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Immune response dynamics of recent and chronic small ruminant lentivirus infection in the male reproductive system

Dinâmica da resposta imunológica da infecção recente e crônica por lentivírus de pequenos ruminantes no sistema reprodutor de machos

Renato Mesquita Peixoto[1]*; Alice Andrioli[2]; Raymundo Rizaldo Pinheiro[2]; Kelma Costa de Souza[3]; Juscilânia Furtado Araújo[4]; Ana Lídia Madeira de Sousa[5]; Ana Kelry Carneiro Lopes[6]; Samara Cristina Rocha Souza[7]

Highlights

Immune responses to small ruminant lentivirus (SRLV) vary by infection stage.
Chronically infected bucks have lower immune responses to SRLV.
Anti-SRLV antibody detection may only occur in seminal plasma.
Distinct infection stages change the immune response in bucks.
Seminal plasma Western blot is an alternative diagnosis for SRLV.

Abstract

The objective of this study was to analyze the immune responses of bucks to small ruminant lentivirus (SRLV) with a focus on the reproductive system of males with recent and chronic infection. A total of 12 bucks were selected, six seronegative and six seropositive with chronic natural infection for more than 18 months (chronic infection group). After selecting the animals, the six seronegative males were intravenously inoculated with caprine arthritis-encephalitis virus (CAEV)-Co viral strain at a titer of $10^{-5,6}$ $TCID_{50}$/mL. After viral inoculation, this group was called the recent infection group and was monitored weekly with the chronically infected group for 180 days with blood serum and seminal plasma Western Blot (WB) analysis. Of the animals with chronic SRLV infection, 18.94% (50/264) showed anti-SRLV antibodies in at least one

[1] Prof. Dr., Centro Universitário Vale do Salgado, UNIVS, Icó, CE, Brazil. E-mail: renatomiraima@gmail.com
[2] Drs., Researchers, Empresa Brasileira de Pesquisa Agropecuária, Embrapa Caprinos e Ovinos, Sobral, CE, Brazil. E-mail: alice.andrioli@embrapa.br; rizaldo.pinheiro@embrapa.br
[3] Profª Drª, Universidade Estadual do Piauí, UESPI, Corrente, PI, Brazil. E-mail: kelma_zoo@hotmail.com
[4] Profª Drª, Faculdade IEducare, FIED, Tianguá, CE, Brazil. E-mail: juscilania.furtado@fied.edu.br
[5] Profª Drª, Faculdade Educar da Ibiapaba, FAEDI, Ipu, CE, Brazil. E-mail: analidiams10@yahoo.com.br
[6] PhD Student in the Pos-Graduate Program in Zootechnics, Universidade Federal de Viçosa, UFV, Viçosa, MG, Brazil. E-mail: kelry_lopes@yahoo.com.br
[7] Master Student in the Post-Graduate Program in Veterinary Sciences, Universidade Estadual do Ceará, UECE, Fortaleza, CE, Brazil. E-mail: samaracr.rocha@gmail.com
* Author for correspondence

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of the samples, and 81.06% (214/264) were negative. Anti-SRLV antibodies were detected in 27.27% (36/132) of the blood serum samples from this group, while 10.60% (14/132) were reactive in the seminal plasma WB test. The animals inoculated with CAEV-Co became seropositive after the third week of viral inoculation. In this group, 31.06% (41/132) of seminal plasma samples had anti-SRLV antibodies, and of these, 70.73% (29/41) coincided with blood serum results. Of the remaining 29.27% (12/41), the seminal plasma sample of only three animals (RIA2, RIA3, and RIA5) had anti-SRLV antibodies. One of the animals with a recent infection presented anti-SRLV antibodies only in seminal plasma samples, possibly due to virus compartmentalization. Intermittent viral shedding was observed in both biological samples, regardless of the infection stage. The immune response in bucks with recent SRLV infection is more significant than in chronically infected animals. Regardless of the stage of infection, there is a fluctuation in antibody levels, therefore, this creates a risk of false-negative samples when performing the diagnosis.
Key words: Antibodies. SRLV. Small Ruminant. Breeder. Western Blot.

