embryosardegna, tecnologia, riproduzione e fertilità, Perfugas (SS), Italy.

*Correspondence: scala@uniss.it

Keywords: sheep, macrocyclic lactones, anthelmintic resistance.

INTRODUCTION

To date, anthelmintic resistance (AR) in sheep has emerged as a significant global health concern. Reports of this phenomenon, particularly concerning gastrointestinal nematodes (GIN), are increasing, and various studies are being conducted to mitigate its progression. These studies explore approaches such as targeted selective treatment, genetic selection, grazing management, nutritional supplementation, phytotherapy, and more (Maurizio et al., 2023). The first step to prevent AR is the ongoing monitoring of treatment effectiveness within the area. Therefore, this report presents the results of a monitoring study aimed at assessing the efficacy of different formulations of macrocyclic lactones (MLs) for the treatment of GIN in Sardinian sheep.

MATERIALS AND METHODS

Between 2022 and 2023, we conducted an evaluation of the efficacy of 12 anthelmintic treatments against gastrointestinal nematodes (GIN) in Sardinia, Italy. These treatments involved various formulations of MLs administered at recommended doses by pharmaceutical manufacturers (6 treatments with subcutaneous (SC) injectable ivermectin, 1 with oral ivermectin, 1 with injectable moxidectin, 3 with oral moxidectin, and 1 with injectable eprinomectin). The efficacy of the treatment was calculated on the basis of the faecal reduction egg count (FECR) of time T14 in comparison with time T0, according to the indications provided by the W.A.A.V.P. (Coles et al., 1992). This evaluation utilized data obtained from individual qualitative-quantitative coprological analyses conducted using McMaster's method, with a supersaturated NaCl solution, at both D0 and D14. In cases where efficacy was less than 90%, coprocultures were initiated to identify the GIN species suspected of displaying resistance.

RESULTS AND CONCLUSIONS

All treatments administered with ivermectin via SC demonstrated AR, as per the parameters outlined by Coles et al. (1992) (% efficacy from 94% (95%CI= 91-95) to 38% (95%CI= 32-44)), as well as the one with eprinomectin by SC (% efficacy of 52.2%, 95%CI= 49.9-54.5). In these cases, coprocultures conducted at D14 identified primarily *Trichostrongylus*, with some presence of *Teladorsagia*. In contrast, there was no observed resistance against moxidectin when administered via SC, with an efficacy of 93% (95%CI= 91-95). The treatments administered through oral formulations (drench) of ivermectin and moxidectin were highly effective, with efficacy rates ranging from 99% to 100%.

These findings underscore the critical importance of choosing the appropriate administration route for macrolides in the treatment of GIN in sheep of Sardinia. The surveillance results indicate a decrease in efficacy, particularly for ivermectin, signalling an onset situation of AR in the region. Consequently, continuous monitoring of treatment efficacy for GIN in sheep flocks in Sardinia is essential, and strict adherence to measures aimed at preventing this phenomenon becomes crucial.

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Isolation of ESKAPE bacteria in healthy cattle, antibiotic resistance, and potential zoonotic risk

Anna-Rita Attili¹, Francesca Gigli¹, Alessandro Guerrini¹, Francesca P. Nocera², Corrado M. Corradini¹, Filomena Fiorito², Claudia Cerracchio², Luisa De Martino², Vincenzo Cuteri^{1*}

¹School of Biosciences and Veterinary Medicine, University of Camerino, Matelica, Italy

²Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Napoli, Italy

*Correspondence: Vincenzo.cuteri@unicam.it

Key words: ESKAPE, Healthy cattle, Zoonosis.

INTRODUCTION

The inappropriate and unnecessary use of antibiotics and the limited availability of new antimicrobial agents have contributed to the increase in antimicrobial resistance. *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species are included into the ESKAPE group, bacteria known to be the most significant causes of nosocomial infections.

Particularly problematic and representative of multidrug-resistant bacteria are the ESKAPE pathogens, the name of which is due to the fact that they 'escape' the effects of antibiotics and can therefore pose a global threat to human health. As the role of healthy or otherwise asymptomatic animals as carriers of ESKAPE bacteria is poorly investigated, we tested the presence of ESKAPE isolates in healthy cattle and assessed their resistance to some antimicrobials.

MATERIALS AND METHODS

A prospective epidemiological study was conducted on dairy farms in the Marche and Campania regions. A total of 180 different samples were taken: eye, nasal, ear and oral swabs, milk, and faeces from healthy cattle. Standard cultures were performed in aerobiosis on nutrient and selective agar media and all the different colonies obtained were identified by MALDI-TOF MS. The susceptibility of ESKAPE isolates to a panel of 30 antibiotics belonging to different classes was assessed using the Kirby-Bauer and E-test methods (EUCAST, 2023). As suggested by Magiorakos et al. (2012), MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR to at least one agent in all but two or fewer antimicrobial categories, and PDR as non-susceptibility to all agents in all antimicrobial categories.

RESULTS AND CONCLUSIONS

Enterococcus faecium (n=17), *Staphylococcus aureus* (n=5), *Klebsiella pneumoniae* ssp. *pneumoniae* and *Klebsiella pneumoniae* ssp. *ozaenae* (n=15), *Acinetobacter baumannii* (n=7), *Pseudomonas aeruginosa* (n=7), and *Enterobacter cloacae* (n=12) were isolated. ESKAPE bacteria were susceptible to carbapenems, except for *P. aeruginosa* strains, polymyxins, sulphonamides, aminoglycosides, glycopeptides (vancomycin and teicoplanin), and *S. aureus* to β -lactams. No PDR strains were recorded, whereas 43 MDR (68.2%, n=63) and 18 XDR (28.6%) were observed. The nose and mouth of healthy cattle represent the anatomical sites of highest zoonotic risk (RR=28.55; AR=0.93) for transmission of *A. baumannii*, *K. pneumoniae*, and *E. cloacae*.

The preliminary data obtained enable the acquisition of epidemiological information on the spread of ESKAPE pathogens from the healthy cattle population and to stimulate all international organisations involved in the One Health approach so that there is increased surveillance of antimicrobial resistance also in healthy animals to avoid the transmission of ESKAPE bacteria.

