

SEROPREVALENCE OF SMALL RUMINANT LENTIVIRUS INFECTION IN A GOAT FLOCK IN EASTERN ROMANIA

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ABSTRACT. Small ruminant lentiviruses (SRLVs) pose significant economic challenges to goat farming and have a detrimental impact on animal welfare. The objectives of this study were to deepen our comprehension of SRLV infections and their implications for small ruminant farming. Within the studied herd, the detected seroprevalence of SRLV was notably high at 91.92% (95.0% CI: 86.5%–97.3%). This finding strongly suggests the enduring presence of SRLV infection within the goat farm, spanning multiple years. Antibodies against SRLVs were detected across all selected age categories and breeds, irrespective of the presence or absence of clinical signs. The data gleaned from this study allow significant implications, raising concerns about the potential for future clinical outbreaks resulting from SRLV infections in eastern Romanian goat herds. Given these findings, the imperative for the continuous surveillance of SRLV infections

becomes evident. Ongoing vigilance and monitoring are essential to mitigate the risk of disease transmission, minimise economic losses and safeguard the welfare of goats within the region.

Keywords: caprine arthritis encephalitis; goat; lentivirus; Maëdi-Visna.

INTRODUCTION

Small ruminant lentiviruses pose significant economic challenges for goat population in Romania. Within this group of viruses, the Maëdi-Visna virus and the caprine arthritis-encephalitis virus (CAEV) stand as notable prototypes, capable of infecting both sheep and goats. Recently, these viruses have been collectively categorised as small ruminant lentiviruses (SRLVs), belonging to the Retroviridae family.



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Their classification as SRLVs underscores their shared characteristics and pathogenic potential in small ruminants. One of the key characteristics that make SRLVs particularly challenging to manage is their remarkable genetic diversity. This diversity poses obstacles in diagnosing and understanding the viruses' epidemiology and pathogenesis. Additionally, SRLVs are recognised for their ability to trigger a multifaceted and gradual humoral immune response (Ramirez *et al.*, 2013). This intricate immune response pattern further complicates the accurate diagnosis of SRLVs infection, making it essential to develop more precise diagnostic tools and strategies. This remarkable characteristic contributes to the distinctive nature of lentiviral infections. In susceptible hosts, the course of infection is marked by a gradual and irreversible progression as a consequence of virus persistence. Lentiviruses integrate their genetic material into the host's genome, specifically within the chromosomes of cells that are critical to the animal's defense system, notably monocytes/macrophages (Jarosław *et al.*, 2021; Minguijón *et al.*, 2015). Monocytes and macrophages play a vital role in defending the host against pathogenic microorganisms, making them a prime target for lentiviral integration. Consequently, the lentiviral infection exerts a profound influence on these antigen-presenting cells, although the precise immunopathological mechanisms that underlie this process remain the subject of ongoing research and investigation. These immunopathological mechanisms

involve critical components of the immune system, with professional antigen-presenting cells serving as a primary focal point. Lentiviral infections modulate the activity of these cells, including their production of cytokines – signalling molecules that play pivotal roles in immune responses (Larruskain and Jugo, 2013). Additionally, the virus manipulates the B- and T-cell immune responses. Antibodies directed against certain viral proteins are produced in large quantities but cannot eliminate the viruses protected by the infected cells and therefore have no effect on the proviruses integrated into these cells (Blacklaws, 2012). Moreover, these antibodies do not have significant neutralising activity against the virus. Together with a capacity for *in vivo* mutation, the virus easily escapes the immune defense.

Small ruminant lentiviruses exhibit a notable mode of transmission, primarily spreading from does to their offspring through the ingestion of infected colostrum or milk (Kaba *et al.*, 2022). This transmission route underscores the importance of addressing SRLV infections in breeding does as it can perpetuate the virus within herds. Direct contact with various body secretions presents another avenue for potential infection. These secretions may contain free viral particles as well as infected macrophages or epithelial cells, all of which can serve as sources of viral dissemination (Jacob-Ferreira *et al.*, 2023). In particular, the virus has been detected in saliva, raising concerns about the possibility of transmission through nose-to-nose contact and highlighting the importance of maintaining good hygiene practices within herds (de Souza

et al., 2015). Additionally, the use of breeding rams introduces another potential infection route. Rams can carry the virus and may transmit it to does during mating. Therefore, the careful selection and monitoring of breeding rams play a crucial role in preventing the further spread of SRLVs within a herd (Adjadj *et al.*, 2020). Understanding these various transmission modes is essential for implementing effective control measures and biosecurity practices to curb the prevalence and impact of SRLV infections in small ruminant populations.