Resumo

O objetivo desse estudo foi analisar a resposta imunológica aos lentivírus de pequenos ruminantes (LVPR) com enfoque no sistema reprodutor de machos com infecção recente e crônica. Para isso, foram selecionados 12 reprodutores caprinos, sendo seis soronegativos e seis soropositivos com infecção natural crônica há mais de 18 meses (grupo com infecção crônica). Após seleção dos animais, os seis machos soronegativos foram inoculados com a cepa viral do vírus da artrite encefalite caprina (CAEV)-Co, título $10^{-5,6}$ $TCID_{50}$/mL, por via intravenosa. A partir da inoculação viral este agrupamento passou a ser denominado de grupo com infecção recente e juntamente com o grupo com infecção crônica foram acompanhados, semanalmente por 180 dias, com análise dos testes de Western Blot (WB) no soro sanguíneo e plasma seminal. Nos animais com infecção crônica para LVPR, 18,94% (50/264) apresentaram anticorpos anti-LVPR em pelo menos uma das distintas amostras, e 81,06% (214/264) tiveram resultados negativos. Das amostras de soro sanguíneo do referido grupo, em 27,27% (36/132) detectou-se anticorpos anti-LVPR, enquanto que no plasma seminal 10,60% (14/132) foram reagentes no teste de WB. Nos animais inoculados com o CAEV-Co, ocorreu a soroconversão após a terceira semana da inoculação viral. Nesse grupo, 31,06% (41/132) das amostras de plasma seminal tiveram anticorpos anti-LVPR, sendo que dessas 41, 70,73% (29/41) coincidiram com resultado das amostras de soro sanguíneo. Nos 29,27% (12/41) restante, houve a detecção somente no plasma seminal e eram amostras provenientes de três animais (AIR2, AIR3 e AIR5). Em um dos animais com infecção recente, só foi identificado anticorpos anti-LVPR em amostras de plasma seminal, possivelmente em função da compartimentalização do vírus. Intermitência viral foi observada em ambas as amostras biológicas, independentemente do estágio de infecção. Conclui-se que a resposta imunológica em reprodutores com infecção recente LVPR é mais acentuada do que em animais cronicamente infectados. E, independentemente do estágio da infecção há uma flutuação nos níveis de anticorpos, sendo, portanto, um fator de risco, em virtude da existência de amostras falso-negativo ao realizar o diagnóstico.
Palavras-chave: Anticorpos. LVPR. Pequeno Ruminante. Reprodutor. Western Blot.

Introduction

Caprine arthritis encephalitis (CAE) is an infectious-contagious disease responsible for productive and economic losses in goat farming caused by the small ruminant lentivirus (SRLV), composed of caprine arthritis encephalitis virus (CAEV) and Maedi-Visna virus (MVV), also called caprine and ovine lentivirus, respectively (Azevedo et al., 2017). These non-oncogenic viral agents have a long incubation period, belong to the Retroviridae family, are characterized by having two single strands of positive RNA, having an envelope containing gp135 (glycoprotein), and p28 in the capsid, which is the protein that induces the formation of antibodies by the immune system (Minguijón et al., 2015). They are viruses commonly known to cause chronic inflammation in numerous tissues and organs and to influence the host's immune system, but without causing deficiency (Jarczak et al., 2019). The main pathogenic characteristics of these viruses relate to their tropism for cells of the phagocytic mononuclear system, which persists for a prolonged period inside monocytes and macrophages (Minguijón et al., 2015).

Anatomical, physiological, phagocytic, and inflammatory barriers function as immune system defense agents against viral infections, with the antibody-mediated humoral immune response being one of the most effective mechanisms to combat viral agents (Soares et al., 2014).

Agarose gel immunodiffusion (AGID) and indirect enzyme-linked immunosorbent assay (ELISA) are the tests recommended by the World Organization for Animal Health to diagnose this disease (World Organization of Animal Health [OIE], 2008). However, advanced SRLV control programs use the Western Blot (WB) test as it is more sensitive and specific and is consequently more accurate compared to other serological tests (Peixoto et al., 2018a; Azevedo et al., 2019; Damasceno et al., 2020; Peixoto et al., 2021). It should be noted that, according to epidemiological data, the prevalence can reach up to 50%, depending on the type of breeding system and other risk factors (Azevedo et al., 2017).

