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Detection and isolation of caprine lentivirus in goat umbilical cord cells

Detecção e isolamento de lentivírus caprino em células do cordão umbilical de cabras

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Highlights

40% (6/15) of the cords were positive for caprine lentivirus.
Cell destruction and presence of syncytium varied from mild to intense.
SRLVs are present in mesenchymal cells of goat umbilical cord.

Abstract

Small ruminant lentiviruses (SRLVs), which include caprine and ovine lentiviruses, cause serious damage to the health of their hosts, considerably reducing production and increasing culling. The intrauterine route may be an important route of transmission of SRLVs, as they have already been detected in neonates. Furthermore, umbilical cord cells show permissiveness to the multiplication of these viruses in vitro. Thus, this study aimed to detect and isolate caprine lentivirus from mesenchymal cells of Wharton's

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jelly from goat umbilical cord. Fifteen umbilical cords were collected from eight goats (seven positive and one negative for SRLV via nPCR) that underwent cesarean section and immersed in 0.9% saline solution. Subsequently, the Wharton's jelly was removed and cultured in enriched minimum essential medium (MEM) in an incubator at 37 °C and 5% CO2 for 63 days. The medium was changed every seven days, and trypsinization and collection of the supernatant for nested polymerase chain reaction (nPCR) were performed every 21 days. Three samples were randomly selected for DNA sequencing based on the positive nPCR results to identify the viral isolate. 40% (6/15) of the cords were positive for caprine lentivirus (Lentivirus capartenc), previously called caprine arthritis-encephalitis virus (CAEV). One out of the six positive cords remained positive since the first supernatant collection. All samples, except for the lost plots, showed cell destruction and the presence of syncytium ranging from a very mild to an intense level. We also found that 26% (4/15) of the offspring, with blood collected at birth, presented positive nPCR results for caprine lentivirus. The DNA sequences, when aligned, presented homology with each other, with the standard strains CAEV Co and MVV K1514, and with some Brazilian isolates described in the literature. Therefore, caprine lentivirus may be present in mesenchymal cells of Wharton's jelly from the umbilical cord of naturally infected goats in the form of proviral DNA, posing a risk of fetal contamination.
Key words: Cell culture. Proviral DNA. Wharton's jelly. SRLVs. Congenital transmission.

Resumo

Os lentivírus de pequenos ruminantes (LVPRs), os quais englobam os lentivírus caprino e ovino, causam danos graves à saúde dos seus hospedeiros, diminuindo consideravelmente a produção e aumentando o descarte. A via intrauterina pode ser uma importante via de transmissão de LVPRs, uma vez que já foram detectados em recém-nascidos. Ademais, células do cordão umbilical apresentam permissividade à multiplicação desses vírus in vitro. Assim, objetivou-se detectar e isolar lentivírus caprino a partir de células mesenquimais da geleia de Wharton de cordão umbilical de cabras. Coletou-se 15 cordões umbilicais de oito cabras (sete positivas e uma negativa para LVPR via nPCR) submetidas à cesariana, os quais foram imersos em solução salina a 0,9% tratada. Em seguida, retirou-se a geleia de Wharton para cultivo em meio essencial mínimo (MEM) enriquecido, em estufa a 37°C e 5% de CO2, por 63 dias. Realizou-se troca de meio a cada sete dias, e a cada 21 dias realizou-se tripsinização e coleta do sobrenadante, destinado à reação em cadeia de polimerase nested (nPCR). A partir dos resultados positivos na nPCR foram escolhidos, aleatoriamente, três amostras para sequenciamento de DNA com finalidade de identificar o isolado viral. Observou-se que 40% (06/15) dos cordões foram positivos para lentivírus caprino (Lentivirus capartenc), anteriormente denominado vírus da artrite encefalite caprina (CAEV). Dos seis cordões positivos, um permaneceu positivo desde a primeira coleta do sobrenadante. Todas as amostras, com exceção das parcelas perdidas, apresentaram destruição celular e presença de sincício variando de um nível muito leve a um nível intenso. Constatamos também que 26% (04/15) das crias, com sangue coletado ao nascimento, apresentaram resultados positivos de nPCR para lentivírus caprino. As sequências de DNA quando alinhadas, revelou homologia entre si, e com as cepas padrões CAEV Co e MVV K1514 e com alguns isolados brasileiros descritos na literatura. Conclui-se que lentivírus caprino pode estar presente em células mesenquimais da geleia de Wharton de cordão umbilical de cabras naturalmente infectadas na forma de DNA pró-viral, oferecendo o risco de contaminação fetal.
Palavras-chave: Cultivo celular. DNA pró-viral. Geleia de Wharton. LVPRs. Transmissão congênita.

