ORIGINAL ARTICLE

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Profile of extracellular matrix metalloproteinase in healthy and infected Toggenburg goats with small ruminant lentivirus in Southeast Brazil

Perfil das metaloproteínas de matriz extracelular em cabras Toggenburg sadias e infectadas por lentivírus de pequenos ruminantes no Sudeste brasileiro

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ABSTRACT: Small ruminant lentiviruses (SRLV) are difficult to diagnose due to their escape mechanisms. Therefore, proteomics is an alternative in the search for biomarkers through extracellular matrix metalloproteinases (MMPs), enzymes related to the immune response. In this sense, this study aimed to analyze the profile of MMPs in healthy and infected Toggenburg goats with chronic SRLV infection in Southeast Brazil. Five positive and five negative goats for SRLV were selected using the agar gel immunodiffusion (AGID) microtechnique, western blot (WB), and nested polymerase chain reaction (nPCR). All animals were submitted to blood collection by puncture of the jugular vein, followed by centrifugation to obtain blood plasma, protein quantification by the Bradford method, one-dimensional electrophoretic separation (1D), and identification of protease activity by zymography and confirmation via reverse zymography in the presence of MMP-2 through the action of tissue inhibitors (TIMP-2). The analysis of protein bands was performed using descriptive statistics and densitometry values for zymography were subjected to the Shapiro-Wilk test to determine normality. Little difference was observed in the occurrence of protein bands between groups. Regarding MMPs, no differences were observed in the expression of proMMP-9, MMP-9, and MMP-2 in animals affected by SRLV. TIMP-2 inhibited proMMP-2 and MMP-2 in all animals. Thus, the profile of protein bands does not change in healthy goats with chronic SRLV infection. The TIMP-2 expression allowed proving the existence of MMP-2 in animals chronically infected by SRLV via reverse zymography.

KEYWORDS: Biomarkers; MMPs; SRLV; zymography.

RESUMO: Lentivírus de pequenos ruminantes (LVPR) demonstram diagnóstico complexo devido seus mecanismos de escape. Desse modo, a proteômica apresenta-se como alternativa na busca por biomarcadores através das metaloproteinases da matriz extracelular (MMPs), enzimas ligadas a resposta imunológica. Assim, objetivou-se analisar o perfil das MMPs em cabras Toggenburg sadias e com infecção crônica por LVPR no Sudeste brasileiro. Selecionou-se cinco cabras positivas e cinco negativas para LVPR utilizando: microtécnica de imunodifusão em gel de agarose (MIDGA), *Western Blot* (WB) e reação em cadeia da polimerase *nested* (*n*PCR). Todas foram submetidas à coleta de sangue por punção da veia jugular, seguido de centrifugação para obtenção do plasma sanguíneo, quantificação proteica pelo método Bradford, separação via eletroforese unidimensional (1D), e identificação da atividade das proteases por zimografia e confirmação via zimografia reversa na presença da MMP-2 por meio da ação de inibidores teciduais (TIMP-2). A análise das bandas proteicas ocorreu através de estatística descritiva e para a zimografia os valores de densitometria foram submetidos ao teste de Shapiro-Wilk para determinar a normalidade. Observou-se pouca distinção na ocorrência das bandas proteicas entre os grupos. Em relação as MMPs, não houve diferenças na expressão da proMMP-9, MMP-9 e MMP-2 nos acometidos por LVPR. Observou-se que a TIMP-2 inibiu a proMMP-2 e MMP-2 em todos os animais. Dessa forma, o perfil de bandas proteicas não se altera em cabras sadias e com infecção crônica por LVPR. Através da expressão da TIMP-2 foi possível comprovar a existência da MMP-2 em animais cronicamente infectados por LVPR via zimografia reversa.

PALAVRAS-CHAVE: Biomarcadores; MMPs; LVPR; zimografia.