In general, lentiviruses have numerous mechanisms to escape from the immune system, which help them camouflage through mutations (Peixoto et al., 2021). These genetic variations change the antigens present in the viral capsid, leading to the incompetence of already formed antibodies (Lorenzi & Coelho-Castelo, 2011). Moreover, the process of virus migration from the bloodstream to other organs - viral compartmentalization (Ramírez et al., 2012) - is another factor that has hindered the diagnosis and consequently the control of the disease, and is one of the possible causes of intermittent viral shedding both in blood (Azevedo et al., 2019) and in semen (Peterson et al., 2008).

The lactogenic route through the ingestion of milk and colostrum with the virus and the direct contract between animals and their contaminated secretions are among the main transmission routes of SRLV (Pisoni et al., 2010). The virus can also be transmitted by contaminated semen, whether by artificial insemination or natural breeding (Turchetti et al., 2013), as sperm quality is not affected by the presence of the virus (Paula et al., 2009).

Therefore, to guarantee the sanitary standard of buck semen and optimize SRLV

control programs, it is essential to know the immune response of male reproducers at different infection stages. The objective of this study was to analyze the immune responses of bucks to SRLV with a focus on the reproductive system of males with recent or chronic infections.

Material and Methods

It is worth noting that this research was developed according to the ethical principles of animal experimentation, and was approved by the Animal Ethics Committee of Vale do Acaraú State University - CEUA UEVA (protocol no. 010.12), following the guidelines of the National Council for the Control of Animal Experimentation (CONCEA, Law 11794 of October 8, 2008) and other subsequent normative resolutions.

Location

The present study was conducted at Embrapa Goats and Sheep, located in the municipality of Sobral, a semiarid region of the Ceará, Brazil, 3° 42′ South latitude and 40° 21′ West longitude, at an altitude of 83 meters.

Experimental animals

The study included 12 Saanen and Anglo-Nubian goats aged three to four years, selected by their serological status for SRLV, of which there were six (four Saanen and two Anglo-Nubian) seropositive by WB with natural infection for more than 18 months, and six seronegative (all Saanen), selected after three consecutive tests at 30-day intervals (Rodrigues et al., 2014) by blood WB and Nested Polymerase Chain Reaction (*n*PCR) (Marinho et al., 2018). The animals were initially divided into two groups (SRLV negative and positive) with six animals each, which were all found to be in good health after a general clinical examination. The animals were kept during the whole experimental period in intensive production conditions, housed in collective stalls of a partially covered pen.

Viral inoculation

After dividing the animals to compose the respective experimental groups, the negative bucks were intravenously inoculated with 1 mL of the caprine arthritis-encephalitis virus (CAEV)-Co viral strain at a titer of $10^{-5,6}$ $TCID_{50}$/mL in the minimal essential medium. After inoculation, this group was called the recently infected group and was monitored weekly by WB tests until the experimental group became completely seropositive.

Blood and semen collection and processing

Throughout the experimental period, the animals were subjected to weekly artificial semen collection using a short artificial vagina and two females (one per group) with estrus induced by the administration of 1 mL of estradiol cypionate 48 hours before semen collection. Thus, 22 semen collections were performed over 180 days, totaling 264 samples, 132 from the chronic infection group and 132 from the recent infection group.

The collected semen was centrifuged in a refrigerated microcentrifuge for 30 minutes at 1500 g to separate the seminal

plasma, which was stored in 1.5 mL tubes and kept in a freezer at -20 °C for subsequent WB testing.

Immediately after each semen collection, blood was collected through jugular vein puncture using a vacuum system with 5 mL tubes without anticoagulant, which was then centrifuged for 10 minutes at 3000 g to separate the blood serum. This material was frozen in 1.5 mL tubes at -20 °C also for subsequent WB testing.

Antigen preparation

The WB test antigen was produced at the Virology Laboratory of Embrapa Goats and Sheep. Viral multiplication was performed according to the methodology described by Dantas et al. (2008). The standard CAEV-Co viral sample was inoculated in semiconfluent monolayers of the caprine nictitating membrane (13th passage), in A150 plastic bottles with viral suspension with 200 syncytial formation doses/mL. The supernatant was clarified by centrifugation at 3,300 g at 4 °C and treated by protein precipitation with 40% polyethylene glycol 8000 (PEG-8000) for 18h at 4 °C to a final concentration of 8%. Subsequently, the suspension was centrifuged at 4 °C at 12,000 g for 60 min, the pellet was then resuspended in TNE buffer (10.0 mM Tris-HCl, pH 7.4; 10.0 mM NaCl; 1.0mM ethylenediamine tetraacetic acid (EDTA), 1/10 of the original viral suspension volume). The precipitate underwent ultracentrifugation in a sucrose cushion (25%) at 42,000 g for 120 minutes at 4 ºC. The pellet was suspended in phosphate buffered saline (PBS) (0.05M; 0.15M NaCl; pH 7.4) and stored at -80 ºC until laboratory tests were carried out. The total protein concentration was determined by the Bradford method (Bradford, 1976).