Introduction

Small ruminant lentiviruses are a group of diseases composed of Caprine Arthritis-Encephalitis (CAE) and Maedi-Visna (MV), which have as etiological agents the species *Lentivirus capartenc,* known as caprine lentivirus or caprine arthritis-encephalitis virus (CAEV), and the species Lentivirus ovivismae, known as ovine lentivirus or Maedi-Visna virus (MVV). Together, these viral entities are known as small ruminant lentiviruses (SRLVs). SRLVs are currently classified in the great kingdom *Riboviria,* kingdom *Pararnavirae,* phylum *Artververiricota,* class *Revtraviricetes,* order *Ortervirales,* family *Retroviridae,* subfamily *Orthoretrovirinae,* and genus *Lentivirus* (International Committee on Taxonomy of Viruses [ICTV], 2024). They are a heterogeneous group of lentiviruses that infect goats and sheep worldwide, affecting animal health, production and, consequently, increasing culling rates in herds (Leroux et al., 2010). Other lentivirus species can infect humans, such as human immunodeficiency virus (*Lentivirus humimdef1* or HIV), non-human primates (*Lentivirus simimdef* or SIV), felids (*Lentivirus felimdef* or FIV), bovids (*Lentivirus bovimdef* or BIV), and equids (*Lentivirus equinfane* or EIAV) (Minguijón et al., 2015).

After exposure, SRLVs lead to a persistent, multisystemic and, in most cases, inapparent infection (Highland, 2017; Minguijón et al., 2015). The progressive worsening of the lesions is slow due to the chronicity of small ruminant lentiviruses, causing weight loss, weakness, and even death of the infected animal. The clinical signs of the infection are classified into arthritic, respiratory, mammary and, to a lesser extent, nervous forms (Narayan & Cork, 1985; Peretz et al., 1993). When circulating within the herd, SRLVs cause productive and economic losses due to reduced live weight gain and decreased milk production and quality, leading to economic losses (Azevedo et al., 2017).

Direct contact and the lactogenic route are the main transmission pathways of these viral agents (Pisoni et al., 2010; Souza et al., 2015) and, therefore, the separation of offspring at birth with the provision of heat-treated colostrum has been recommended as the primary measure in control programs (Ministério da Agricultura Pecuária e Abastecimento [MAPA], 2004; Peretz et al., 1993). However, new cases have still emerged even adopting these procedures (Alcindo et al., 2020), and research has revealed that neonates have been positive for SRLVs even under monitoring (J. F. Araújo et al., 2020; Hasegawa et al., 2017; Rodrigues et al., 2017).

The literature shows evidence of SRLV infection in neonates (Rodrigues et al., 2017), as well as in the female reproductive tract (Cavalcante et al., 2013; Hasegawa et al., 2017; Lamara et al., 2002). The embryo or fetus are believed to become infected during passage through the oviduct and uterus, or during birth when the fetus passes through the vaginal canal (Fieni et al., 2003). Another recognized form of SRLV transmission is the transplacental route (J. F. Araújo et al., 2020; Hasegawa et al., 2017). The exact mechanisms of this transmission have not yet been elucidated and the risk of transplacental transmission is considered low, but its occurrence can compromise disease control (Cortez-Romero et al., 2013).