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Importance and variety of mixed infections in diarrhoeic neonatal calves from North-western Spain

Cynthia López-Novo^{1*}, Alberto Prieto¹, David García-Dios¹, José Manuel Díaz-Cao¹, Susana Remesar¹, Gonzalo López-Lorenzo¹, Gonzalo Fernández¹, Patrocinio Morrondo¹, Pablo Díaz¹

¹Department of Animal Pathology (INVESAGA Group), Faculty of Veterinary Sciences, Campus Terra, Universidade de Santiago de Compostela, 27002 Lugo, Spain

*Correspondence: clnovo@hotmail.com

Keywords: neonatal calf diarrhoea, co-infections, Spain

INTRODUCTION

Neonatal calf diarrhoea (NCD) is a very common disorder in calves under 30 days of age leading to significant economic losses for cattle farmers. It is a multi-aetiological syndrome since a wide variety of bacterial, viral and parasitic pathogens may be involved. Although mixed infections are very common and often related to more severe NCD outbreaks, most studies focus on a single enteropathogen. The identification of the aetiological agents involved in an outbreak plays a key role in implementing the most adequate control measures. However, the routine laboratory diagnosis of NCD usually includes only enterotoxigenic *Escherichia coli*, *Rotavirus*, *Coronavirus* and *Cryptosporidium parvum*. Furthermore, unravelling major co-infections is essential to identify synergistic or antagonistic interactions between enteropathogens. Therefore, the aim of this study was to determine the importance of mixed infections in NCD as well as the most common co-infections.

MATERIALS AND METHODS

Faecal samples from 420 diarrhoeic calves up to 30 days old were collected on farms in northwestern Spain. *Escherichia coli* and *Salmonella* spp. were isolated by plating on selective media. Subsequently, pathogenic pathotypes of *E. coli* were identified by qPCR of the eight virulence factors, while *Salmonella* spp. was confirmed using a commercial serum agglutination test. *Rotavirus A*, *Coronavirus*, *Torovirus*, *Norovirus* and *Nebovirus* were directly detected from faecal samples by qPCR. Both *C. parvum* oocysts and *Giardia duodenalis* cysts were identified by a commercial immunofluorescence antibody assay and *Eimeria* oocysts were detected using the McMaster floatation technique.

RESULTS AND CONCLUSIONS

Mixed infections were identified in most samples (82.1%), being especially frequent in animals aged between three (94.3%) and four (95.7%) weeks. Up to seven pathogens were identified in a single sample although associations comprising two (27%), three (33.6%) or four (21.4%) agents were predominant. It is worth noting that some enteropathogens were detected exclusively or almost exclusively in combination with others, such as *Torovirus* (100%), *Nebovirus* (98.9%) and *G. duodenalis* (98.6%). Finally, 172 different combinations of pathogens were found; among them, the most frequent included one or more viruses and *C. parvum* (20.9%).

Our results show that most of NCD cases are the result of mixed infections. The great diversity of combinations detected in this study suggests that a higher number of enteropathogens should be included in routine laboratory analyses to obtain a more complete aetiological diagnosis.

Furthermore, the frequent association between *C. parvum* and various viral agents suggests that the immunosuppressive role of viruses may favour infection with the protozoan.

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30 years of melatonin use in Sardinian sheep: considerations

Sebastiano Luridiana¹, Maria Consuelo Mura¹, Mortadha Ouadday¹, Giovanni Cosso¹, Ben Smida Boubaker¹, Vincenzo Carcangiu^{1*}

¹Department of Veterinary Medicine, University of Sassari, Via Vienna 2, 07100, Sassari, Italy

²National School of Veterinary Medicine, Sidi Thabet, University of Manouba, La Manouba 2010, Tunisia

*Correspondence: vcarcangiu@uniss.it

Keywords: Melatonin implant, Sarda ewes, seasonal reproduction

INTRODUCTION

In order to ensure the conservation of the species, the majority of animal species, including small ruminants, tend to give birth in spring, a more favourable period for the survival of their offspring. Melatonin is a hormone secreted mainly by the pineal gland during the hours of darkness, and therefore, its production follows a circadian and circannual pattern in accordance with the pattern of the photoperiod. Its secretion rhythm is the organic signal to the hypothalamic-pituitary-gonadal axis on the progress of the seasons.

MATERIALS AND METHODS

Melatonin administration resulted in a significant increase in GnRH and LH secretion during anoestrus in sheep with an improvement in reproductive activity. The use of melatonin in the form of slow-release subcutaneous implants to control reproductive seasonality in sheep and goats is now a widespread practice throughout the world. These implants contain 18 mg of melatonin and determine high plasma concentrations of melatonin, for 24 hours for approximately 60 days, without suppressing the endogenous secretion of the pineal hormone. Therefore, the implants mimic a short photoperiod by making the melatonin signal active on the hypothalamus. In Sardinia, melatonin implants have been used for about 30 years and have shown to improve reproductive efficiency in spring, ensuring lambings in Autumn to achieve a longer lactation period.

RESULTS AND CONCLUSIONS

Over the course of these years, the method has been developed by identifying the factors that can influence the effect of melatonin on reproductive activity. One of the factors influencing the response to treatment is the distance between lambing and the moment of melatonin administration. In fact, treatments carried out before 3 months from the previous lambing appear to influence reproductive efficiency in a non-optimal way. Two other factors that influence the reproductive response to melatonin treatment are the BCS level and the month of treatment. The BCS around 3 is what guarantees optimal responses in terms of fertility and concentration of lambings. Treatments carried out from April onwards give greater fertility responses than those carried out in February and March. However, animals treated with melatonin always show better reproductive performances than untreated animals regardless of the physiological state of the animals. In conclusion, melatonin treatment in Sarda sheep improves reproductive efficiency in spring and therefore can be considered a valid method to rationalize reproduction.

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Experimental Bluetongue virus (BTV) infections in sheep using two different inoculum sources mimic fatal natural disease

Davide Pintus^{1*}, Rosario Scivoli¹, Vanessa Herder², Angela Maria Rocchigiani¹, Caterina Maestrale¹, Roberto Bechere¹, Annalisa Oggiano¹, Massimo Palmarini², Ciriaco Ligios¹, Giantonella Puggioni¹

¹ Istituto Zooprofilattico Sperimentale della Sardegna, Sassari,

² MRC-University of Glasgow Centre for Virus Research, Glasgow

*Correspondence: davide.pintus@izs-sardegna.it

Keywords: Bluetongue, rams, experimental infection

INTRODUCTION

Bluetongue virus (BTV) is a Culicoides-borne arbovirus of the genus *Orbivirus* of the family *Reoviridae* that causes an infectious hemorrhagic disease in wild and domestic ruminants. BTV infection can be asymptomatic in most animals, but can lead to fatal disease, particularly in sheep (Maclachlan et al., 2006. J Comp Pathol 141(1):1-16). The variable clinical consequences of bluetongue have been well described in several BTV epidemics around the world, and testicular degeneration has also been reported in rams, with serious consequences for reproductive management (Puggioni et al., 2018. J Virol. 12;92(19)).

Until the late 1800s, experimental infection studies were conducted with different inocula to understand the pathogenesis, virulence, and interaction between BTV and host (Coetzee et al., 2014. Virus Res. 182:21-34).

We investigated whether a strain of BTV serotype 1 isolated from severe clinical cases in rams produced different clinical-pathological outcomes after experimental infection using two different inoculum sources.

MATERIALS AND METHODS

Two groups of rams were experimentally infected using a field strain of BTV serotype 1 (BTV-1_{IT2006}) isolated in 2006 in Sardinia (Italy).