Western blot

Blood serum and seminal plasma samples were subjected to WB test following the methodology by Rodrigues et al. (2014), at a dilution of 1:50 for blood serum and 1:8 for seminal plasma.

Initially, the samples underwent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with concentration and separation gels at 4% and 12.5%, respectively. Next, the proteins contained in the gel were passively transferred to nitrocellulose membranes (NM) and subsequently blocked with 0.3% PBS Tween.

After blocking, the MNs were subjected to blood serum or seminal plasma, according to the aforementioned dilutions. Then, the labeled anti-IgG conjugate was added at a dilution of 1:15,000 (SIGMA® - A 5420). The antigenic proteins present in the NM were revealed with a solution of 4-Chloro-1-naphthol (SIGMA® - C 8890) and 3,3' Diaminobenzidine (DAB - SIGMA® - D 5637), with 30% H2O2 and protected from light. The reaction was blocked with the addition of distilled water.

Statistical analysis

The Pearson's correlation test was performed in the statistical analysis and the results were subjected to the Chi-square test (X2) with an established value of ($p < 0.05$) and Yates's correction, using the EPI-INFO statistical software version 6.0.

Results and Discussion

The immunological dynamics analyzed in blood serum and seminal plasma of goats with chronic SRLV infection are presented in Table 1. Of the six chronically infected animals (CIA) that composed this group, only two presented anti-SRLV antibodies (CIA1 and CIA3), with several detections occurring throughout the experimental period. The remaining bucks remained negative for WB tests over the 22 collections. Table 1 also shows that only some CIA had antibodies against SRLV detected in seminal plasma samples (CIA3), and these positive results always coincided with the blood serum sample collected on the same day.

The evaluation of the percentage of antibody detection in biological samples of bucks with chronic SRLV infection evidenced that 18.94% (50/264) of the biological samples collected had anti-SRLV antibodies in at least one of the samples, while 81.06% (214/264) of the samples had negative results. As for the blood serum samples, 27.27% (36/132) detected the presence of anti-SRLV antibodies, while in seminal plasma, only 10.60% (14/132) were positive.

SRLV chronically infected animal present no immune deficiency but do present inadequate immune system functioning (Blacklaws & Harkiss, 2010), which culminates in intermittent viral shedding in blood (Azevedo et al., 2019) and semen (Peterson et al., 2008). This fact was also shown in the present study through the oscillation of the immunological dynamics of chronically infected animal throughout the experimental period; however, this immune change is common in viral diseases (Stonos et al., 2014).

Cytokines commonly regulate the duration and stage of the immune response via specific cell receptors (Jarczak et al., 2016). In animals with persistent infection by SRLV there is potential deregulation of their expression by the action of these viral agents (Ravazzolo et al., 2006). Thus, some interferons are only expressed at the beginning of viral infection, becoming deficient in prolonged conditions (Jarczak et al., 2019), that is, with oscillation of immunological titles in persistent cases caused by retroviruses. Thus, cytokine expression instability causes immune system disorders, making immunodiagnosis difficult and partly justifying the occurrence of a high number of negative samples from a chronically infected animal.

