Article

Small Ruminant Lentivirus Infection in Sheep and Goat in North Portugal: Seroprevalence and Risk Factors

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Abstract: Small ruminant lentivirus (SRLV) are infected and transmitted among ovine and caprine species. This disease is a severe problem for small ruminant production, not only for animals' wellbeing but also for the herd's efficiency. The main aim of this research was to quantify the seroprevalence and associated risk factors for SRLV infection in the north region of Portugal. Collected samples from a total of 150 herds, of which 129 (86.0%; 95% CI: 80.67% - 91.33%) had at least one seropositive animal. Out of 2607 individual blood samples, 1074 (41.2%) were positive for SRLV. The risk factors associated with SRLV infection were: specie (caprine), age (> 2 years old), herd size (> 100 animals), production system (intensive), production aptitude (milk), type of activity (professional), participation in livestock competitions (yes), buy replacement young ewe (yes) and rearing (natural). This knowledge empowers the implementation of effective preventive measures. Overall, biosecurity measures should be promoted and implemented to aim reducing viral transmission, with the main goal of reducing the prevalence of this disease. Completely, we understand that government authorities should promote and audit voluntary control and eradication programs in small ruminant herds in the region studied.

Keywords: sheep; goat; lentiviruses; SRLV; seroprevalence; risk factors

1. Introduction

Small Ruminant Lentivirus (SRLV) infection is a disease that affects ovine and caprine species caused by a group of phylogenetically co-related viruses (Family Retroviridae, genus *Lentivirus*). Originally, Maedi-Visna concept was used to describe the ovine disease, while Caprine arthritis-encephalitis to caprine disease. Nowadays, SRLV infection is worldwide accepted to describe different clinical and histopathological manifestations developed by the same viral aetiology [1]. Phylogenetic studies prove that SRLV can be divided into 5 genotypes, A to E, with subgroups in some [2].

Seroprevalence studies have shown that SRLV infection is present worldwide [3]. Having a heterogeneous distribution, it has significant variations between continents and even in different regions in the same continent [4]. Seroprevalences described in several studies are challenging to compare due to the different sensibility and specificity of the used diagnostic tests as well as the criteria used to define disease and sampling [5]. Remarkably, the high prevalence of SRLV in individual and herds of various European countries is notorious, this might be explained by the high

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density of the ovine population and intensive production systems [4]. Also, in the caprine population, the studies show high prevalence percentages of this infection [6].

SRLV transmission from infected progenitors to offspring may occur through milking with colostrum and milk [7,8]. This kind of transmission, though important, seems to have a minor role in spreading these viruses because the offspring may be infected with contact with other infected animals and truly not though milking [9,10]. Adult animals can be infected by inhaling viral particles from the secretions of infected animals [11], being described as the main possible transmission route in intensive production systems [12]. During pasturage, transmission seems to be extremely low, fact that favours extensive production systems [13,14]. Also, semen seems to be a possible route of virus transmission by mating and artificial insemination techniques [15,16]. However, it is unclear if this results in female or offspring infection [17].

SRLV infection develops as a progressive, inflammatory, and wasting disease that provoke chronic lesions that affects animals' health and prime to austere economic losses [8]. Affected individuals are persistently infected [1]. This disease may affect different organs such as the lung, central nervous system, mammary gland, and joints [18]. When the lung is affected, it is common to observe tachypnea and respiratory distress due to the developed interstitial pneumonia [19]. Clinical signs are initially detected with exercise, with affected individuals remaining behind when the herd moves. Both respiratory and neurological syndromes can lead the animal to progressive cachexia and subsequent death after a long period of illness [20]. Joint disease can cause lameness by affecting the carpal and tarsal joints [19]. The affection of the mammary gland results from the development of indurative mastitis [21]. Thus, animals with this syndrome are prematurely slaughtered due to suboptimal production [18]. The nervous form is less frequent and may present weakness and ataxia of the posterior limbs [22]. Clinical examination and post-mortem findings can be helpful for the Veterinarian when suspecting the presence of this infection in a herd. However, an early diagnosis should not be based on these, as most affected animals are asymptomatic and may develop clinical signs late after primoinfection [23,24]. This fact makes it difficult to establish an early suspicion of the entry of the infection in a herd, delaying the diagnosis of SRLV infection that should have been established previously with laboratory tests.