Given the absence of any available treatment or vaccine to protect healthy individuals, it becomes evident that infected animals can serve as reservoirs of the virus, thereby facilitating its transmission to other healthy animals within the same herd. The inability to intervene medically underscores the importance. The percentage of affected animals may vary among goat families, emphasising a significant genetic factor contributing to the disease (Minguijón *et al.*, 2015). The clinical manifestations associated with SRLV infections are multifaceted, with distinct patterns emerging among different age groups. In young animals, the prominent clinical presentations often revolve around encephalitis and interstitial pneumonia, whereas in the case of adult goats, arthritis and mastitis are more commonly observed. These diverse clinical signs reflect the complex nature of SRLV infections and their ability to affect various organ systems (Shuralev *et al.*, 2021).

The primary aim of this study was to comprehensively assess the

seroprevalence of SRLVs within a substantial goat farm setting. Beyond merely determining the prevalence, the study also sought to meticulously evaluate the risk factors associated with SRLV transmission within the flock. Furthermore, it aimed to elucidate the broader impact that SRLV infections have on the overall health and productivity of the infected herd.

MATERIALS AND METHODS

Sample collection

Serum samples for this study were obtained from a goat farm ($n = 2,800$) in eastern Romania which was suspected to be infected with small ruminant lentiviruses. The goat farm included animals from three different breeds: Alpine, Saanen and Murciano-Granadina. During 2022, 99 blood samples from 95 females and four males were collected. Young animals (older than 6 months) and adults were included in this study, both with and without clinical signs. After collection, blood samples were centrifuged at $1,200 \times g$ for 10 min, and the serum was stored in the freezer at -20°C until use.

Serological testing

All samples were analysed for Maëdi-Visna virus/caprine arthritis encephalitis virus (MVV/CAEV) antibodies using a commercially available ELISA kit (ID Screen[®] MVV/CAEV indirect ELISA, Innovative Diagnostics France). The diagnostic kit is specifically designed to identify antibodies directed against MVV or VAEV in the serum or plasma of both sheep and goats. This diagnostic kit uses a carefully selected panel of

peptides derived from the transmembrane envelope protein (TM) as well as the glycoprotein 135 (gp135) and capsid protein p25, which are vital components of both MVV and CAEV. By incorporating this combination of viral antigens into the test, sensitivity and diagnostic accuracy are enhanced. This is especially critical given that the humoral immune response varies depending on the stage of the infection. Notably, antibodies targeting gp135 tend to predominate in animals that are in the chronic phase of the infection. However, during the early stages of infection, antibodies against capsid antigens, specifically p25, become more prevalent. Thus, by including a diverse array of viral antigens in our diagnostic panel, we can effectively capture the dynamic nature of the humoral immune response throughout the course of MVV and CAEV infections. Samples were analysed, and the results were interpreted following the manufacturer's instructions. The diagnostic performance of the tests is represented by sensitivity (Se) values above 90% or specificity (Sp) values above 95% (Nowicka *et al.*, 2014).

Statistical analysis

The statistical analysis carried out in this study encompassed a comprehensive evaluation of seroprevalence at the individual animal level, complemented by the calculation of a robust 95% confidence interval to provide a reliable estimate of the results. Confidence limits for apparent prevalence were determined using the methods outlined by Brown *et al.* (2001). True prevalence estimates were computed following the methodology

outlined by Rogan and Gladen (1978). To begin, a univariate analysis was conducted, employing the *chi-Square* test as a fundamental statistical tool. This initial step aimed to explore and elucidate the potential relationships between each independent variable under consideration, namely age category and breed, and the outcome variable of seropositivity. Subsequently, a meticulous selection process was employed to identify predictors with statistical significance. Variables with a *p-value* less than 0.50 in the univariate analysis were singled out as candidates for inclusion in a binary logistic regression model.