Table 1
Immune response of bucks with chronic SRLV infection by WB using blood serum and seminal plasma samples collected weekly over 180 days in the Brazilian northeastern semiarid region

Experimental Week	Animals											
	CIA1		CIA2		CIA3		CIA4		CIA5		CIA6	
	Serum	Seminal Plasma	Serum	Seminal Plasma	Serum	Seminal Plasma	Serum	Seminal Plasma	Serum	Seminal Plasma	Serum	Seminal Plasma
1	-	-	-	-	+	+	-	-	-	-	-	-
2	-	-	-	-	+	-	-	-	-	-	-	-
3	-	-	-	-	+	-	-	-	-	-	-	-
4	-	-	-	-	+	-	-	-	-	-	-	-
5	+	-	-	-	+	+	-	-	-	-	-	-
6	+	-	-	-	+	+	-	-	-	-	-	-
7	+	-	-	-	+	+	-	-	-	-	-	-
8	+	-	-	-	+	-	-	-	-	-	-	-
9	+	-	-	-	+	-	-	-	-	-	-	-
10	+	-	-	-	+	-	-	-	-	-	-	-
11	+	-	-	-	+	-	-	-	-	-	-	-
12	+	-	-	-	+	-	-	-	-	-	-	-
13	+	-	-	-	+	+	-	-	-	-	-	-
14	+	-	-	-	+	+	-	-	-	-	-	-
15	-	-	-	-	+	+	-	-	-	-	-	-
16	-	-	-	-	+	+	-	-	-	-	-	-
17	-	-	-	-	+	+	-	-	-	-	-	-
18	-	-	-	-	+	+	-	-	-	-	-	-
19	+	-	-	-	+	+	-	-	-	-	-	-
20	+	-	-	-	+	+	-	-	-	-	-	-
21	+	-	-	-	+	+	-	-	-	-	-	-
22	+	-	-	-	+	+	-	-	-	-	-	-

CIA = Chronically infected animal; SRLV = Small ruminant lentivirus; WB = Western Blot.

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Santos et al. (2019) propose that the innate immune system acts in seminal plasma in the acute phase of SRLV infection through the expression of proteins such as clusterin, cystatin C, serum albumin, zinc-alpha-2 glycoprotein, and prostaglandin. According to Sabatte et al. (2011), the clusterin found in the seminal plasma of humans has a high binding affinity with the DC-SIGN cellular receptor, competing with HIV-1, while cystatin C, expressed in the cervical mucosa of HIV-1 resistant women (Burgener et al., 2008), acts in cell protection and control before an inappropriate proteolytic cascade. These proteins, present in the seminal plasma of goats, can potentially act in SRLV infection for belonging to the same family of HIV lentiviruses. This fact may partially explain why there was a reduction in the infectious process and, consequently, in the amount of anti-SRLV antibodies in the chronically infected animal.

The concomitant blood serum and seminal plasma detection in only one of the chronically infected animal possibly occurred due to individual humoral response profile, which differs among animals of the same species (Rachid et al., 2013). Furthermore, SRLV infection resistance is directly related to the host genetics, which is a determinant of infection progression (Larruskain & Jugo, 2013).

Table 2 shows blood serum and seminal plasma WB results of bucks with recent SRLV infection. All recently infected animals (RIA) were positive in at least one of the biological samples over the experimental period, which differed from what occurred with the CIA. Seroconversion started after the first week in 33.3% of the animals (2/6) inoculated with CAEV-Co, and 83.3% (5/6) were totally seropositive after the third week. The detection of anti-SRLV antibody in seminal plasma of 33.3% (2/6) animals started after the third week. RIA3 was the only one identified as SRLV positive through antibody detection in seminal plasma samples.

Table 2 shows that 31.06% (41/132) of the seminal plasma samples were positive, and of these, 70.73% (29/41) coincided with serum sample results, which were also positive. The remaining 29.27% (12/41) were detected only in the seminal plasma of three animals (RIA2, RIA3, and RIA5).

The percentage antibody detection values in biological samples of RIA showed higher antibody production at the beginning of the infection. It is noteworthy that 43.93% (116/264) of the different samples collected were positive, 10.98% (29/264) in both, 56.82% (75/132) only in blood serum, and 9.09% (12/132) only in seminal plasma samples. It is important to emphasize that no anti-SRLV antibodies were detected in 56.06% (148/264) of serum or seminal plasma samples collected.