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INTRODUCTION

Small ruminant lentiviruses (SRLV) are composed of caprine arthritis-encephalitis virus (CAEV) and maedi-visna virus (MVV), also called caprine and ovine lentiviruses, respectively, which are responsible for cross-infection (MINGUIJÓN et al., 2015), that is, the same viral subtype infecting both species. It has been elucidated by phylogenetic analyses (SHAH et al., 2004) and reported in other studies (SOUZA et al., 2015; SOUZA et al., 2018; LIMA et al., 2018). This interspecies transmission is caused by failures in the virus RNA transcription due to reverse transcriptase (CALLADO; CASTRO; TEIXEIRA, 2001), and these agents are responsible for causing diseases that lead to production and economic losses (GUILHERME et al., 2017).

Although the ingestion of contaminated colostrum and/or milk is one of the main routes of transmission of SRLV (AZEVEDO et al., 2017), the proximity of healthy animals to sick ones favors contagion through secretions (SILVA et al., 2017). Thus, among production systems, the intensive production model tends to have a higher occurrence of infected animals (NASCIMENTO-PENIDO et al., 2017). Furthermore, animals of dairy breeds have a higher prevalence of the disease than native mixed-breed animals (TEIXEIRA et al., 2016).

Direct (nested polymerase chain reaction – nPCR) (TU et al., 2017) and indirect (agar gel immunodiffusion – AGID, indirect enzyme-linked immunosorbent assay – ELISA-i, and western blot – WB) techniques are used in the diagnosis of animals infected by SRLV (RODRIGUES et al., 2018). However, there is a consensus on the need to associate different methods and even different biological samples for an accurate diagnosis (HASEGAWA et al., 2017; PANNEUM; RUKKWAMSUK, 2017; AZEVEDO et al., 2019; ARAÚJO et al., 2020), mainly due to escape mechanisms, which include genetic diversity, the potential for viral mutation, and intermittency and viral compartmentalization, which characterize these viral agents (PEIXOTO et al., 2021).

Thus, the early and accurate identification of sick animals is of paramount importance. In this context, proteomics has been an alternative in the search for biomarkers related to the inflammatory processes of the disease, aiming to identify the animals carrying these viral agents more accurately (BEZERRA JÚNIOR et al., 2017).

Based on this premise, some proteases, by mediating the inflammatory response, are in the group of these possible biomarkers (MANICONE; MCGUIRE, 2008), standing out extracellular matrix metalloproteinases (MMPs), which are zinc-dependent enzymes synthesized in the form of the zymogen, controlled by the action of tissue inhibitors (TIMPs), in which four types have already been found, as follows: TIMP-1, TIMP-2, TIMP-3, and TIMP-4 (CHAUDHARY et al., 2013).

The functions of MMPs are diverse, such as wound healing (CALEY; MARTINS; O'TOOLE, 2015), embryo formation (BLANCO et al., 2017), tissue regeneration (LI et al., 2020), and blood vessel formation (WEBB et al., 2017). There are 24 types of MMPs, which are divided into six groups. Among them, MMP-2 (Gelatinase A) and MMP-9 (Gelatinase B) are of greatest interest, as they are the main ones involved in pathological processes (JABŁOŃSKA-TRYPUĆ; MATEJCZYK; ROSOCHACKI, 2016), with reports of increased activity in SRLV-positive goat breeders (BEZERRA JÚNIOR et al., 2015).

However, studies of these MMPs in animals affected by SRLV are still preliminary (GALIZA et al., 2020). Thus, there is a need to expand investigations in this field of research to identify and validate the possible biomarkers associated with SRLV infections and, consequently, develop confirmatory diagnostic techniques. In this context, this study aimed to analyze the profile of MMPs in healthy and infected Toggenburg goats with chronic SRLV infection in Southeast Brazil.

MATERIAL AND METHODS

The study was carried out in a privately owned dairy herd in Southeast Brazil. The collected samples were analyzed at the Embrapa Goat and Sheep, in the municipality of Sobral, state of Ceará, Brazil, upon approval by the ethics committee for the use of experimental animals (CEUA) of this institution under protocol number 013/2015.