RESULTS

SRLV seroprevalence in sampled flock. Out of the 99 tested animals, 91 were identified as positive for SRLV antibodies. The outcomes of the MVV/CAEV indirect ELISA test conducted on the goat samples unveiled an overall seroprevalence of 91.92% (95.0% CI: 86.5–97.3%). The true prevalence of the infection based on the known Se and Sp values of the diagnosis kit was 102.2 (95.0% CI: 93.9%–106.8%). Notably, this implies that a vast majority of the tested goats within the study population exhibited detectable antibodies against SRLVs. All goats aged 3 years and older were positive for SRLV-specific antibodies. This age-specific pattern highlights the cumulative nature of SRLV infections over time, with older animals being more likely to have encountered and developed an immune response to the virus. Furthermore, antibodies against SRLVs were detected across all selected

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age categories and breeds included in this study (Table 1). This broad distribution of seropositivity emphasises the widespread nature of SRLV infections within the sampled goat population, transcending age and breed distinctions.

Table 2 provides a comprehensive overview of the seropositive animals, with a breakdown according to the two independent variables: age and breed. In addition to this descriptive information, the results of the univariate analysis are presented, shedding light on the associations between each independent variable and seroprevalence. A notable observation emerging from the univariate analysis is the significant relationship between age and seroprevalence. Conversely, when it comes to breed, univariate analysis did not reveal any significant differences in

the seroprevalence of SRLV infection among the three breeds raised within the farm. This suggests that, at least in the context of this study, breed alone may not be a decisive factor in determining the likelihood of SRLV infection.

Clinical signs identified in the sampled flock

In the course of our investigation within the goat farm under study, we also detected clinical cases associated with SRLV infection. Among these cases, joint lesions emerged as a predominant concern among infected goats. These lesions manifested as arthritis, primarily affecting the carpus (knee joint) and, albeit less frequently, the tarsus (hock joint). Notably, this joint involvement often exhibited a bilateral pattern (Figure 1).

Table 1 – Prevalence of anti- SRLV antibodies in goats within the tested farm

Age of the animals	Seropositive animals for MVV/CAEV antibodies			Seronegative animals for MVV/CAEV antibodies	
	Number of animals	Breed			Number of animals
		Alpine	Saenen	Murciano-Granadina	
< 2 years	14	5	9	1	4
2 years	26	7	17	1	4
3 years	25	8	9	8	0
> 4 years	26	14	1	11	0
Total	91	34	36	21	8

Table 2 – Percentages of seropositive cases and relationship between each independent variable and SRLV infection in goats

Variables	No. of animal tested	Seroprevalence (%)	95% Confidence interval	p-value
Age				
< 2 years	18	14.1	7.2 to 21	0.117
2 years	30	26.2	17.6 to 34.9	
3 years	25	25.3	16.7 to 33.8	
> 4 years	26	26.2	17.6 to 34.9	
Breed				
Alpine	38	89.5	79.7 to 99.2	0.692
Saenen	38	94.7	87.6 to 101.84	
Murciano-Granadina	23	91.3	79.8 to 102.82	



Figure 1 – Clinical manifestations in SRLV infection often revolve around the development of arthritis, primarily affecting the carpal joints. (A) Lameness. (B) Enlargement and outward displacement of the carpal joints in an adult goat

These progressive joint hypertrophies led to a significant reduction in mobility, occasionally culminating in complete ankylosis, where the joint becomes immobile and fixed. The pathological hallmark of these lesions includes the thickening of peri-articular tissues, along with the calcification of the joint capsule and tendons. It should be noted that these joint lesions primarily afflicted animals aged 2 years and older. This age-specific pattern of occurrence underscores the progressive nature of SRLV-associated arthritis and its potential to significantly impact older members of the goat herd. These clinical findings highlight the debilitating effects of SRLV infections, particularly the profound discomfort and reduced mobility experienced by affected animals.

DISCUSSION

Small ruminant lentivirus infections are widespread, with documented cases occurring on every continent and in most

countries, although it is important to note that some regions still lack comprehensive epidemiological data despite the potential presence of the virus. In developed European countries, particularly those with intensive dairy production systems, elevated rates of SRLV infection have been reported. This phenomenon is likely linked to specific farming practices, including the adoption of mechanical milking techniques and the distribution of colostrum and mixed milk. These practices inadvertently facilitate the transmission of the virus within goat farms, contributing to its prevalence (Illius *et al.*, 2020).

The real economic impact of the spread of SRLVs within the farming sector remains incompletely evaluated, although it has significant direct and indirect consequences. Direct losses are readily observable and include factors such as an increased need for replacement animals due to the culling of goats displaying clinical signs of infection, diminished growth rates in

lambs, shortened lactation periods and reduced milk production in infected animals (Reina *et al.*, 2009). These direct losses are detrimental to the financial viability of goat farming operations.