Table 2
Immune response of bucks with recent SRLV infection by WB using blood serum and seminal plasma samples collected weekly over 180 days in the Brazilian northeastern semiarid region

Experimental Week	Animals											
	RIA1		RIA2		RIA3		RIA4		RIA5		RIA6	
	Serum	Seminal Plasma	Serum	Seminal Plasma	Serum	Seminal Plasma	Serum	Seminal Plasma	Serum	Seminal Plasma	Serum	Seminal Plasma
1	-	-	-	-	-	-	-	-	-	-	-	-
2	+	-	+	-	-	-	-	-	-	-	-	-
3	-	-	+	-	-	-	-	-	+	-	+	-
4	-	-	+	-	-	+	+	+	+	-	+	-
5	-	-	+	-	-	-	+	-	+	-	+	-
6	-	-	-	-	-	-	+	+	+	+	+	-
7	-	-	-	+	-	+	+	-	+	+	+	-
8	-	-	-	-	-	-	+	+	+	+	+	-
9	-	-	-	+	-	-	+	+	+	-	+	-
10	-	-	-	-	-	+	+	+	+	-	+	-
11	+	-	-	-	-	+	+	-	+	-	+	-
12	+	-	-	-	-	+	+	-	+	-	+	-
13	+	+	-	-	-	+	+	-	+	+	+	-
14	+	-	-	-	-	-	+	+	+	+	+	-
15	+	+	-	-	-	-	+	+	+	-	+	-
16	+	+	-	-	-	-	+	-	+	-	+	+
17	+	-	-	-	-	-	+	-	+	-	+	+
18	+	-	-	-	-	-	+	-	+	+	+	-
19	+	+	-	-	-	-	+	-	-	+	+	+
20	+	+	-	+	-	+	+	-	-	-	+	+
21	+	+	-	-	-	-	+	-	+	+	+	+
22	+	+	+	-	-	+	+	+	+	+	+	+

RIA = Recently infected animal; SRLV = Small ruminant lentivirus; WB = Western Blot.

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Recently infected animal showed greater immune response both in blood serum and seminal plasma samples as a consequence of antibody production. Bertoni et al. (1994) used immunoprecipitation and radiolabeling to identify blood serum antibodies against viral proteins one week after experimental infection with a CAEV-Co sample.

Some recently infected animal only showed antibodies in seminal plasma samples. This is probably due to viral compartmentalization, in which lentiviruses circulating in the bloodstream migrate to the reproductive organ and pass the blood-testicular barrier (BTB), increasing local cytokine expression, which is active throughout the viral pathogenesis (Larruskain & Jugo, 2013). Although this type of barrier behaves partially as an immunological obstacle, preventing the entry of leukocytes and immunoglobulins in the testicular compartments. Preleptotene and spermatogenic spermatocytes present in the seminiferous epithelium tend to trigger an immune response against different viruses (Mital et al., 2011).

The comparison of anti-SRLV antibody detection in biological samples between the chronic and recent infection groups (Table 3) showed a statistically significant difference both between blood serum and seminal plasma samples from CIA ($p \leq 0.001$ - $\chi^2=37.76$), as well as between biological samples from RIA ($p \leq 0.05$ - $\chi^2= 3.9059$).

Table 3
Comparative analysis of the immune response by blood serum and seminal plasma WB in SRLV chronically infected animal and recently infected animal

		Positive Results
Chronic Infection	Blood Serum	36 (27.27%) a
	Seminal Plasma	14 (10.60%) b
	Total Samples	50 (18.93%)
	$X^2 = 37.76$ $P < 0.001$	
Recent Infection	Blood Serum	75 (56.82%) a
	Seminal Plasma	41 (31.06%) b
	Total Samples	116 (43.94%)
	$X^2 = 3.9059$ $P < 0.05$	

Means followed by different letters in the same line differ by the Chi-square test (X^2); SRLV = Small ruminant lentivirus; WB = Western Blot.

Different immunoglobulin productions between the biological samples may have had an effect, since the seminal plasma preferentially produces immunoglobulin A (IgA), which is the main immunoglobulin present in external secretions. In contrast, the blood serum of animals infected by SRLV is characterized by the synthesis of immunoglobulin G (IgG), and to a lesser extent, of immunoglobulin M (IgM) (Trujillo et al., 2004). Additionally, IgA levels in seminal plasma were essential for detection only in this type of biological sample.

A similar viral compartmentalization situation was reported by Ramírez et al. (2009), with 16% of a total of 94 samples being WB reactive only in the seminal fluid of animals carrying SRLV. In this context, at certain times, the virus tends to be located in specific organs in infected animals, which results in a restricted antibody concentration.

Cavalcante et al. (2013) detected pro-viral DNA and free virus in oocyte and uterine fluid samples of goats infected with SRLV, but the same animal did not present blood serum antibodies by WB. The present study showed similar cases, with different biological samples from the same animal presenting divergent results, demonstrating that the immunological dynamics may change according to virus compartmentalization in the animal.