The experiment was conducted from October 2019 to February 2020. A herd of approximately 100 was used, composed of Toggenburg goat dams with the dairy aptitude and over one year of age, which had already reached puberty and sexual maturity, which were the criteria adopted to have uniform lots and reduce error. Five positive (animals with chronic infection) and five negative goats (healthy animals) for SRLV were selected. The absence and presence of infection were confirmed by three diagnostic tests: the agar gel immunodiffusion (AGID) microtechnique, according to the methodology by Gouveia (1994), western blot (WB), according to Rodrigues et al. (2014), and nested polymerase chain reaction (nPCR), advocated by Marinho et al. (2018), totaling ten animals, which came from an intensive production system.

All animals underwent a single blood collection by puncturing the jugular vein using the Vacutainer® system, through the use of 10-mL tubes with anticoagulant (ethylenediaminetetraacetic acid – EDTA). After collection, the samples were centrifuged at 3000 g for 10 minutes in a centrifuge (Excelsa® II Model 206-BL, São Paulo, Brazil) at room temperature to obtain blood plasma. Subsequently, these biological samples were transferred to 2.5 mL tubes and stored in a freezer at –20 °C until the tests were carried out.

Blood plasma was submitted to quantification of total proteins through the Bradford method (1976), in triplicates, using a previously established standard curve containing known concentrations (0, 3, 5, 8, 10, 13, 18, 15, and 20 mg) of bovine serum albumin (BSA). The samples were read in a spectrophotometer (Gehaka model UV/Visível – UV 330G, São Paulo, Brazil) at a 595 nm absorbance.

Subsequently, the blood plasma samples were submitted to 1D (one-dimensional) electrophoresis (LAEMMLI, 1970) using sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) at 12.5%. The blood plasma samples were solubilized in a specific SDS buffer (concentration buffer, 10% SDS, 2-mercaptoethanol, and Milli-Q water q.s.) before being placed in the wells of the polyacrylamide gel to allow a final concentration of 1 µg/mL. Subsequently, 7 µL aliquots were taken and added to the wells of the gels.

Blood plasma samples were added to the wells alternately, that is, from positive and negative animals. Furthermore, all procedures were performed in duplicates. The second well was destined to place 10 µL of standard molecular weight (Bio-Rad, Hercules, CA, USA – 200.0, 116.2, 97.4, 66.2, 45.0, 31.0, 21.5, 14.4, and 6.5 kDa), serving as a reference for identifying the weights of protein bands from the blood plasma samples. The wells that remained empty were filled with 10 µL of sample buffer.

After this step, the gel was submitted to an electrophoresis run (300 V, 20 W, 40 mA), lasting approximately one hour (adapted from LAEMMLI, 1970) in a vertical electrophoresis tank (SE260 Mighty Small II Deluxe, Hoefer). The run was maintained at 20 mA in the first 10 minutes to allow the proteins to concentrate at the bottom of the well and begin migration. Later, it was increased to 40 mA.

At the end of electrophoresis, the gels were transferred to recipients containing Coomassie Brilliant Blue G-250 dye and stained for two hours under stirring. Then, the gels were bleached with hot Milli-Q water through two consecutive 30-minute washes to remove excess dye. Subsequently, they were digitized (Epson model EU-88 A3 Transparency Unit, Anaheim, California) to visualize the protein bands in the blood plasma of the experimental animals.

For the zymography analysis, gelatin (8 mg/mL) was added to the 1D SDS-PAGE gels (adapted from KUPAI et al., 2010) to serve as a substrate for degradation by proteases. The samples were solubilized in specific zymogram buffer (Tris, sucrose, bromophenol blue, SDS, and Milli-Q water q.s.) and added to the wells of the gels. These gels were later transferred to the vertical cuvette to undergo the electrophoresis run. After the run, the gels were transferred to a container and washed twice consecutively for 30 minutes in a renaturing solution (Triton X-100 2.5%) to remove the SDS. Thereafter, the gels were incubated overnight in development buffer (50 mM Tris, pH 7.8–8.0, 150 mM NaCl, and 10 mM $CaCl_2$) at 37 °C in a drying oven.