One group was injected intradermally with the blood of rams clinically infected with BTV, and the other group was infected with the same viral strain after being cultured in insect cells (KC). At 7- and 10-days postinfection (dpi), rams were humanely suppressed, and a wide range of tissues were sampled for virological, histopathological and immunohistochemical examinations during necropsy.

RESULTS AND CONCLUSIONS

After infection with the two different types of BTV inoculum, the experimental infected rams reproduced the severe clinical signs and BTV lesions observed in the field, with two fever peaks (> 40 °C) at 3 and 6-7 dpi and an increase in the severity of clinical signs at 7 dpi.

Furthermore, as observed in the field, all infected rams showed a clinical disease characterized by involvement of the genital tract.

No pronounced difference in induction and progression of viremia, as determined by real-time RT-PCR, was observed in both groups of animals.

Macroscopically, the gastrointestinal tract, pulmonary artery, and papillary muscle of the left ventricle showed hyperemia and hemorrhage. Microscopically, the histological lesions reflected the gross changes detected in the organs.

By immunohistochemistry, BTV-NS2 protein was found in vascular endothelial cells of several organs up to 10 dpi and in the lymph nodes and spleen, where it also appears to be associated with macrophages and dendritic cells.

Interestingly, irreversible degeneration of the tubular germinal epithelium of the testis was observed in all infected animals.

Reliable experimental infection models are considered an important research tool to develop and facilitate pathogenetic studies of bluetongue. We found that BTV KC inoculum maintains the testicular tropism determined in the field and that BTV blood inoculum does not appear to enhance the pathological findings of natural infection of the same serotype. BTV KC inoculum is a valid alternative to the use of infected blood in the field because it faithfully reproduces the natural disease, increases biosafety, and standardizes protocols to eliminate the presence of bias in experimental conditions.

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Could a locomotion score be useful in the type trait assessment of the Italian Mediterranean Buffalo?

Damiano Altieri¹, Yuri Gombia², Dario Rossi², Massimo Neri², Francesca Carnovale¹, Rossella Di Palo¹

¹ Dipartimento di Medicina Veterinaria e Produzioni Animali - University of Naples Federico II, Italy

² Associazione Nazionale Allevatori Specie Bufalina – Caserta Italy

*Correspondence: damiano.altieri@unina.it

Keywords: Locomotion score, Buffalo, Type traits

INTRODUCTION

A correct morphology of the Italian Mediterranean Buffalo is considered a fundamental requirement to ensure greater welfare and longevity of the animals (Booth, C. J., et al., 2004). Strong limbs and feet are traits that have been included in selection indices for dairy breeds (Miglior, F., et al. 2005) and have recently been included also in the selection index used by the National Association of Buffalo Breeders (ANASB). The type trait assessment of IMB include 3 linear limb descriptors: hock angle, heel height and fetlock strength. The aim of this study was to define a linear scale for a locomotion score and to verify how this can be useful to complete the description of limbs and feet in the type trait evaluation of the IMB.

MATERIALS AND METHODS

The type trait evaluation was assessed for 4311 IMBs registered with ANASB and a locomotion character was measured for each of them on a linear scale from 1 to 50. Statistical analyses were performed by IBM SPSS statistics 29.0 (2023); Pearson's correlation test was used to assess the relationships of locomotion with the other descriptors related to skeletal structure and limb conformation. Linear regression analysis using the stepwise method was carried out in order to assess how the overgrown claw (OGC), open claw (OPC) and corkscrew claw (CC) defects (ICAR 2022), could influence locomotion together with the various characters relating to skeletal structure and limbs and feet. Claw defects were included as dummy variables. Age at assessment was also included in the regression analysis.

RESULTS AND CONCLUSION

Significant correlations were found between locomotion score and linear traits for structure but with not relevant r values (< 0.09) while significant and higher r values were found for limb conformation: hock angle ($r = -0.35$; $P < 0.01$), fetlock strength ($r = 0.39$; $P < 0.01$) and heel height ($r = 0.35$; $P < 0.01$). The factors included in the equation were: age at assessment, OGC and CC defects, all the descriptors for limb, height at withers and chest width. The R^2 of the overall model was 0.51. A closed hock angle penalized the locomotion score by -0.37 points for each point of its linear value, while each linear point of fetlock strength and heel height added 0.22 points to the linear value of locomotion. The presence of OGC defect reduced the locomotion score by 1.9 points while the presence of the CC penalized it by 0.84 points. The study results seem to confirm the locomotion score a useful aid in defining limb functionality, as it appears to summarise the information related to the three limb descriptors combined with the claw defects. However, part of the variability in the locomotion score

was explained by other components, including the environmental effect and other factors that need to be better characterised on more data.

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Parasitological Evaluation of Diet Supplementation with Hemp (*Cannabis sativa* L.) Inflorescences in goat and *in vitro* Usage of Different Cannabis Extract: a preliminary study

Ruggero Amato^{1*}, Paola Vitiello¹, Antonio Bosco¹, Laura Rinaldi¹

¹Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, 80100, Napoli, Italy

*Correspondence: ru.amato@studenti.unina.it

Keywords: cannabis; dairy goats; parasites

INTRODUCTION

The worldwide increased difficulty to counteract gastrointestinal nematode (GIN) infections in ruminants, due to progressing anthelmintic resistance, has led to the evaluation of other alternative helminth control options, mainly from plants. *Cannabis sativa* L. is an annual plant of the family Cannabaceae which grows roughly anywhere, and it includes various botanical subspecies [1]. Some varieties of *Cannabis sativa* have gained particular interest in the last years for their low content in tetrahydrocannabinol (THCA) (<0.2–0.3%), a chemical compound responsible for some psychoactive effects, which makes this cultivar suitable for legal cultivation in Europe [2]. For its nutritional characteristics this plant has been studied as feasible feed supplement in various animal species [3-4] but there are no studies which examine the possible anti-helminthic effect of its fito-complex and its purified molecules (such as CBD or Cannabidiol). Hemp is rich in bioactive compounds such as natural antiparasitic (alkaloids, sesquiterpenes, terpenes and terpenoids) which could be used as natural allies in the battle against anthelmintic resistance. The use of plant-based feed supplements rich in bioactive compounds could be an important strategy to find another way of use for by-products or co-products and at the same time to control the parasitic burden and also has been seen to be useful to improve the quality of goat milk and their derived products [5-6] in a win-win situation.

MATERIALS AND METHODS

For the purpose *Cannabis sativa* variety Kompolti (*in vivo*) and three different extracts (*in vitro*: full-spectrum; broad spectrum and purified CBD) are being used in the trial. Twelve goats (Camosciata delle Alpi), after kidding, were equally divided into two groups (G: Grazing and GH: grazing and hemp), homogeneous for milk yield in the previous lactation, parity and live weight. For three months, all goats were fed on a permanent pasture and received 700g/head/day of concentrate (barley, oats and faba bean); diet of group GH was supplemented with 20 g/head/day of hemp inflorescences with 3% of CBD. Faecal samples were collected individually from rectal ampulla and tested using FLOTAC technique (7). Faecal samples were also used for the Egg Hatching Test (EHT); EHT efficacy profile of *Cannabis sativa* L., as percentage of eggs unhatched (mean of triplicates), the positive control (TBZ) and negative control (deionized water/DMSO 0.5%) was evaluated in this preliminary study.

