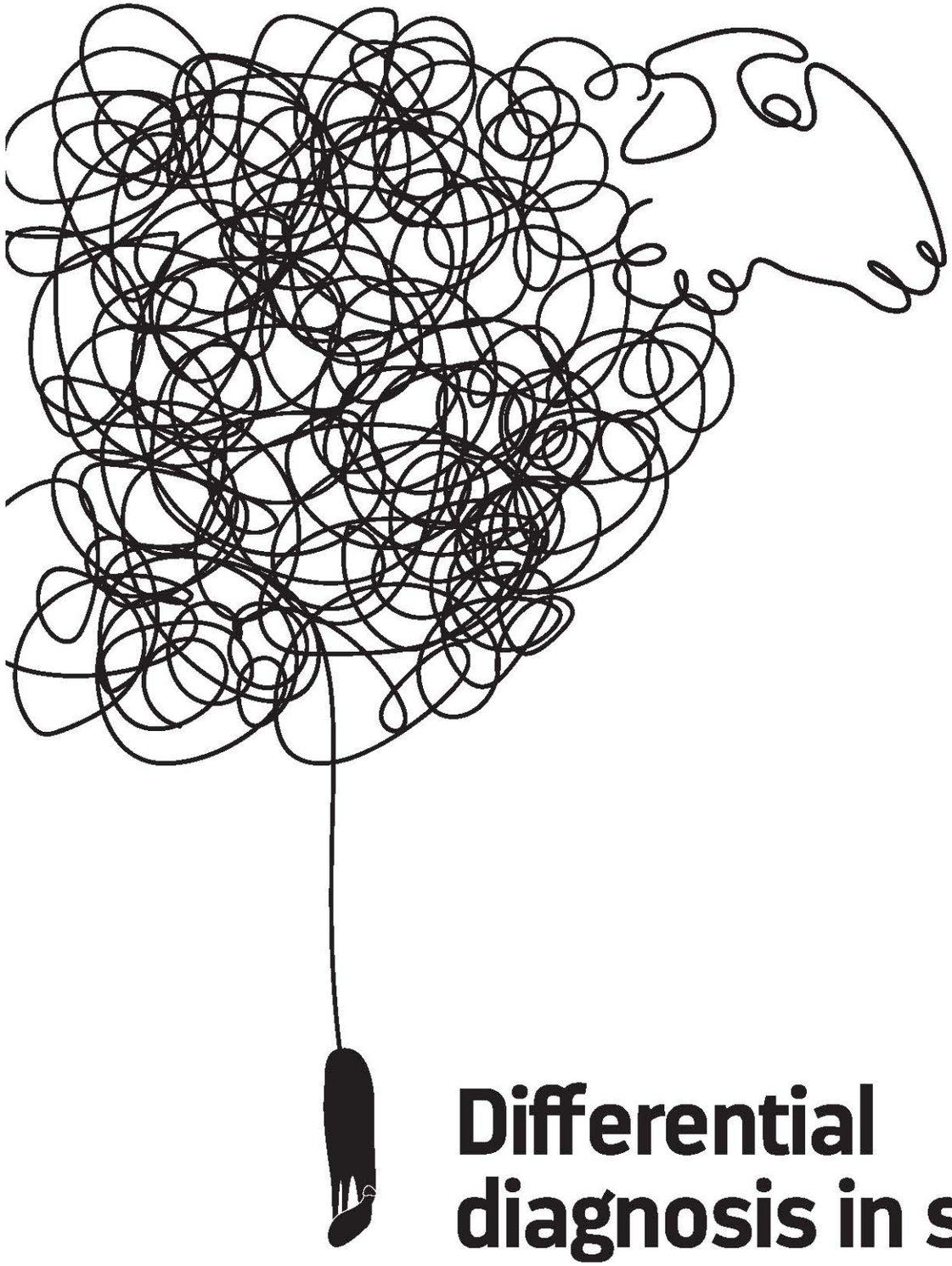

Differential diagnosis in sheep

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Seroprevalence and risk factors associated with small ruminant lentivirus infection in the north-eastern Portugal. OVISPID project results

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Keywords

Small Ruminants Lentiviruses; Seroprevalence; Risk factors; Intersticial pneumonia, Mastitis; Encephalitis; Arthritis.

Introduction

Small ruminant lentivirus (SRLVs) is a group of phylogenetically co-related viruses transmitted between caprine and ovine (Cirone *et al.* 2019). Previously, due to different clinical signs developed by each species, caprine arthritis-encephalitis virus was associated merely with caprine and Maedi-Visna virus with ovine species.