In addition to direct losses, indirect losses are incurred due to limitations on the exchange of breeding stocks and semen, impacting the genetic diversity and quality of goat populations. These constraints on breeding practices have implications for the long-term sustainability and genetic health of goat herds. Whilst the economic ramifications of SRLV infections in the farming sector are multifaceted and not yet fully quantified, it is evident that these infections exert a substantial toll, both directly and indirectly.

Close proximity among animals residing in overcrowded barns represents also a significant factor that is likely to facilitate the transmission of infections. When animals are densely housed, the opportunities for direct contact and the sharing of common spaces increase, which, in turn, heightens the risk of disease transmission within the herd (Blacklaws *et al.*, 2004).

Furthermore, the management practices employed on a farm can exert a profound influence on the prevalence of infections. These factors collectively shape the disease landscape within a herd, directly impacting the frequency and severity of disease outbreaks. Taking all these factors into account, it becomes clear that the interaction among animals, their genetics and farm management practices represent a dynamic and complex network that determines the overall health and disease

resilience of livestock (Broughton-Neiswanger *et al.*, 2010).

The eradication of SRLVs only requires the prevention/elimination of infected animals since there is currently no vaccine or therapeutic treatment. Different control programmes based on voluntary work and offering a qualification system have been set up in recent decades in different countries against SRLV infections (WOAH, 2023). Seroprevalence studies conducted in various European countries have typically relied on voluntary control programmes as a primary data source. These programmes, initiated by farmers and veterinarians, involve the systematic monitoring and testing of animals within specific herds or regions to assess their serological status regarding SRLV infections. One conspicuous finding that emerges from these seroprevalence studies is the notable disparity in the prevalence of SRLV infections across different countries. The variation in infection rates becomes readily evident when comparing data from one area to another. This regional heterogeneity underscores the complex interplay of factors influencing the spread and persistence of SRLVs within small ruminant populations.

Furthermore, the findings elucidated in the studies conducted by Arsenault *et al.* (2003) and Huttner *et al.* (2010) emphasise a noteworthy trend: the seroprevalence rate of small ruminant lentiviruses tends to be higher in larger flocks, those encompassing more than 250 animals, as opposed to smaller flocks comprising fewer than 100 animals. This observation underscores the potential for SRLV

infections to exhibit an increased presence in larger and more densely populated livestock groups. One of the unique challenges posed by lentiviruses is their protracted incubation period. This extended period of silent infection can lead to the widespread dissemination of the virus within a farm or across an entire region before any clinical cases become apparent. The insidious nature of lentiviral infections is further underscored by the gradual onset and progression of clinical symptoms in affected animals. The inconspicuous initiation and slow evolution of the disease make early detection and intervention particularly challenging. Given that the primary target organs of SRLVs encompass the joints, lungs, central nervous system and mammary glands, a meticulous clinical examination should encompass a thorough assessment of these predilection sites. Detecting any potential lesions or abnormalities in these areas is crucial for early diagnosis and effective disease management. This is why comprehensive surveillance, including routine testing and monitoring, is paramount, especially in large-scale farming operations.

In response to the significant economic losses caused by SRLV infections, several European countries have taken proactive measures to control and, in some cases, eradicate the virus (Illius *et al.*, 2020; Pérez *et al.*, 2010). These initiatives often operate on regional or national scales and involve a multifaceted approach. Prominent examples of countries implementing such plans include France, Switzerland, the Netherlands, England, Finland, Norway, Italy and Spain. One key facet

of these control and eradication efforts is the establishment of officially designated disease-free breeding grounds. These proactive measures not only safeguard the health and productivity of livestock but also contribute to the broader goal of reducing the prevalence and impact of SRLV infections on a regional or national level.

The seroprevalence findings obtained in our study provide strong indications that SRLV infections have had a longstanding presence in the studied goat farm, extending their impact over several years. Notably, Romania has seen limited published research on the seroprevalence of SRLV infections until recently. Potârniche *et al.* (2018) conducted a comprehensive 3-year survey in Sibiu County, revealing a consistent increase in SRLV seroprevalence year after year. Building upon this, Potârniche *et al.* (2020) expanded their investigation to encompass 13 Romanian counties, ultimately estimating a true individual-level seroprevalence of SRLVs at 31%. These studies shed light on the evolving landscape of SRLV infections within the country. In the southeastern region of Romania, Gurău *et al.* (2015) reported a significant SRLV seropositivity rate of 38.46% in Brăila County, further emphasising the regional variability in infection rates. Similarly, in northeastern Romania, Mihai *et al.* (2018) conducted a comprehensive screening of 412 symptomatic and asymptomatic goats across four counties, revealing a true individual-level seroprevalence of 28.1%. These studies collectively highlight the widespread presence of SRLV infections in various regions of

Romania and emphasise the necessity of continued surveillance, comprehensive research and targeted control measures to effectively manage the impact of SLRV infections.