RESULTS AND CONCLUSIONS

Results show there were no significant differences between G and GH group in the *in vivo* test; the *in vitro* test shows that the most efficient product was full spectrum cannabis extract (0.004mg/mL) in deionized water with 94.3±3.5 % of eggs unhatched followed by purified CBD (0.001mg/mL) in DMSO 0.5% with 91.3±7.3 % of eggs unhatched, the broad spectrum extract was the less efficient

with 89±1 eggs unhatched. Further studies are needed to better understand the possibilities of cannabis usage as helminthic-control product.

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Sustainable approaches to parasite control in ruminants

Antonio Bosco

Department of Veterinary Medicine and Animal Production, University of Naples Federico II

*Correspondence: antonio.bosco@unina.it

Keywords: Gastrointestinal helminths, ruminants, sustainable parasite control

Although the use of chemical anthelmintics is still the most common approach used by farmers to control helminths in livestock, alternative or complementary methods are also available, which include nutritional, immunological and biological interventions, as grazing management, nutritional supplementation, genetic selection, biological control and vaccination (Charlier et al., *Advances in Parasitology*, 2022). Among these approaches, the use of natural bioactive compounds is also considered a valid option due to their anti-parasitic properties. Natural bioactive compounds, such as plant extracts and plant-derived compounds can be used as herbal medicines or nutritional supplementations, or as models for the synthesis of new drugs (Hoste et al., *Parasite*, 2022).

In particular, many plants are used in ethnoveterinary medicine for parasite control in small ruminants (Castagna et al., *Veterinary Sciences*, 2021) and strong and rooted ethnoveterinary knowledge is available in the country.

Furthermore, another alternative aspect to parasite control can be the genetic selection of some ruminant breeds. Indeed, host resistance (i.e. the ability to mount an effective immune response) and resilience (i.e. the ability to produce even under worm challenge) to parasite infections are largely influenced by genetic factors, and can vary among species, breeds and individuals (Maurizio et al., *Parasitology*, 2019). Resistance/ resilience to helminths may increase productivity under conditions of high parasite infection risk and, because this leads to less frequent treatments, reduce selection pressure for AR. Therefore, studies demonstrating differences in resistance/resilience of ruminants to helminths can help develop appropriate strategies to control parasitism by using resistant breeds and improving sustainable rearing of local breeds (Zanzani et al., *BMC Journal – Veterinary Research*, 2014).

It seems necessary an overall strategy to change the attitude of ruminant farmers and veterinary practitioners, towards a holistic approach to parasite control, integrating a careful use of chemical drugs with the adoption of new phytotherapeutic compounds and other alternative and integrative practices.

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Application of RNA technologies for improved control of cystic echinococcosis

Elena Ciccone^{1*}, Paola Pepe¹, Nunzio Antonio Cacciola^{1,2}, Ciro Campanile², Antonio Bosco¹, Maria Paola Maurelli¹, Gaetano Oliva¹, Jacopo Guccione¹, Laura Rinaldi¹

¹Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Via F. Delpino 1, Naples, 80137, Italy

² Institute of Genetics and Biophysics “A. Buzzati-Traverso”, National Research Council (IGB-CNR), Via P. Castellino 111, Naples, 80131, Italy

*Correspondence: elenaciccone@unina.it

Keywords: Cystic echinococcosis, non-coding RNAs, new targets.

INTRODUCTION

Cystic echinococcosis (CE) is a worldwide parasitic disease caused by the larval stages of the tapeworm *Echinococcus granulosus*. The life cycle of this cestode includes canids as definitive hosts and a wide range of domestic and wild mammals and humans as intermediate hosts. This parasitic zoonosis is widespread in Mediterranean countries, including Italy, and poses a significant medical and veterinary problem due to its impact on human and animal health. To date, the control of CE has been a public health priority due to the difficulties in diagnosis and treatment. Although therapeutic agents exist, e.g. benzimidazoles to treat intermediate hosts, these approaches have shown little efficacy. Therefore, novel chemotherapy alternatives and drug targets are urgently needed to improve control programmes against CE.

MATERIALS AND METHODS

Non-coding RNAs such as small interfering RNAs (siRNAs), microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have been identified as potential diagnostic targets and therapeutic candidates for parasitic infections. Previous studies have shown that some miRNAs, including mir-71, are involved in parasite development and hydatid cyst fertility. Mir-71 has also been analysed at the organism level in a larval-stage cestode model, demonstrating its central role in the establishment of successful infection in the experimental host. In addition, the diagnostic ability of circulating mir-71 in the peripheral blood of infected sheep was also demonstrated, highlighting the importance of these miRNAs also for the early diagnosis of CE. In line with this, this study aims to improve the knowledge of RNAs regulating important target genes and pathways by identifying the non-coding RNAs involved in each stage of the parasitic life cycle, in order to discover potential new targets for diagnosis and development of a new therapeutic approach against CE. This will be achieved through a methodological approach consisting of different work packages involving the collection and analysis of hydatid cysts, eggs and adult parasites, as well as careful procedures for the extraction and analysis of RNA.

RESULTS AND CONCLUSIONS

The results of this study could form the basis for the development of innovative strategies based on RNA technology to control CE and reduce the global health burden. Finally, the novel

approaches developed using *Echinococcus* as a “model parasite” could also be used to control other parasitic infections.

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Use of perennial plants in the fight against gastrointestinal nematodes of sheep

Alessandra Falzarano¹, Antonio Bosco², Pierpaolo Scarano¹, Antonello Prigioniero¹, Maria Paola Maurelli², Laura Rinaldi², Giuseppe Cringoli², Giovanni Quaranta^{3,4}, Salvatore Claps⁵, Rosaria Sciarrillo^{1*}, Carmine Guarino¹

¹ Department of Science and Technology, University of Sannio, Benevento, Italy

² Department of Veterinary Medicine and Animal Production, CReMoPAR - Centro Regionale per il Monitoraggio delle Parassitosi, University of Naples Federico II, Naples, Italy

³ Department of Mathematics, Computer Science and Economics, University of Basilicata, Potenza, PZ, Italy

⁴ MEDES Foundation – Mediterranean Sustainable Development Foundation, Sicignano degli Alburni, SA, Italy

⁵ CREA-ZA – Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca Zootecnia e Acquacoltura, Bella Muro, PZ, Italy

*Correspondence: sciarril@unisannio.it

Keywords: gastrointestinal nematodes (GINs), anthelmintic resistance, phytotherapy (edible plants)

INTRODUCTION

Gastrointestinal nematodes (GINs) are a serious problem in ruminant pastures worldwide. They generate production losses, from the point of view of both the food chain and animal mortality. This study provides preliminary results concerning the use of pasture plants in the Campania region (of southern Italy) to control GINs in sheep.