Among the different laboratory methods that can be used, we can include serological techniques, such as agar gel immunodiffusion tests (AGID) and enzyme-linked immunosorbent tests (ELISA) and molecular techniques, such as PCR and RT-PCR [25]. Blood serum is the sample of choice to perform serological tests. However, other biological samples, such as milk, can also be used [26,27]. ELISA test is a method that offers optimal results, being economical and easy to perform. Compared to ELISA, AGID tests are very specific but are less sensitive [28]. However, the heterogeneity of this group of viruses, the late seroconversion and the fluctuating antibody response determine important difficulties in the detection of SRLV [29]. Molecular tests are also useful in the diagnosis, especially for early detection of infection (before seroconversion) and as a complement to previous tests [7,30]. However, the low viral load in patients with latent infection and the high viral genetic heterogeneity decrease the PCR sensitivity. Therefore, no gold standard test for diagnosis has yet been defined [25]. In this sense, and to improve the detection of infection, a combination of different laboratory tests should be used to detect the maximum number of infected animals [24,31]. For example, some control programs resort to performing sequential tests, usually ELISA tests, followed by a confirmatory test, for example AGID [28].

Studies that address economic losses resulting from SRLV infection are scarce, with limited and incomplete information. However, authors generally agree that these are particularly significant for small ruminant producers [32]. The harmful impact on production indices and, above all, the high rate of early culling of animals due to the development of lesions and reduced production are identified as the points of most significant economic loss for herds [33,34]. The diversity of small ruminant herds can influence the negative economic impacts that the disease can have. Dairy herds seem to be the most affected by these negative impacts. In these, the development of the infection can decrease the amount of milk produced by infected animals [35,36] and negatively affect quality parameters and cheese yield [37,38]. Consequently, if there is lower milk production and quality, the offspring will also have a lower growth rate [39]. Furthermore, the health and well-being of animals

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affected by this disease are seriously compromised. There is, however, no major direct relationship between infection and the natural death of animals [32].

Livestock farming, specifically sheep and goats, provides an important economic, social, and cultural contribution to human beings since the housetraining of these species. Also, this activity has similar importance in the north region of Portugal. Despite the recent appearance of more industrialized farms, most small ruminant farms still carry out traditional management practices. Human activities have likely influenced the ecology of diseases such as SRLV infection [40]. It is essential to note how certain anthropogenic factors, such as international trade and husbandry practices, may play an important role in the spread of this disease. This knowledge, particularly of risk factors, can support the development of more effective control programs [41]. In many countries, veterinary health authorities have implemented eradication programs, some voluntary. They are generally based on (i) the removal of newborns immediately after birth; (ii) the slaughter of positive animals in periodic screenings; and (iii) the segregation of the herd into positive and negative animals [42]. So far, these have allowed an extreme decrease in the prevalence of this infection [41,43]. It is crucial to convey to livestock producers the most valuable aspects of these programs, namely, to emphasize the increase in the overall profitability of the farm [44]. In the absence of an effective vaccine or treatment, the only approach is to implement programs of this nature that should be encouraged worldwide. Also, at the level of livestock holdings, they should be encouraged preparing, and implement them considering the particularities of each herd and production system.

This way, the main objective of this study was to study the seroprevalence and potential risk factors associated with SRLV infection in sheep and goat farms in north of Portugal.

2. Materials and Methods

2.1. Data collection

The sample size was calculated based on the list of Bragança district 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. The number of animals to be sampled was estimated using the formula n = (1.96)2 p (1 p)/d2 [45]. 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 aged at least six months old were collected during technical visits from official veterinarians of the local health units. The sampling procedures and the laboratory tests were performed from September 2019 to February 2023. A herd was defined as SRLV 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.

2.2. Serological 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 -20ºC until analysis.

Serological analysis was performed at Zamora Provincial Animal Health Laboratory. Infection by small ruminant lentiviruses (SRLV) 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 [46].

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2.2. 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 with $p < 0.001$. A univariate analysis was performed between the independent variables according to the association between the causes of failure and the potential risk factors. Odds ratio (OR) values were estimated, and 95% confidence intervals (CI) were calculated.

3. Results

3.1. Seroprevalence of SRLV

A total of 151 small ruminant herds from north region of Portugal participated in this study. Table 1 represents the SRLV seroprevalence results from individuals and herds as well. Overall, a serological investigation was made in 2607 samples of ovine and caprine species from a total of 150 herds (samples from a single herd were lost).

Table 1. SRLV Seroprevalence of individual and herds in the north region of Portugal.