Several seroprevalence studies have shown that SRLVs are present worldwide except in Australia and New Zealand (Lago *et al.* 2012).

In research conducted in 1995, SRLVs seroprevalences were detected in 81.6% of the Portuguese herds and in 34.4% in the animals studied (Fevereiro, 1995).

In research conducted in 1995, SRLVs seroprevalences were detected in 81.6% of the Portuguese herds, and 34.4% of the animals studied (Fevereiro, 1995).

SRLVs cause systemic infection in ovine and caprine species, leading to lung, central nervous system, mammary gland and joint clinical presentations (Minguijón *et al.* 2015). These infections cause chronic, insidious and progressive diseases, seriously affecting animal health and causing economic losses (Michiels *et al.* 2018). Associated respiratory distress and neurological syndromes may evolve to cachexia and death. Joint and chronic mammary infections may lead to the disability with different grades of mobility impairment (primarily seen in goats) or decrease milk production leading to lambs and goat kids' malnutrition (Minguijón *et al.* 2015). Animals showing these clinical signs are often prematurely slaughtered.

Vertical transmission occurs through infected milk and colostrum ingested by young animals. Horizontal transmission occurs due to airborne particles spread through the air inhaled by these animals, being one of the main transmission routes in intensive production systems. SRLVs transmission can also occur via milking equipment, mainly in goats (Leginagoikoa *et al.* 2006; Villoria *et al.* 2013; Junkuszew *et al.* 2016).

Diagnosis is based on clinical, analytical, and post-mortem lesions evaluation. Nevertheless, most animals are asymptomatic and clinical signs may only develop years later after infection (Barquero *et al.* 2013). Laboratory methods are essential and may include serological (agar gel immunodiffusion and ELISA) and molecular techniques (PCR and RT-PCR) (Reina *et al.* 2009).

In the northeast of Portugal, small ruminant production has economic and social importance, and animals are produced using mostly traditional methods.

The main aim is this study is to conduct a research about seroprevalence and risk factors associated with SRLVs infection in sheep and goats in the north-eastern of Portugal.

Materials and methods

Data collection

The sample size was calculated based on the list of Trás-os-Montes' small ruminant herds registered at the official animal health database PISA.net. The sample size was calculated from the population data in 2019. Only herds with a minimum of 20 animals per herd were included in the study. All eligible herds agreed to participate in the study. The number of animals to be sampled was estimated using the formula n = (1.96)2 p (1 p)/d2 (Thrusfield, 2013). This sample size provides a 95% confidence level for an expected prevalence of 15%. Herds sampled were proportionally allocated according to the number of herds in the 12 counties under study. The number of samples taken per herd was 14-19. This sample size provides a 95% confidence level for an expected prevalence of 1% per herd and allows a compromise between the cost and precision of the estimates. Samples in the herds were randomly collected with aleatory numbers taken for a list of animals in each herd. The blood samples from sheep and goats ageing at least six months old were collected during technical visits of official veterinarians of the local health units. The sampling procedures and the laboratory tests were performed from September 2019 to February 2022. A herd was defined as SRLVs seropositive if at least one seropositive animal was present. Risk factors and health management protocols were recorded in a questionnaire in all small ruminant herds.

Serology analysis

Blood samples (10 ml) were collected from each animal by jugular venipuncture into 10-ml tubes (Vacutainer®, Becton Dickinson, Plymouth, UK) with a clot activator. Blood samples were allowed to clot at ambient temperature. Then, the serum was obtained by centrifugation at 200X g for 10 min and stored at -20°C until analysis.

Serological analysis was performed at Zamora Provincial Animal Health Laboratory. Infection by small ruminant lentiviruses (SLRVs) of each sample was determined by a commercial indirect ELISA test (ID Screen® MVV / CAEV Indirect) following the manufacturer's instructions. ELISA test is based on the use of a mixture of peptide antigens resulting in superior test performance, separating positive and negative results with high sensitivity and detecting all genotypes (including A, B and E) with high specificity.

Statistical analysis

Data collected were recorded in Microsoft Office Excel® (Office 365). Answers to the questionnaire of each farm were matched to the laboratory results through their official herd code identification, respecting the typology of the question. Variable analysis was performed using the chi-square test ($\chi 2$) to verify the association between the variables. JMP® Statistical Discovery version 7 software was used for this analysis. A significant effect was considered to $p<0.05$, a very significant effect with $p<0.01$ and a highly significant effect for $p<0.001$. A univariate analysis was performed between the independent variables according to the association between the causes of failure and the possible risk factors. Odds ratio (OR) values were estimated, and 95% confidence intervals were calculated.