MATERIALS AND METHODS

Sixteen species of wild and cultivated perennial plants present in seminatural pastures were sampled. All species were extracted with the conventional maceration technique, using three different solvents (i.e., H₂O, EtOH, and an EtOH:H₂O (8:2) mixture) in order to extract different bioactive compounds. The total phenolic content (TPC; assessed via the Folin–Ciocâ lteu assay) of all extract samples was preliminarily characterized. Each sample was aliquoted across six different concentrations and an in vitro egg hatching test (EHT) was conducted to evaluate the ovicidal effect on sheep GINs.

MATERIALS AND METHODS

The results indicated that *Cichorium intybus* L. and *Foeniculum vulgare* Mill. extracts greatly inhibited egg hatching within 48 hours of exposure, showing efficacy ($\geq 62.6\%$) at the three higher concentrations when compared with the other plants. The use of extracts of wild and cultivated perennial plant species in pastures can be a valid alternative to the use of synthetic anthelmintic drugs, which can generate problems, such as anthelmintic resistance, in the long term. Looking forward, further in vitro studies that evaluate the in vitro effect of these extracts on ruminant cell cultures, and field application through in vivo studies, would likely confirm the results obtained from preliminary in vitro EHTs. All these studies should be aimed at evaluating the therapeutic potential and future applicability of specific plant cultures in pastures to achieve sustainable pest control.

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First morphometric and molecular identification of liver flukes in sheep in Northwest Tunisia

Ines Hammami^{1, 2*}, Lavina Ciuca³, Maria Paola Maurelli³, Rihab Romdhane¹, Limam Sassi¹, Nadia Farhat¹, Alain Kouam Simo⁴, Laura Rinaldi^{2,3}, Mourad Rekik⁵, Mohamed Gharbi¹

¹Laboratoire de Parasitologie, École Nationale de Médecine Vétérinaire de Sidi Thabet, Univ Manouba, Sidi Thabet, Tunisia.

²MUNA - Mediterranean and Middle East Universities Network Agreement.

³Unit of Parasitology and Parasitic Diseases, Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Napoli, Italy.

⁴Faculté des sciences de la santé, Université des Montagnes, Bangangté, Cameroun.

⁵International Center for Agricultural Research in the Dry Areas (ICARDA), Amman, Jordan.

*Correspondence: ineshammami4421@gmail.com

Keywords: *Fasciola hepatica*, Sheep, Tunisia

INTRODUCTION

Fasciolosis is a worldwide zoonosis caused by Fasciola trematode parasites, mainly *Fasciola hepatica* (Linnaeus, 1758) and *Fasciola gigantica* (Cobbold, 1856) (Itagaki et al., 2022). Liver flukes primarily infect domestic ruminants, although wild herbivores and other mammals, including humans, can be also infected (Mas-Coma et al., 2009; Sabourin et al., 2018; Evack et al., 2020; Omar et al., 2021). This infection is widespread in Tunisian livestock especially in the northwest and southwest of the country. Despite its significant impact, few studies underlined only the prevalence of fasciolosis in Tunisian sheep while the morphology and the occurrence of the Tunisian species of *Fasciola* have never been studied. Therefore, the present study aimed to characterize the Tunisian liver flukes by morphometric and molecular analyses.

MATERIALS AND METHODS

A total of 335 flukes were collected from sheep livers in Sejnane slaughterhouse in Northwestern Tunisia during March 2021. Five morphometric parameters were determined for all the liver flukes, as follows: (i) total fluke length, (ii) ventral sucker to the tail length, (iii) oral to ventral sucker length, (iv) abdomen diameter and (v) tail diameter and the ratio length/width of the body.

The final output of the morphometric evaluation of the liver flukes included in the present study was then compared with available data of *Fasciola gigantica* morphology. Flukes' molecular identification was first carried out by amplification of a 680 bp sequence of ITS1 gene followed by a restriction fragment polymorphism (RFLP) assay and second by sequencing and phylogenetic analysis of a 500 bp sequence of the ITS2 gene.

RESULTS AND CONCLUSIONS

Morphometric measurements showed that the mean of the total body length of the adult flukes was 21.1 ± 2.7 mm and the mean ratio length/width of the fluke body was 2.32 ± 0.96 mm. Moreover, the morphometric assessment revealed that the mean of abdomen diameter and the tail diameter of the *Fasciola* specimens were 10.14 ± 3.04 mm and 6 ± 2.22 mm, respectively. The mean of all morphometric parameters was significantly different between *F. gigantica* and Tunisian *Fasciola* spp. (<0.001). PCR-RFLP analysis revealed only one profile consisting of

three bands of approximately: 370, 100, and 60 bp indicating *Fasciola hepatica*. Neither *F. gigantica* nor an intermediate RFLP pattern was detected in all digested amplicons. Therefore, all specimens collected from sheep livers in the current study belonged to *F. hepatica*.

The *F. hepatica* sequence described in this study (OQ457027 and OQ457028) was 99.58 – 100% homologous to *F. hepatica* sequences published in the GenBank.

In conclusion, morphometric results were in agreement with those of the molecular and phylogenetic analyses revealing the existence of only *F. hepatica* in northwest Tunisia. Moreover, our results will be useful to compare other morphometric and molecular profiles of *Fasciola* species from different areas of Tunisia. Further, studies using the same methodology are now required to confirm the existence of *F. gigantica* in other regions of Tunisia.

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- University of Naples Federico II, Department of Veterinary Medicine and Animal Production.

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Characterization of non-conventional feedstuff for ruminants' nutrition: case of cashew and pineapple by-products

Dieu donné Kiatti^{1*}, Alessandro Vastolo¹, Bossima Ivan Koura², Monica Isabella Cutrignelli¹, Serena Calabrò¹

¹University of Naples Federico II, Naples, Italy

²Université Nationale d'Agriculture, Ketou P.O. Box 43, Benin

*Corresponding author: Dieu donné Kiatti. E-mail: dieudonne.kiatti@unina.it

Keywords: *in-vitro* degradability, west-Africa, livestock nutrition

INTRODUCTION

The extensive system, the most common livestock system in west Africa, depends mainly on the natural pasture which is scarce during the dry season and fluctuates in terms of quantity and quality throughout the year. The variability of the nutrient source negatively affects animal performance. To limit the consequences, the breeders use alternative feed resources: shrub and trees fodders, agricultural and industrial waste. Unfortunately, some wastes are not or less used in ruminants' nutrition due to the presence of secondary compounds, their short self-life, or lack of knowledge in nutritive characteristics. This is the case of cashew apples and pineapple, which by-products are thrown after nut and fruits harvesting and processing. The objective of the study was to characterize the cashew apples and pineapple by-products (CABP and PBP, respectively) in terms of chemical composition, nutritive value, and *in vitro* degradability to encourage their utilization in ruminants' diets.