The umbilical cord has a direct connection between the placenta and the fetus (Bankowski, 1999), maintaining the vascular connection between both, consisting of an essential structure in intrauterine life. Pathologies associated with the umbilical cord can have significant consequences for the fetus (E. Araújo et al., 2006). Wharton's jelly surrounds the veins and arteries of the umbilical cord and is formed by connective tissue composed of mesenchymal cells immersed in a basal substance (Bankowski, 1999). Research has shown that these cord cells can replicate SRLVs in vitro (Dias et al., 2016; Martins et al., 2017).

However, the mechanisms acting throughout this vertical transmission are still unknown. This study aimed to detect and isolate caprine lentivirus from mesenchymal cells of Wharton's jelly from goat umbilical cords.

Material and Methods

The study was conducted in the dairy herd of Embrapa Goats and Sheep, in the city of Sobral, located in the northern region of the State of Ceará, Brazil. Importantly, this research was duly approved by the Ethics Committee on the Use of Animals (CEUA) of Embrapa Goats and Sheep (protocol No. 010/2018), following the guidelines of the National Council for the Control of Animal Experimentation (CONCEA, Law No. 11794 of October 8, 2008) and other subsequent normative resolutions. Eight pregnant goats belonging to the herd were randomly selected to compose the group of animals.

Blood collection and diagnostic test

The eight breeding does were tested for positivity for caprine lentivirus in the final third of gestation and on the day of cesarean section (140 and 147 days of gestation, respectively). For this purpose, blood was collected from all goats by puncture of the jugular vein, using 5-mL vacuum tubes with ethylenediaminetetraacetic acid (EDTA) anticoagulant. The samples were then subjected to deoxyribonucleic acid (DNA) extraction, as previously described by Sousa et al. (2018). The DNA samples were subjected to nested polymerase chain reaction (nPCR), according to Marinho et al. (2018). This same procedure was performed on the offspring at the time of cesarean section.

Umbilical cord collection

The umbilical cord was collected from 15 neonates during cesarean section surgery from all eight does. For the surgical procedure, the goats were subjected to high epidural anesthesia with 1% lidocaine and infiltration in the incision region (left flank) in an inverted L (up to a maximum of 6.0 mg/kg).

After careful exposure of the gravid uterus, a cut was made to remove the kid (Dias et al., 2016). Then, the umbilical cords were clamped with sterile hemostatic scissors and cut into 6- to 10-cm sections. Once collected, each cord fragment was kept in a 0.9% sodium chloride (NaCl) solution supplemented with 10% beta-lactam and aminoglycoside antibiotic (penicillin and streptomycin, PS), 2% antifungal (amphotericin, A), and 1% aminoglycoside antibiotic (gentamicin, G).

Umbilical cord cell culture

Wharton's jelly was removed from each cord At the Laboratory of Virology of Embrapa Goats and Sheep, cultured in 24-well plates containing minimum essential medium (MEM) plus 10% fetal bovine serum (FBS), supplemented with 1% PS, 1% A, and 1% G, and cultivated in an incubator at 37 °C and 5% CO2 for 63 days. The subculture of explants of each sample were divided into four wells of the plate. The medium was changed every seven days and trypsinization was performed every 21 days. Four supernatant collections were performed during the culture period, the first after seven days and the others every 21 days from the beginning of the culture, which were stored in a freezer at −80 °C for nPCR. Cell monolayers were observed daily under an inverted microscope for the presence of cytopathic effect (CPE) and syncytia. The plates were stained with crystal violet (0.1%) after 63 days of culture to visualize cytopathic effects, such as cell destruction and the presence of syncytia.

Extraction of proviral DNA from cell supernatant and nPCR

The supernatants from Wharton's jelly cell monolayer trypsinization were used for DNA extraction by employing proteinase K and ethyl alcohol (Sigma-Aldrich®, USA), according to the methodology by Sousa et al. (2018). The DNA samples were subjected to a first PCR. The expected 297 base pair (bp) product of the first PCR was then subjected to a second (nPCR) to amplify a final 185 bp fragment of proviral DNA, corresponding to the gag gene of SRLVs. All oligonucleotide primers were produced based on the CAEV-Co standard sequence with GenBank accession No. M33677.1 (Saltarelli et al., 1990) (Table 1).