When the virus compartmentalizes in the reproductive organ of infected males, either in free or pro-viral form, it triggers antibody redirection to these regions, possibly due to its high concentration. As this occurs, the semen of SRLV infected bucks becomes an important route of disease transmission within the herd, with high potential for viral dissemination (Paula et al., 2009) due to the caprine lentivirus transmissibility via semen (K. C. Souza et al., 2013; Hasegawa et al., 2017), impairing the use of bucks with the high genetic standard (Peixoto et al., 2018b). In addition, goat breeders traditionally share animals between farms (T. S. Souza et al., 2010), which becomes an aggravating viral transmission factor by the reproductive route.

In general, the presence of anti-SRLV antibodies in seminal plasma was more variable compared to the immune response in blood serum. This can be attributed to the immunologically privileged microenvironment in the testicles provided by the BTB, which is resistant to inflammatory responses and protects any potential antigens from the action of the immune system (Qu et al., 2019). Additionally, testicular cells secrete several immunoregulatory molecules that inhibit macrophage migration, and consequently, regulate and block the immune response in the testicular region (Winnall et al., 2011).

It should also be considered that the virus tends to become quiescent in infected animals over time, probably with pro-viral DNA not integrated or integrated without activation (Cavalcante et al., 2013). It is likely that the polymorphism of certain genes that compose structural regions of the virus present variability that hinders its detection by the host immune system throughout the progression of the disease, thus helping the SRLV evade the immunological barriers (Li et al., 2014).

These facts consequently tend to make the diagnosis difficult, even when using a more sensitive technique such as the WB (Peixoto et al., 2021), which has been proven to be a good tool for the early diagnosis of

anti-SRLV antibodies and is used in several studies on this theme (Peixoto et al., 2018a; Sousa et al., 2019; Araújo et al., 2020; Damasceno et al., 2020).

The Pearson's correlation between the biological samples of both groups in the detection of anti-SRLV antibodies (Table 4) showed a moderate correlation between blood serum and seminal plasma samples in CIA (0.5870), as well as between the seminal plasma of RIA and blood serum of CIA (0.5062), and between seminal plasma samples from both experimental groups (0.4020).

Table 4
Correlation between the detection of anti-SRLV antibodies in biological samples of SRLV chronically infected animal and recently infected animal

Correlation	BsCI	BsRI	SpCI	SpRI
BsCI	--			
BsRI	0.0179	--		
SpCI	0.5870	0.1222	--	
SpRI	0.5062	0.3597	0.4020	--

BsCI = blood serum chronic infection; BsRI = blood serum recent infection; SpCI = seminal plasma chronic infection; SpRI = seminal plasma recent infection; SRLV = Small ruminant lentivirus; WB = Western Blot.

Table 5 compares blood serum and seminal plasma WB test data. The percentage of accuracy was 69.69% of the results and the Kappa index was 0.33.

Table 5
Comparative analysis of blood serum and seminal plasma samples from SRLV chronically infected animal and recently infected animal analyzed by WB

		WB Blood Serum		
WB Seminal Plasma		Positive	Negative	Total
	Positive	43	12	55
	Negative	68	141	209
	Total	111	153	264

Accuracy: 69.69%; kappa index = 0.33; SRLV = Small ruminant lentivirus; WB = Western Blot.

It is important to note that seminal plasma WB detected 12 positive samples only in this type of material, with no detection in blood serum. Furthermore, the presence of an animal in which anti-SRLV antibodies were detected only in seminal plasma shows the risk of false-negative tests resulting in keeping infected animals in the herd.

Therefore, the immunological diagnosis using seminal fluid WB can help in the diagnosis of SRLV recently infected animal, in which this value tends to be high, but also in chronically infected animal samples.

Conclusion

The immune response in recently infected animal is more pronounced than in the chronically infected animal. Regardless of the infection level, there is a variation in the levels of antibodies, which is a risk factor due to the existence of false-negative samples in the tests.

Considering the results obtained and the fact that bucks have a high turnover rate in farms, increasing the potential for SRLV dissemination, it is recommended to perform the WB test both in blood serum and seminal plasma to diagnose the disease, especially in insemination centrals.

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