	Herds		**Animals**	
	Analysed (*n*)	Positive (%)	Analysed (*n*)	Positive (%)
Sheep	107	92 (85.98)	2035	778 (38.23)
Goats	32	26 (81.25)	572	296 (51.75)
Mixed	11	11 (100)	-	-
Total	150	129 (86)	2607	1074 (41.20)

One hundred and twenty-nine (129) herds had at least one positive animal to SRLV, with an apparent prevalence of 86.0% (95% CI: 80.67% - 91.33%). Considering the sensitivity (91.70%) and specificity (98.90%) of the diagnostic test used the actual prevalence in this region is 93.71% (95%CI: 89.98% - 97.44%). When analysing herds, it was verified that 92 ovine herds (85.98%), 32 caprine herds (81.25%) and 11 mixed herds (100%) were positive for SRLV.

In each herd, an average of 17 (17.38±1.28) blood samples were drained. The distribution of the herds was as follows: 21 herds (14.00%) didn't have any positive animal; 7 (4.67%) had less than 10% of positive animals; 66 (44.00%) had between 10 and 50%; 49 (32.67%) between 50 and 90% and 7 (4.67%) more than 90% of positive animals. From a total of 2607 collected samples, 1047 showed positive results in the diagnostic test; therefore, the estimated prevalence was 41.20% (95% IC: 39.32% - 43.07%) and the actual prevalence of 44.26% (CI 95%: 42.36% - 46.15%). In each specie, 778 ovine (38.23%) and 296 caprine (51.75%) were positive.

3.2. Risk factors analysis

Numerous factors that could influence SRLV infection in small ruminants in this region of Portugal were analysed and show in Table 2. These potential risk factors were identified using a questionnaire into small ruminant producers in the region.

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Table 2. Potential risk factors associated with SRLV infection in the North of Portugal

Variable	Analysed (*n*)	Seroprevalence (%)	*p* value	Odds ratio
Specie				
Caprine	572	296 (51.75)	< 0.0001	1.73 (1.44-2.09)
Ovine	2035	778 (38.23)		
Breed				
Exotic	1415	588 (41.55)	0.6898	-
Autochthonous	1192	486 (40.77)		
Age				
> 2 years old	1735	818 (47.15)	< 0.0001	2.15 (1.80-2.55)
< 2 years old	872	256 (29.36)		
Herd size				
> 100 animals	1572	718 (45.67)	< 0.0001	1.60 (1.36-1.86)
< 100 animals	1035	356 (34.40)		
Production system				
Intensive	55	43 (78.18)	< 0.0001	5.29 (2.77-10.07)
Semiextensive	2552	1031 (40.40)		
Production aptitude				
Milk	868	435 (50.12)	< 0.0001	1.73 (1.47-2.04)
Meat	1739	639 (36.75)		
Mixed herd				
Yes	192	71 (36.98)	0.2239	-
No	2415	1003 (41.53)		
Producer with training in animal production				
No	2174	876 (40.29)	0.0372	-
Yes	433	198 (45.73)		
Producer knows the disease				
Yes	382	218 (57.07)	< 0.0001	-
No	2225	856 (38.47)		
Type of activity				
Professional	2256	983 (43.57)	< 0.0001	2.21 (1.71-2.84)
Hobby	351	91 (25.93)		
Participation in livestock competitions				
Yes	319	151 (47.34)	0.0180	1.33 (1.05-1.68)
No	2288	923 (40.34)		
Contact with other herds				
Yes	1561	667 (42.73)	0.0564	-
No	1046	407 (38.91)		
Buy replacement young ewe				
Yes	495	250 (50.51)	< 0.0001	1.60 (1.31-1.94)
No	2112	824 (39.02)		
Rearing				
Natural	2552	1059 (41.50)	0.0375	1.89 (1.03-3.44)
Artificial	55	15 (27.27)		
Performs artificial insemination			< 0.0001	-

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Yes	68	57 (83.82)		
No	2539	1017 (40.06)		
Mating with males from other herds				
Yes	200	73 (36.50)	0.1784	-
No	2407	1001 (41.59)		
Unhealthy animals' isolation				
No	1815	750 (41.32)	0.8627	-
Yes	792	324 (40.91)		
Regular veterinary care				
Yes	263	159 (60.46)	< 0.0001	-
No	2344	915 (39.04)		