In most cases, only a small proportion of infected animals exhibit noticeable clinical signs, which often leads to an underestimation of the disease's overall significance by livestock owners. This phenomenon is particularly challenging because it can mask the true extent of the problem, leaving many infected animals undiagnosed and untreated. The emergence of clinical signs and the consequent economic losses are intimately connected to the seroprevalence rate within a given population (Gufler *et al.*, 2005; Peterhans *et al.*, 2004). When the seroprevalence rate is high, there is a greater likelihood of clinical cases emerging, resulting in economic repercussions such as reduced productivity, increased veterinary costs and, potentially, the culling of affected animals.

Implementing a complete culling of animals affected by a disease can inadvertently result in the loss of significant genetic advancements achieved through meticulous selection processes within genetic improvement programmes. These programmes aim to enhance desirable traits in livestock, such as increased milk production, superior meat quality or disease resistance. The frequency at which testing is needed to confirm a herd's disease-free status can vary considerably among countries. Typically, this interval falls within the range of every 6, 12 or

24 months, depending on the specific protocols and regulations in place. The choice of the testing frequency reflects a balance between the need for regular surveillance to ensure the absence of disease and the practicality of conducting such tests on a recurring basis (Kalogianni *et al.*, 2020). The control plans, based on the principle of the impermeability of the placental barrier and the thermolability of the viruses, consist of separating the young from birth and feeding them with heat-treated colostrum (heating at 56°C for 1 hour) or substitute colostrum (bovine or commercial colostrum) and/or artificial milk (Berriatua *et al.*, 2003).

The implementation of proactive prophylactic measures has yielded valuable insights into the management of SRLV infections. A notable example is the experience in Switzerland over the last decade, as documented in a study by Thomann *et al.* (2017). By diligently applying these preventive measures, Switzerland has achieved a discernible reduction in SRLV seroprevalence rates. This serves as a testament to the effectiveness of strategic disease management strategies in curbing the impact of SRLV infections within the country.

Similarly, Spain has made significant strides in addressing SRLV infections, successfully lowering both seroprevalence rates and the frequency of seroconversion events. This achievement extends across various types of sheep farms, including those categorised as extensive or semi-intensive, and encompasses diverse meat and dairy breeds. Biescas (2006) has documented these noteworthy

advancements, highlighting Spain's commitment to mitigating the prevalence and impact of SRLV infections within its small ruminant populations. These examples underscore the potential for proactive measures to yield tangible results in controlling and managing SRLV infections. They serve as compelling evidence of the positive outcomes that can be achieved through concerted efforts in disease prevention, monitoring and strategic intervention.

Nonetheless, the effectiveness of these control plans is confronted with certain limitations. First and foremost, some of the diagnostic tests in use may not fully capture the complexity of SRLV infections, potentially resulting in underdiagnosis or misinterpretation of the disease status.

In light of these challenges, it becomes clear that optimising the control and management of SRLV infections requires a multifaceted approach. This approach should not only address diagnostic shortcomings and encompass a broader understanding of transmission dynamics but also consider the socio-economic factors influencing breeder engagement and cooperation. By recognising and proactively addressing these limitations, a more resilient and comprehensive strategy for mitigating the impact of SRLV infections in goat populations can be developed.

CONCLUSIONS

Infections of farming goats by SRLVs have far-reaching consequences, extending beyond the individual animals to have a profound impact on both the economic viability of goat farming and the welfare of the infected livestock.

Economically, SRLV infections are associated with substantial losses, including weight reduction and decreased milk production. The results of this study indicate the potential for future clinical outbreaks stemming from SRLV infections in eastern Romanian goat herds. This underscores the ongoing and crucial need for the vigilant surveillance of SRLV infections, with the aim of not only minimising economic losses but also ensuring the health and welfare of goats within the industry. The multifaceted nature of these challenges necessitates a holistic approach, taking into account both economic and ethical imperatives to effectively manage SRLV infections in goat populations.

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