Then, the gels were stained with Coomassie Brilliant Blue G-250 Colloidal for two hours under stirring. Then, the dye excess was removed with hot Milli-Q water twice consecutively for 30 minutes (adapted from NEUHOFF et al., 1988). At the end of these stages, the activity of the proteases present in the blood plasma of goat dams was identified by the observation of clear bands present in the gels, which were digitized (Epson model EU-88 A3 Transparency Unit, Anaheim, California) for band visualization.

Two blood plasma samples were randomly selected for reverse zymography, one from a naturally positive animal and another from a negative animal for SRLV. TIMP-2 (Merck, at a concentration of 0.1 µg/uL) was added to them (adapted from HAWKES; LI; TANIGUCHI, 2010).

An aliquot of 7 µL of sample was placed in a 2.5-mL tube with the zymogram buffer and 2 µL of TIMP-2 was added. Then, the material was stirred for subsequent removal of 9 µL and addition to the well of the gel. A specific well of the gel was designed to add 5 µL of molecular weight standard (Figure 1). The proof of MMP-2 was conducted using the two samples in the gel with zymogram buffer only, and two other samples from the same animals using TIMP-2.

The experimental design was completely randomized. The analysis of the bands in the gels was performed by the software GelAnalyzer 19.1, version 2010, to determine the densitometry of the protein bands obtained from 1D electrophoresis and zymography. Descriptive analysis by the band frequency table was used for the protein bands identified through 1D electrophoresis. The presence or inhibition of MMP-2 was evaluated for reverse zymography analysis.

In the zymography analysis, the densitometry values of MMPs were submitted to the Shapiro-Wilk (W) test to determine the normality of the groups. Subsequently, the t-test was adopted to determine significance (5% significance level), as the data showed a normal distribution. All analyses were performed using the software PAST 4.03 (HAMMER; HARPER; RYAN, 2001).

RESULTS AND DISCUSSIONS

The molecular weights (MW) found for the protein bands ranged from 18 to 176 kDa in the group of positive animals and 18 to 178 kDa in the group of negative animals. Thus,

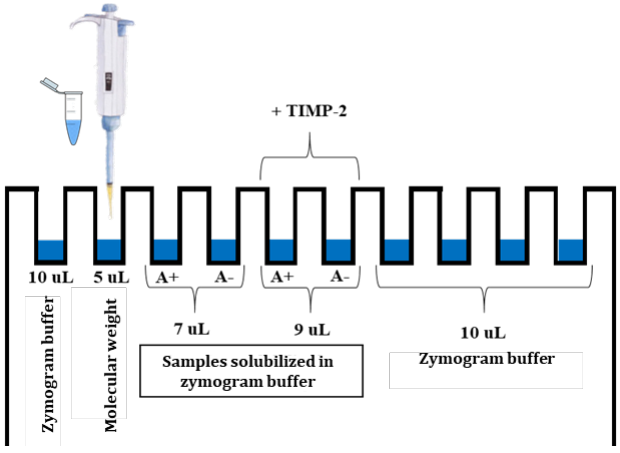

Figure 1. Schematization of the zymogram gel with the addition of TIMP-2 in blood plasma samples from animals chronically affected or not by small ruminant lentivirus (SRLV). A+: Animal positive for SRLV; A–: Animal negative for SRLV.

there were no differences in the number of bands between both groups, with 26 bands below 50 kDa and 23 above 50 kDa.

Bands between 15 and 30 kDa were identified in 100% of the positive and negative animals for SRLV. Furthermore, the high MW bands (>100 kDa) were also present in all animals, regardless of their health status. Bands between 80 and 90 kDa presented the lowest frequency (20%) in both groups. In contrast, MW bands between 90 and 100 kDa showed a frequency of 60% in positive animals and 80% in negative animals (Table 1). This frequency distribution shows the profile of possible proteins in animals infected by SRLV.

Little distinction was observed in the occurrence of protein bands between groups. This occurrence possibly occurred because the experimental animals were asymptomatic at the time of blood collection although SRLV was present in their organism. It was expected, as one of the characteristics of SRLV is that some animals may not show symptoms (JUSTE et al., 2020).