MATERIALS AND METHODS

To achieve this aim, five parts of CABP (whole, up, down, middle, and pomace) and PBP (crown, bud end, peel, core, and pomace) were collected respectively in Sudan and Guinea zone of Republic of Benin and dried. The samples were analyzed at the Department of Veterinary Medicine and Animal Production. The AOAC method was used for chemical composition (Official Methods of Analysis, 18th, 2005, USA) while the *in vitro* fermentation technique was utilized to determine the by-products degradability (Koura et al., 2022. Animals, 12: 1-16). The metabolizable energy (ME) content was estimated (Menke et al., 1988. Anim. Res. Dev, 28: 7-55).

RESULTS AND CONCLUSIONS

As results, all by-products were low in dry matter (DM), highlighting the difficulty in preserve them. For cashew and pineapple, the individual by-products showed significant differences ($p < 0.001$) except ME for CABP ($p > 0.05$). Low variation of crude protein (CP) was observed in all by-products with the highest values in the pomaces (9.48 and 8.45% DM, in CABP and PBP respectively). The ether extract (EE) and acid detergent lignin (ADL) were lower in PBP while the CABP showed the highest ADL (8.03 and 11.52% DM, middle and pomace respectively). In addition, crown, bud end and peel showed the highest structural carbohydrates content (NDF: 43.32, 39.44, 30.17% DM respectively) whereas the core and pomace showed the highest ME (7.88 and 7.79 MJ/kg DM respectively). Regarding the organic matter degradability rather varied between by-products: from 56.14% (crown) to 86.55% (core) in PBP and from 43.63% (pomace) to 73.73% (middle part) in CABP. In conclusion, some by-products (pineapple: crown, bud end, and peel; cashew: pomace) could be used as fiber source, whereas other ones (pineapple: core and pomace; cashew: whole, up, down, and pomace) as energy source. All of them could be useful during the dry season in West Africa to support the farmers in guarantying the animals' performance throughout the year.

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Identification of Genetic Regions Associated with Resistance to Gastrointestinal Nematodes in Comisana Sheep using a Genome-wide Association Study based on EBV Ranking

Christian Persichilli^{1*}, Stefano Biffani², Gabriele Senczuk¹, Marika Di Civita¹, Antonio Bosco⁴, Giuseppe Cringoli⁴, Silverio Grande³, Fabio Pilla¹

¹ University of Molise, Department of Agriculture, Environment and Food Science, Campobasso, Italy

² National Council of Research, Institute for Agriculture Biology and Biotechnology, Milan, Italy

³ National Sheep and Goat Breeders Association, Rome, Italy

⁴ University of Naples Federico II, Department of Veterinary Medicine and Animal Production, CREMOPAR, Naples, Italy

*Correspondence: christian.persichilli@unimol.it

Keywords: Gastrointestinal Nematodes (GINs); Sheep Resistance; Case-control GWAS

INTRODUCTION

Gastrointestinal Nematodes (GINs) have significant economic, environmental, and animal welfare implications in small ruminants. This study is aimed to identify genetic regions responsible for sheep resistance to GINs (McRae et al., 2014; Rashid et al., 2019; Sanchez et al., 2004).

MATERIALS

AND

METHODS

Fecal samples were collected from 642 Comisana sheep over three years to assess Fecal Egg Counts (FEC) with the FLOTAC technique. Using pedigree data and $\log_n(\text{FEC}+2)$ as phenotypes, Estimated Breeding Values (EBVs) for GIN resistance were estimated by a BLUP animal model. The EBVs in the 99.95th and the 0.05th percentile were used to identify the most and the least genetically resistant individuals to GINs, later genotyped with the Illumina OvineSNP50 beadchip. Using the software PLINK (Purcell et al., 2007) a case/control GWAS was performed. A threshold for the 0.005% most significant FDR corrected p-values was chosen. Using the R package GALLO (Fonseca et al., 2020), QTLs associated with the significant SNPs were annotated and enriched. With the ToppGenes utility (Chen et al., 2009), genes associated with the SNPs have been enriched for KEGG pathways, using a threshold of 0.05 FDR.

RESULTS

AND

CONCLUSIONS

As a result, 18 significant SNPs involving 13 genes were identified on 12 chromosomes. Among these, many are involved in the physiology or pathology of the gastrointestinal tract (the UGT1A* family, KIF6, LOXL2, CALN1 and TWISTNB). Others play a role in adaptive processes and production traits (LOXL2, GPC6, MYT1 and SS18L1). The QTLs Classes found to be associated with the significant SNPs were *Meat and Carcass*, *Milk*, *Production*, *Wool*, *Health*, *Reproduction*, and *Exterior*. Among 6 QTLs in the Health Class, 5 were associated with traits related to FEC. Enrichment analysis of the found genes highlighted 11 significant pathways classifiable as *involved in the regulation of the immune response*, *involved in drug metabolism and detoxification*, and *involved in other metabolic processes*. Previous research has linked some of the discovered pathways to GIN resistance, such as *Ascorbate and aldarate metabolism* and *Glucuronate pathway (uronate pathway)*, which are involved in the regulation of immune response during parasitic infections in sheep (Wang et al., 2022; Prasanphanich et al., 2013) and *Metabolism of xenobiotics by cytochrome P450*, known to be involved in the metabolism of anthelmintic drugs (Kellerová et al., 2019).

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***In vitro* evaluation of grape pomace in the diet of adult sheep**

Alessandro Vastolo, Martina Nocerino, Dieu donné Kiatti, Antonio Bosco, Serena Calabrò, Monica I. Cutrignelli, Laura Rinaldi

University of Napoli Federico II, Napoli, Italy

Key words: *agroindustrial by-products, small ruminant, feed ration*

INTRODUCTION

Fruit and vegetable by-products are the most abundant waste, with residues accounting for 40-50% of total waste. Industrial processes are the main source of these by-products. Food waste has a strong environmental impact, exacerbating the problem of climate change because food production that ends up being lost is also responsible for a significant amount of greenhouse gas emissions. In this regard, grape pomace are results from the wine and juice industry and that is costly to dispose of. Besides, due to high fibre concentration, grape residue could be an alternative feed ingredient to partially replace the forage portion in the diet of ruminants (Gómez-Cortés et al., 2018, Food Res. Int. 113: 36-42); . The aim of this study was to evaluate the nutritional characteristics of grape pomace using the *in vitro* gas production technique.