Results and discussion

A total of 1,774 animals from 102 herds were tested (Table 1), of which 75 were sheep herds, 18 goat herds and 9 mixed sheep and goats herds.

In this study, 91 herds (89.22%) were seropositive to SRLVs. By specie, 66 (88.00%) of the ovine, 16 (88.89%) of caprine and 9 (100%) of mixed herds were seropositive to SRLVs.

The animal seroprevalence was very high, with 42.00% (n=745) of the animals being seropositive to SRLVs. In 1,306 sheep blood samples, 519 were positive (39.74%). Of the 309 caprine blood samples, 160 were seropositive (51.78%), and in 159 blood s amples collected from mixed herds, 66 (41.51%) were positive to SRLVs.

These results suggest that SRLVs infection is endemic in the studied region, with seroprevalences similar to those found in previous studies in the Iberian Peninsula (Fevereiro, 1995; Alba *et al.* 2008; Pérez *et al.* 2010; Kaba *et al.* 2013).

Analysis of the odds ratio (OR) for being seropositive to the potential risk factors was studied and is presented in Table 2.

There was an association between SRLV infection and animal age, farmer skill levels, and veterinary advice.

Animals older than 2 years showed a higher prevalence and were 2.17 times more likely to be infected (OR=2.17; IC95%: 1.76-2.66) by SRLVs than those younger than 2 years. This association corroborate the results previously published (McGuire, 1987; Sobrinho *et al.* 2010). There is a higher probability of being infected by another animal through different horizontal routes in a long animal's life.

When analysing breeder skill levels, we observed higher infection prevalence among those who raise small ruminants' as their primary activity. When comparing with the control group (i.e. "secondary activity"), the main activity group had 2.12 times more probability of being exposed to infection (OR=2.12; IC95%: 1.56-2.86). The association between infection by SRLVs and animal production according to breeder skill level is unclear and difficult to explain. Local market practices, such as regular nonofficial trade (i.e. temporary animal exchanges) among breeders, animal shows/

Table 1. Animal and herd SRLVs prevalence in North-Eastern Portugal between September 2019 to February 2022

	TOTAL	SHEEPS	GOATS	MIXED
Farms	102	75	18	9
Positive	91	66	16	9
% Positive	89.22	88.00	88.89	100.00
Animals	1774	1306	309	159
Positive	745	519	160	66
% Positive	42.00	39.74	51.78	41.51

Table 2. Risk factors associated with SRLVs infection in the North-Eastern of Portugal

	POSITIVITY TO SRLV		
	Prevalence	p	OR
Age of the animal			
< 2 years old	185 (24.63%)	< 0.0001	2.17
> 2 years old	566 (75.37%)		
Farmer skill levels			
Secondary activity	65 (8.66%)	< 0.0001	2.12
Main activity	686 (91.34%)		
Veterinary advice			
Yes	110 (14.65%)	< 0.0001	1.99
No	641 (85.35%)		

OR: odds ratio.

competitions, and likely further intensification and/or production pressure on these farms should be taken into account in the future.

Finally, we noticed that herds with no regular veterinary advice showed higher seroprevalence and were 1.99 times more likely to be exposed to infection (OR=1.99; IC95%: 1.47-2.70) than those who had regular veterinary advice. This highlights the importance of specialised veterinary advice in the application of good production practices and biosecurity measures for diseases control.

Small ruminant management practices in this region are standardised, characterised by dual-purpose (i.e. meat and milk) small farms mainly in a semi-extensive regime. Thus, some risk factors identified in other studies (i.e. intensive husbandry systems, high animal density, large herd size, etc.) may not have been identified, due to the inexistence/small number of this husbandry systems or due to some known bad management practices (i.e. poor milking practices, non-pasteurisation of milk/colostrum and prolonged exposure to infected animals, etc.) that are common across all herds studied.

Conclusions

SRLVs infection is a significant problem on small ruminant production in the Portuguese region of Trás-os-Montes. Animal age (older than 2 years), breeder skill levels (main activity) and absence of veterinary advice were associated with odds of SRLVs infection. The results of this study emphasise the need for breeders to regularly screen and eliminate seropositive animals from the herds. The present study results should be considered in developing an efficient SRLVs control program in Portugal.

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