In addition, the lack of protein distinction between groups can also be attributed to the age factor. Lima (2015) observed that serum levels of total proteins of lambs infected with caprine lentivirus did not differ from healthy ones from 390 days of life, which demonstrates little influence of lentiviruses on protein metabolism in adult animals. This situation corroborates the present study, in which animals over one year were used. Furthermore, Ceciliani et al. (2012) mentioned that many proteins can increase or reduce their concentration in inflammatory diseases, especially proteins expressed during the acute phase, whose expression occurs according to the profile of the immune response promoted by the etiological agent.

Importantly, acute phase proteins (APP) are divided into two groups: positive APP and negative APP (CECILIANI et al., 2012). Thus, albumin (66 kDa), a protein that is part of the negative APP group, has a decreasing content in response to an inflammatory process (COSTA et al., 2010). However, it did not happen in the present research because the protein expression in the gels in the range of 60 to 70 kDa, in which it would be inserted, was similar in both groups. It is assumed that there was no change in albumin contents, as the animals were clinically healthy, even without mastitis. These data corroborate the results by Fonteque et al. (2010), who evaluated Saanen goats (seronegative for SRLV) with compelled mastitis and observed a reduction in the albumin contents in the blood only of animals affected with mastitis because there was a displacement of proteins to the mammary gland, which, consequently, reduces albumin in the blood, which is more present in this fluid.

Similarly, haptoglobin (45 kDa), positive APP, considered one of the best markers of inflammatory processes (THULASIRAMAN et al., 2013), did not have a marked expression in this study, as positive animals had a lower occurrence of proteins from 40 to 50 kDa, a weight range in which haptoglobin would show higher activity. This fact also corroborates the asymptomatic condition demonstrated by animals positive for SRLV. Nielsen et al. (2004) studied cows with mastitis and extramammary inflammatory disorders and observed high haptoglobin contents in the blood serum and milk only in animals that presented mastitis due to the dispersion of these proteins of the mammary gland, where it is produced, into the bloodstream. The concentration of this protein is also high in animals with subclinical mastitis (Safi et al., 2009).

Moreover, transferrin (85 kDa) (negative APP) was possibly found at baseline concentrations in all experimental animals, as proteins with molecular weight within the range of 80 to 90 kDa had the same occurrence, regardless of the infectious condition. In general, this situation is attributed to the function of this protein in capturing ferric ions as defense mechanisms against pathogenic microorganisms that use these substrates (MURATA; SHIMADA; YOSHIOKA, 2004). For

Table 1. Percentage of occurrence of protein bands at intervals according to molecular weight in gel electrophoresis in sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) at 12.5% in samples of blood plasma from Toggenburg goat dams chronically carriers or not of small ruminant lentivirus (SRLV).

	Protein band	Molecular weight (kDa)	Percentage of occurrence	
			Positive	Negative
<50 kDa	B1	0–15	0% (0/5)	0% (0/5)
	B2	15–30	100% (5/5)	100% (5/5)
	B3	30–40	60% (3/5)	60% (3/5)
	B4	40–50	40% (2/5)	60% (3/5)
	B5	50–60	60% (3/5)	40% (2/5)
	B6	60–70	60% (3/5)	60% (3/5)
>50 kDa	B7	70–80	80% (4/5)	80% (4/5)
	B8	80–90	20% (1/5)	20% (1/5)
	B9	90–100	60% (3/5)	80% (4/5)
	B10	>100	100% (5/5)	100% (5/5)

instance, iron in HIV-1 is an important substrate used by regulatory proteins that use this element to carry out the assembly of virions, in addition to helping to complete the viral cycle. Additionally, iron at high concentrations in the intracellular medium (macrophages) contributes to higher HIV-1 transcription and replication (KHAN; CHEN; GEIGER, 2020). Thus, there is a drop in the transferrin concentration in an infectious process due to its integration into the immune system, preventing invaders from using iron, a fact that attributes to it antibacterial and antiviral function (CONTIN et al., 2015).