MATERIAL AND METHODS

The grape pomace was supplemented (10% of DM) with a control diet for dairy sheep. The experimental diet and control diet (CTR: TMR without by-product) were incubated in a serum flask with buffered sheep rumen fluid at 39°C under anaerobic conditions for 120h (Theodorou et al., 1994, Anim. Feed Sci. Technol. 48:185–197). The rumen liquors were collected at slaughterhouse according to EU legislation from six healthy sheep (age: 5-6 months; weight 45-50 kg). The gas produced was recorded 21 times through 120 h of incubation with a manual system. The cumulative volume of gas produced at 120 h of incubation was related to incubated organic matter (OMCV, ml/g). At the end of the incubation period, the fermentation liquor was analysed for pH using a pH meter. The organic matter degradability (OMD, %) was determined by weight difference of the incubated organic matter and the undegraded filtered throughout crucibles and burned in muffle at 550°C. The volatile fatty acids (VFA, mmol/g) were measured by gas chromatography (Vastolo et al., 2019, 9:556).

RESULTS AND CONCLUSIONS

The inclusion of grape pomace to the standard diet reduce ($p<0.05$) the organic matter degradability (OMD) and increase ($p<0.01$) the gas production (OMCV). The diet with grape pomace showed a higher ($p<0.05$) fermentation rate (R_{max} , ml/g). The pH was higher ($p<0.01$) in the diet with grape pomace. The control diet showed a higher ($p<0.01$) level of total fatty acids (VFA) and branched chain fatty acids (BCFA). Moreover, the control diet had a higher amount of propionic, butyric, and iso-valerianic acids. On the other hand, the diet with the inclusion of grape pomace showed a higher ($p<0.01$) level of acetic and valerianic acids, and acetic/propionate ratio. In conclusion, the addition of 10% DM of grape pomace to a standard diet for ruminants could reduce the organic matter degradability and increase the fermentation kinetics. Moreover, the inclusion of grape pomace appears to lead to an increase in acetic acid production. These results could be due to the presence of bioactive compounds, which influenced the fermentation process in which the grape pomace is rich (i.e., polyphenols, tannins) (Ianni et al., 2019, J Sci Food Agric. 99(7):3635-3643).

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The role of the “Sistema Allevatori” in Italian livestock farming

Augusto Calbi

Regional Farmers' Association of Campania and Molise

*Correspondence: direzione@aracampania.it

Keywords: Italian Breeders Association, livestock breeds

The Italian Breeders Association (AIA), legally established on August 2, 1944, and recognized as a Moral Entity by Decree of October 2, 1950, No. 1051, has a technical-economic character and aims to implement all initiatives that effectively contribute to the valorization of livestock breeds and derived products. In pursuit of its institutional objectives, the Association collaborates with the competent Ministry of Agriculture, Food Sovereign, and Forestry and other public administrations and agricultural organizations.

AIA associates 16 first-level local associations operating at the territorial level (Inter-regional, regional, and, exclusively in the Trentino Alto Adige region, provincial associations), among which ACM is in charge of carrying out the technical activities in Campania and Molise Regions.

The Recognized Breeder Associations entrust AIA with the data collection required by the genetic programs. Indeed, the core activity of AIA and the regional Association includes the registration, long-term storage, management, and statistical elaboration of productive and reproductive performances of livestock. The activity is performed according to the rules and standards established by the International Committee for Animal Recording (ICAR).

Nowadays, AIA plays a crucial role in Italy's livestock sector, boasting an association of over 46,000 farmers. The AIA central database contains over 1.3 million records and is the foundation for offering cutting-edge extension services. AIA actively collaborates and engages in significant national and international livestock research projects with leading Italian universities, the National Research Centre (CNR), and various research consortia and institutes. For example, the Livestock Environment Open data project (LEO, URL: www.leo-italy.eu) led by AIA in collaboration with seven partners is creating a linked open data platform, merging existing and novel information on Italian livestock populations. Data are collected routinely on more than 18,000 livestock farms and about 4 million extant animals belonging to six species and 109 breeds and spanning a variety of farming models such as intensive and semi-extensive dairy and beef, organic, mountain, and dry areas. AMC is actively involved in LEO, collecting field data and producing lab analyses. Up to now, the database contains information on 30 million heads. The big Open database produced by LEO counts more than 14 x 10⁹ triplets (Data-ID-Measure), including sixty-three million genetic and genomics indexes produced by the Breeder Association. The applicative cooperation of several running national databases like BDN (National livestock database) and Si@alleva (DHI National database) enriches the information content of the database. Up to now, Leo is by far among the most extensive open data platform in Europe, growing at a rate of about 300 million data per year. LEO open data represent a unique opportunity for a new generation of scientists to engage with big data in livestock and develop advanced solutions applicable worldwide to contrast emerging challenges such as biodiversity loss, the adaptation to climate changes, and the reduction of antimicrobics.

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Artificial insemination in Mediterranean Buffaloes: a field research in southern Italy

Dionisio Del Grosso¹, Concetta Avallone¹, Emanuela Califano¹, Davide Cembalo¹, Luigi Lavorgna¹, Deborah Jessica Mainardi¹, Francesca Salerno¹, Lorenzo Volatile¹

¹Vet Buiatri Salerno

*Correspondence: assbuiatrisalernitani@gmail.com

Keywords: Mediterranean buffalo, artificial insemination, fertility

In the last 20 years, the Italian Mediterranean Buffalo population has significantly increased, becoming one of the most important sources of income in southern Italy thanks to the improvements in the Italian livestock scenario, but mainly to the increased demand and market value of Mozzarella di Bufala Campana DOP cheese, the major product obtained from buffalo milk. Mozzarella cheese is most requested during summer, but our buffaloes are negatively photoperiodic and show a natural increase in fertility during decreasing day length. For many years the solution to this problem has been the deseasonalization of the herd to progressively reduce the loss of fertility during the years but that was not enough to improve buffalo livestock.

Our team-work Vet Buiatri Salerno has conducted a field search to show how artificial insemination can break down the wall represented by the seasonal limits and obtain the same fertility levels. In order to synchronize the buffaloes' heats two different protocols have been used: a spring-summer protocol using progesterone implants and a fall-winter protocol without it.

There have been many upsides to this method, from genetic improvements to the elimination of the risk of sexually transmitted diseases. One of the most impressive advantages is the decrease of the interpartum interval and the increase of the sustainability and productivity of the farm. The research has started in 2016 and two years later 33 farms have been included in a mass artificial insemination project. Over the course of the field study five synchronization protocols have been used and the results have shown that with less than two insemination interventions, it's possible to impregnate more buffaloes artificially than with bulls, with an interpartum interval under 400 days.