Univariate risk factor analysis found a statistically significant association between seropositivity to SRLV and specie (caprine: $p < 0.0001$; OR = 1.73, 95% CI: 1.44-2.09), age (> 2 years old: $p < 0.0001$; OR = 2.15, 95% CI: 1.80-2.55), herd size (> 100 animals: $p < 0.0001$; OR = 1.60, 95% CI: 1.36-1.86), production system (intensive: $p < 0.0001$; OR = 5.29, 95% CI: 2.77-10.07), production aptitude (milk: $p < 0.0001$; OR = 1.73, 95% CI: 1.47-2.04), type of activity (professional: $p < 0.0001$; OR = 2.21, 95% CI: 1.71-2.84), participation in livestock competitions (yes: $p = 0.018$; OR = 1.33, 95% CI: 1.05-1.68), buy replacement young ewe (yes: $p < 0.0001$; OR = 1.60, 95% CI 1.31-1.94) and rearing (natural: $p = 0.0375$; OR = 1.89, 95% CI: 1.03-3.44).

No statistically significant association ($p > 0.05$) was found between seropositivity to SRLV and breed, mixed herds, contact with other herds, mating with males from other herds, and unhealthy animal isolation. Other factors, despite presenting statistically significant association, may act as confounding factors: a producer with training in animal production (yes: $p = 0.0372$), a producer who knows the disease (yes: $p < 0.0001$), performs artificial insemination (yes: $p < 0.0001$) and regular veterinary care (regular: $p < 0.0001$).

4. Discussion

Limited data about SRLV prevalence in sheep and goat populations in Portugal have been published. This study demonstrates that SRLV infection is widespread in the north region of Portugal, affecting 86% of the participating herds and about 41% of the sampled animals. In sheep herds, the verified seroprevalence was 85.98% of positive herds and 38.23% of positive animals. A study carried out in 1995 in Portugal showed a slightly higher prevalence for the region. This study used a smaller sample size and other diagnostic laboratory tests, making comparing difficult [47]. There is some variation in the prevalence data presented in the literature from different regions in comparison with those obtained. In Spain, a neighbouring country, a similar prevalence in sheep has been reported in some studies [5,48,49]. However, other studies have also reported a lower prevalence [3,8,50]. In other continents, prevalence tends to be lower than in Europe [51,52].

In goats, the prevalence obtained in our study was 81.25% of positive herds, and the individual prevalence was 51.75%. Some studies reported a similar prevalence in goat herds [53] and others lower, especially individual prevalence [1,6,54–56]. Some of the low levels of seroprevalences reported in some studies are due to official or voluntary control programs implemented in these countries. It is important to mention that Portugal has never had an official program to control this disease. Some more developed farms in other regions of the country started individual programs on their own initiative with the help of their veterinarians.

Sheep and goat rearing in the north region of Portugal are mainly semi-extensive, with grazing during the day and collection at night in stables or high-density fences. More traditional production methods prevail, and management practices are very standardised. The statistical significance analysis carried out in this study demonstrates that certain risk factors can influence the presence of SRLV infection in herds of small ruminants in this region.

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Specie analysis showed to have an association with SRLV infection. Goats herds showed higher seroprevalence and a greater probability of occurrence of the infection than sheep. Phylogenetic studies are necessary to know the SRLV variants circulating in the region. Some of these appear to be species-specific; however, others transmit between both species [57,58]. Some studies point to breed as a possible risk factor for SRLV infection [48,53,59]. There is evidence that host genetics (breed) may influence its susceptibility/resistance to SRLV infection and disease progression [60,61]. In our region, there are 4 indigenous sheep breeds and 2 indigenous goat breeds; for this reason, we only check whether the analysed herd had one of the indigenous breeds or an exotic breed. We found no statistical association between this distinction and infection. Regarding the age of the animals, we found that those over 2 years old had a significantly higher seroprevalence and were more than twice as likely to be infected. This is in concordance with many studies that reported age as a relevant risk factor [3,52,59]. This may be due to lifetime exposure to the agent that can determine the contagion of animals free of infection at some point [62]. It is added that the late seroconversion, characteristic of this disease, can also influence the laboratory positivity and delay the diagnosis.

Herd size has also been shown to be statistically associated with SRLV infection. Herds with more than 100 animals were more likely to acquire the infection than those with less than 100. This data has been reported in several other epidemiological studies [5,8,52,54,63]. Similarly, intensively reared animals also had a significantly higher seroprevalence and a greater probability of infection. Both risk factors are mentioned to have a relevant influence on SRLV infection in the literature [48,50,59]. It is common that larger herds are also produced more intensively, with greater population density, facilitating the transmission of the virus between animals [13,49]. We also obtained higher seroprevalences in dairy herds compared to meat production herds. Literature needs to be more precise about the influence of productive aptitude. However, it is known that the productive pressure on dairy sheep is much higher than on meat-production herds. Lactating ewes may be immunologically compromised and susceptible to various infections, including SRLV [13]. Literature indicates that SRLV can infect mixed sheep and goat herds more frequently than single-species herds [3,5,52]. However, in our study, there was no statistical association between mixed herds and positivity. As previously mentioned, phylogenetic studies are needed in this region to better understand the SRLV variants present that may influence this data.