The numerical analysis showed no differences ($p>0.05$) relative to the expression of proMMP-9 (762 ± 202 pixels) and MMP-9 (435 ± 102 pixels) in animals from the positive group for SRLV compared to those from the negative group (proMMP-9: 707 ± 188 pixels and MMP-9: 392 ± 179 pixels). Similar to the proMMP-2 (132 ± 45 pixels) and MMP-2 (360 ± 172 pixels), no differences were found between the positive group and the negative group (proMMP-2: 131 ± 44 pixels and MMP-2: 270 ± 193 pixels) (Figure 2).

The absence of distinction in the expression of proteases between healthy and infected groups is justified by the possible chronic infection stage in which the animals with SRLV were. Galiza et al. (2020) observed that goat MMPs return to physiological normality after seroconversion (from the 23rd week post-infection), with a reduction in antibody production. Therefore, these MMPs possibly have higher expression in animals before seroconverting.

However, the literature has shown increased expression of proMMP-9, MMP-9, and MMP-2 in goats affected by SRLV (GALIZA et al., 2020). This fact is expected because the expression of these MMPs, although occurring in healthy and infected animals, tends to increase in sick animals to combat inflammatory processes (GALIZA et al., 2020). This situation has already been reported in HIV-infected patients, who showed higher activity of MMP-2 and MMP-9 in the extracellular matrix, and the excess of these MMPs has led to the disruption of the endothelium, allowing the virus to propagate through the tissues (DIAZ et al., 2011). This spread demonstrates that the virus is replicating, initiating persistent infections in small ruminants, leading to inflammatory and degenerative processes (SILVA; LIMA, 2007).

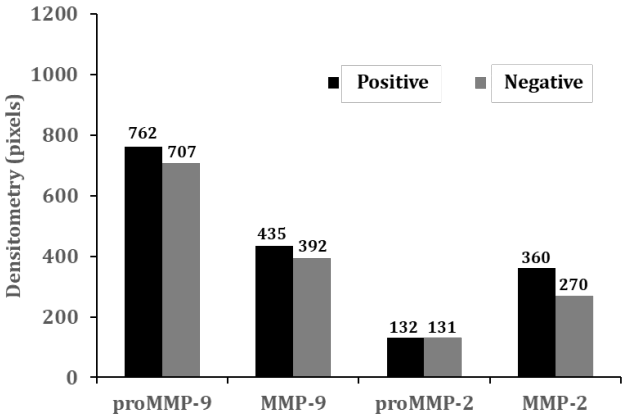

Figure 2. Densitometric mean of proMMP-9, MMP-9, proMMP-2, and MMP-2 in negative Toggenburg goat dams with chronic infection for small ruminant lentivirus (SRLV).

MMP-2 and MMP-9 can be used as biomarkers in inflammatory diseases since they are related to the degradation processes of collagen IV (the main component of the extracellular matrix) and tissue modifications. Furthermore, they have the potential to allow the invasion of inflamed cells, which commonly occurs in metastases (CHEN et al., 2012). The disturbances promoted in the tissue by inflammation will favor the entry of pathogens and, consequently, the entry of inflammatory cells and other plasma proteins due to the release of inflammatory mediators by MMPs, such as cytokines and chemokines. These mediators are responsible for compelling the displacement of leukocytes to inflamed sites (MANICONE; MCGUIRE, 2008). Macrophages will be activated and promote lymphoproliferative lesions in target tissues, especially in the mammary gland, respiratory system, synovial fluid, and choroid plexus with compromised endothelial barrier due to SRLV infection (LOFSTEDT, 2014).

MMP-2 is not much involved in extracellular matrix degradation but in tissue remodeling and cytokine activation. Furthermore, it may also be associated with stimulating endothelial cells to invade tissues (ZHANG et al., 2020). In wounds, MMP-9 acts in the phase in which there is higher cell migration (initial healing) and is more related to the direct degradation of the extracellular matrix and cell invasion (NAGEL et al., 2015). In contrast, MMP-2 acts in the final step, which is the remodeling of the extracellular matrix (CLUTTERBUCK et al., 2010). MMP-9 can breach the blood-brain barrier in Japanese encephalitis virus infections (SHUKLA et al., 2016). In addition, MMP-9 may be responsible for mediating the recruitment of neutrophils to the infected region, for example, the respiratory system resulting from the influenza virus (BRADLEY et al., 2012).