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Risk categorization of antimicrobial and anthelmintic consumption in Italian Mediterranean buffalo farming - IZSME15/22 RC

Gabriele Di Vuolo^{1*}, Giovanna Cappelli¹, Maria Serrapica¹, Chiara Denise Ambra¹, Domenico Vecchio¹, Esterina De Carlo¹, Lucrezia Lucchese¹, Federico Scali², Valentina Lorenzi², Antonio Bosco³, Cristina Lecchi⁴

1 Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy

2 Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy

3 University of Naples Federico II, Napoli, Italy

4 Università degli Studi di Milano, Lodi, Italy

*Correspondence: gabriele.divuolo@izsmportici.it

Keywords: Antimicrobial, Buffalo, Biosecurity

The use of antimicrobials, especially inappropriate antimicrobials (AMU), represents a huge danger in the One Health perspective, due to the increasing antimicrobial resistance, especially of multi-resistant microorganisms. The World Health Organisation (WHO) has classified certain AM agents as 'highest priority critical antimicrobials' (HPCIA) and their use should be restricted and only permitted when strictly necessary. The information on the relationship between animal welfare (AW), biosecurity, antimicrobial use and microorganisms isolated in Italian Mediterranean Buffalo (BMI) is limited. Endoparasitic infestations in farm animals are also a major problem due to their high negative impact on animal welfare, production, animal health and potentially public health. The assessment of antimicrobial consumption and the parallel collection of information related to welfare, biosecurity and resistance levels in isolated micro-organisms will provide the structural basis on which to develop risk categorisation on different farms and to promote the prudent and rational use of antibiotics through the development and adoption of good husbandry practices in BMI. There are currently no studies defining AMR risk in BMI and its correlations with Welfare, Biosecurity and AMU. The objective of this work is to assess AMU in the BMI herd and to categorise herds into risk classes by consumption of the drug. The expected impact on stakeholders is the increase in awareness of the correct application of BA measures with the consequent reduction of AMU. Reduction of AMR risk through conscious and accurate decision-making.

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Innovative and sustainable strategies to control Cystic echinococcosis in the Mediterranean area

Paola Pepe^{1*}, Antonio Bosco¹, Gerard Umhang², Yousra Said³, Rania Baka⁴, Maria Chiara Alterisio¹, Giuseppe Piegari¹, Paolo Ciaramella¹, Orlando Paciello¹, Francesco Antonio D'Orilia⁵, Paolo Sarnelli⁶, Giorgio Saralli⁷, Abdelkarim Laatamna⁸, Samia Lahmar³, Marshall Lightowlers⁹, Peter Deplazes¹⁰, Franck Boué², Smaragda Sotiraki⁴, Laura Rinaldi¹

¹University of Naples Federico II, CREMOPAR, Department of Veterinary Medicine And Animal Production, Naples, Italy;

²Anses, Rabies And Wildlife Laboratory, Malzeville, France;

³National School of Veterinary Medicine, Parasitology, Sidi Thabet, Tunisia;

⁴Hellenic Agricultural Organisation ELGO-DIMITRA, Veterinary Research Institute, Thessaloniki, Greece;

⁵Centro di Riferimento Regionale Sanità Animale (CReSan), Regione Campania, Salerno, Italy;

⁶UOD Prevenzione e Sanità Pubblica Veterinaria Regione Campania, Naples, Italy;

⁷Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, U.o.t. Lazio Sud, Latina, Italy;

⁸Ziane Achour University of Djelfa, Parasitology, Djelfa, Algeria;

⁹Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Department of Biosciences, Melbourne, Australia; ¹⁰University of Zurich, Institute of Parasitology, Vetsuisse Faculty, Zurich, Switzerland

*Correspondence: paola.pepe@unina.it

Keywords: Cystic echinococcosis; sheep; control programs

Cystic echinococcosis (CE) is a severe zoonosis, caused by the larval stage of the tapeworm *Echinococcus granulosus*. This helminth infection is of increasing public health and socio-economic concern due to the considerable morbidity rates that give rise to high economic losses in the public health sector and in the livestock industry. Control programmes against *E. granulosus* are considered long-term measures that require an integrated approach [1]. Usually, they included a combination of several strategies: (i) regulation of slaughter activity and disposal of offal; (ii) prevention of dogs accessing offal; (iii) regularly de-worming of dogs; (iv) public health education and recently (v) the introduction of EG95 recombinant vaccine for protection of lambs against infection with *E. granulosus* [2; 3]. However, despite such control initiatives have been implemented in several countries and regions, resulting in a marked decrease in the incidence of the disease, CE still remains a major health problem worldwide especially in Mediterranean areas [4].

In this context, ECHINO-SAFE-MED (New sustainable tools and innovative actions to control cystic ECHINOcoccus in sheep farms in the MEDiterranean area: improvement of diagnosis and SAFETY in response to climatic changes) project aiming to implement pasture-based livestock farming systems by delivering sustainable and cost-effective tools, as well as innovative strategies to control cystic echinococcosis (CE) in sheep farms in the Mediterranean regions. This will be obtained by the use of high throughput diagnostic, surveillance and control strategies in order to establish guidelines for sustainable CE control to be further extended to other endemic Mediterranean areas. With this as the

main tenet, ECHINO-SAFE-MED has three macro-objectives: *i*) to develop novel diagnostic tools for early detection of cystic echinococcosis in sheep in Mediterranean countries of Europe (i.e. France, Greece and Italy) and transfer these methodologies to Mediterranean areas in North Africa (i.e. Algeria and Tunisia); *ii*) to improve surveillance and control activities for CE in Mediterranean areas through the use of innovative sustainable strategies to be applied in highly endemic areas; *iii*) to strengthen capacity for CE diagnosis, surveillance and control in both Africa and Europe through training and stakeholders' engagements.

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PATHOCENOSIS

(from contagious diseases of antiquity to plagues, epidemics, pandemics and epizootic diseases)

Antonio Pugliese*

University of Messina, Italy

*Correspondence: antonio.pugliese@unime.it

Keywords: plagues, epidemics, pathocenosis

A historical journey from plagues to Covid-19 closely connected to the history of infectious diseases that allows you to examine the various calamitous health events that have affected humans and animals over the centuries. In representing the different causes, it was observed how the diffusion has almost always pursued the same path of contagiousness, as well as the intervention strategies that have been invoked.

Scrolling through the infinite space of time, it can be inferred that man, the last stage of the evolution of the species, has periodically found himself facing and fighting large-scale health catastrophes such as epidemics, which have irrepressibly determined massacres of animals and mortality of its kind.

An endless story that since the dawn of civilization concerns the origins and changes of communicable diseases, as documented by recent paleontological and molecular research.

Public health events, commonly called plagues which, as an expression of an altered balance between organic defenses and the potential of some microbial agents, without neglecting those unconventional agents, such as prions, have altered the ecosystem.

Epidemics of infectious diseases that made their appearance with the transition to agriculture, as documented by prehistoric finds, where hominids brought with them various family pathogens such as parasites, fleas, enterobacteria, staphylococci, and streptococci without neglecting the bites of insects, animal bites, and the consumption of contaminated food, at the basis of certain zoonoses.

Homo sapiens has never raised the problem of confronting nature and paying due attention to the sound principles of the human-nature-environment correlation, a prerequisite for an anthropocentric culture that has no justified reason to exist. A dynamic balance of diseases, under the sign of pathocenosis, allows us to better understand the synergies between them, as in the case of the new coronavirus.

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