Regarding producers, we found that those with training in livestock production and those who knew the disease had higher seroprevalence, which seems contradictory data. However, we can speculate that trained producers tend to have larger herds and in an intensive regime. As well as producers who knew about the disease could have been affected by it in their herd and had been previously diagnosed by their veterinarian. The percentage of producers who do not know about the disease is also high in other studies [8] and, therefore, not knowing about the disease are not motivated to fight it. It was also found that professional producers had a higher prevalence and more than twice the probability of their animals being infected compared to hobby producers. Despite greater knowledge and attention on the part of professional producers, they usually have larger herds and often trade in animals, which can contribute to higher prevalence. Participation in livestock competitions was also shown to be statistically associated with SRLV infection. Livestock contests favour the permanence, in the same space, of animals with different origins and unknown health statuses for some diseases. It is added that these also favour the trade of breeders between herds, which will enter the farm without worrying about screening for the infection [64].

Contact between different small ruminant herds was not associated with infection in this study. Despite this, we know that it can play an important role in the dissemination of several diseases, including SRLV [52]. This is a concern in this region, where herds are often driven through common pastures and spend the night in urban areas where other herds may also be held, posing a risk [63]. Buying animals from other herds may also pose a risk of disease entering the herd [56]. The purchase of replacement young ewes showed a higher seroprevalence and a higher probability of infection in our study. Similarly, the type of rearing was also significantly associated with SRLV infection. Natural rearing with colostrum and milk from positive females is one of the most effective means of transmission considered in the bibliography [65]. We found that performing artificial insemination was significantly associated with the disease. This data is not in line with the literature that mentions

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natural breeding as a possible risk factor [15,53]. Although there is no clear evidence of venereal transmission [17], artificial insemination is usually performed using SRLV-free semen whenever purchased from certified centres. This factor presented in our results may only act as a confounding factor, or the artificial insemination practiced does not follow the most appropriate norms. Farms that had regular veterinary care had a higher prevalence of infection. This contradictory fact may not be accurate because these herds are usually also larger and with more relevant productive pressures.

Other risk factors commonly presented in the literature are difficult to analyse in this region. The standardization of the characteristics of herds and management carried out makes it difficult, on the one hand, to collect other types of data, and, on the other hand, it has reduced the robustness of the potential risk factors that we present.

This study has potential limitations. First the research design, since it is a cross-sectional study which was carried out at a local level in a single region. It is our future goal to include other regions of Portugal to obtain information about SRLV infection in these other regions and as well in different production systems. This study may also present a -1 error due to the high number of variables included in the model and to the number of statistical tests performed. Performing a multivariate analysis instead of a univariate one could also provide greater robustness to our data. Due to these limitations imposed by the study design itself, these results need to be interpreted with care, as it was not possible to clearly identify a cause-and-effect relation.

High seroprevalence verified in this study supports the urge to develop a strategy for implementing effective SRLV control programs. Due to the high costs of implementing an exhaustive control program, initially the reduction and minimization of the risk of infection by SRLV should be promoted through biosecurity measures such as: i) removal of offspring from mothers soon after birth and artificial rearing; ii) separation of infected animals; iii) periodic screening for SRLV; iv) acquisition of animals from certified SRLV-free herds. Later, more drastic measures, such as the culling of seropositive animals, can be implemented, but they are only viable for the low prevalence of infection. However, the motivation of producers is essential for the success of a possible control program. The immediate economic and productive benefits of controlling this disease should be highlighted.

5. Conclusions

In this study, we founded a highly SRLV seroprevalence in sheep and goat herds, concluding that SRLV infection is widespread in small ruminant herds throughout the north region of Portugal. The epidemiological study of risk factors contributes to a greater and better knowledge of the disease. Early detection of this disease is essential, using laboratory tests such as serological tests. Thus, adapted and effective preventive measures can be implemented to reduce viral transmission. This study also should serve to encourage veterinary health authorities to promote and audit voluntary control and eradication programs to control this disease in sheep and goat herds in Portugal.

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