Animals affected by SRLV show a higher expression of MMPs in the inflammatory process, triggering a higher demand for cytokines and chemokines, which will attract more cells, mainly leukocytes, to the affected sites (BEZERRA JÚNIOR et al., 2015; MANICONE; MCGUIRE, 2008), thus leading to the manifestation of clinical symptoms such as inflammation of the mammary gland, arthritis (adults), and damages to the respiratory and neurological systems (encephalomyelitis in goat kids) (HASEGAWA et al., 2016).

A study with goat breeders (Saanen and Anglo-Nubian) affected with SRLV found a higher expression of MMPs (MMP-2 and MMP-9) in symptomatic and asymptomatic animals, indicating a possible association of these MMPs with the infectious inflammatory process (BEZERRA JÚNIOR et al., 2015). Therefore, MMPs can be used as markers of SRLV infection, especially in the early stages, and are still likely to aid in diagnosis even during viral latency, in

which serological tests may indicate false-negative results (GALIZA et al., 2020).

Figure 3 shows that TIMP-2 inhibited proMMP-2 and MMP-2 in all experimental animals, regardless of whether or not they were affected by viral agents. Inhibitory activity occurs when the N-terminal domain of TIMP binds with the catalytic domain of MMPs, or the C-terminal domain of TIMP-2 binds with the hemopexin domain of MMPs (BREW; NAGASE, 2010). This inhibition delays the progression of degradation of extracellular matrix components (ŁANOCHA-ARENDARCZYK et al., 2018).

Alakus et al. (2008) evaluated the expression of TIMP-2 in human stomach cancer and observed a lower expression of TIMP-2 in tissues with metastasis compared to those without metastases. It demonstrates the importance of TIMP-2 in inhibiting MMP-2 (associated with worse survival) in the tissue affected by cancer, preventing degradation of the extracellular matrix, tumor invasion, and the appearance of metastases. Ahn et al. (2019) explained that inhibiting the exacerbated activity of MMP-2 is important because its marked activity promotes tissue damage, which facilitates cell invasion and triggers inflammatory processes. Reducing MMP activity reduces the degree of inflammation, as observed in a lung study of mice that had a delayed respiratory viral infection (VILLERET et al., 2020).

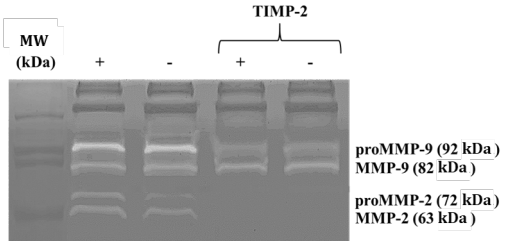

Figure 3. Reverse zymography on 12.5% polyacrylamide gel in blood plasma samples from healthy Toggenburg goat dams with chronic infection by small ruminant lentivirus (SRLV) in Southeast Brazil. MW = Molecular weight; + = Animal positive for SRLV; - = Animal negative for SRLV.

Importantly, the inhibition of an MMP can bring benefits or harm depending on the amount of TIMP. Thus, it is important to only prevent the exacerbated activity of MMPs when it occurs in the presence of disease to maintain their physiological levels and facilitate treatment. Therefore, the balance of MMP-TIMP is desirable for the normalization of the extracellular matrix environment (CLUTTERBUCK et al., 2010). Thus, since TIMP-2 has an MMP-2 inhibitory activity, using it as a possible anti-inflammatory in animals with SRLV can potentially control and/or delay the progression of viral activity. This inhibition would avoid or minimize the severity of clinical signs resulting from the presence of the virus in the animal, preventing discards and reducing production losses.

CONCLUSIONS

The profile of protein bands does not change in healthy Toggenburg goat dams and those with chronic infection by SRLV. The TIMP-2 expression allowed proving the existence of MMP-2 in animals chronically infected by SRLV via reverse zymography. Other proteomic approach techniques may be used in future studies to confirm the findings of the present study and help in the differential diagnosis of lentiviruses.

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