Table 1
Sequences of the oligonucleotide primers used in the nPCR and their respective amplified fragments

Gene Gag	Primers	Sequences 5′ → 3′	Fragments (pb)
1st round	Gag 1	CAAGCAGCAGGAGGGAGAAGCTG	297
	Gag 2	TCCTACCCCCATAATTTGATCCAC	
2nd round	Gag 3	GTTCCAGCAACTGCAAACAGTAGCAATG	185
	Gag 4	ACCTTTCTGCTTCTTCATTTAATTTCCC	

Ultrapure water was used in all nPCR procedures as a negative control and a positive control corresponding to CAEV-Co (standard viral sample kindly provided by the Universidade Federal Rural de Pernambuco, from the Laboratoire Associé de Recherches sur les Petits Ruminants – INRA – ENVL – France).

The nPCR reactions were performed in a thermocycler (BIO-RAD, T100TM Thermal Cycler) in a total volume of 50 µL, containing buffer (10 mM Tris-HCl, 50 mM KCl, and 1.5 mM MgCl2 – Sigma-Aldrich®, USA), 100 µM of each dNTP (Sigma-Aldrich®, USA), 20 pmol of each primer, 2U of Taq Platinum DNA polymerase (Sigma-Aldrich®, USA), 3 µL of sample in the first reaction, and 1 µL of its product in the second reaction.

The nPCR amplification cycles consisted of 94 °C for five minutes, 35 cycles of 94 °C for one minute, 56 °C for one minute, and 72 °C for 45 seconds, followed by a final extension at 72 °C for seven minutes. The amplified samples and controls (positive and negative) were subjected to 2% agarose gel electrophoresis (Sigma-Aldrich®, USA), stained with ethidium bromide (Sigma-Aldrich®, USA) and visualized in an ultraviolet transilluminator (UVP, Benchtop UV Transilluminator M-26) (Marinho et al., 2018).

Sequencing and sequence analysis

Three positive samples visualized in agarose gel electrophoresis were randomly selected for DNA sequencing of nPCR products. These samples came from goat umbilical cord cell cultures. Sequencing was performed using the Sanger method (Applied Biosystems® 3500 Genetic Analyzer). The sequences obtained from each sample were edited and aligned using Geneious software (version 8.1). Subsequently, the consensus sequences were subjected to local alignment using the BLASTn tool to verify their identity with the sequences already deposited in the online databases (GenBank). The consensus sequences of each sample were then aligned with the sequences of the standard strains CAEV-Co (M33677) and MVV K1514 (M10608), and some Brazilian sequences that obtained greater identity with the samples of this study, i.e., BR CNPC (EU300976, EU300977, and EU300978).

Results

Among the eight selected does, both in the nPCR result in the final third of gestation and on the day of cesarean section, seven does were positive (88%) and only one was negative (12%) for caprine lentivirus when the technique was performed with blood samples collected on the respective days. Regarding the nPCR results of the offspring, obtained with blood collected at zero hour, 26% (4/15) presented positivity for caprine lentivirus (animals 02, 06, 07, and 09). Importantly, the only goat that tested negative for this viral agent gave birth to three offspring (animals 01, 02, and 03), one of which (offspring 02) tested positive in the nPCR for caprine lentivirus, both in the blood collected at zero hour and in the umbilical cord.

The migration of umbilical cord cells out of the explants occurred after four to seven days of culture, and the cells gradually multiplied into colonies in the first 21 days of culture. Adjacent colonies interconnected during cell growth, and a monolayer of approximately 80% confluence was obtained. The first signs of CPE caused by caprine lentivirus in some of the samples were observed from the 21st day onwards with cell trypsinization.

Regarding the presence of proviral DNA, 40% (6/15) of the cords were positive for caprine lentivirus, with sample number

six being positive since the first collection of the supernatant (Table 2). Importantly, four samples (01, 02, 03, and 09) were lost during the culture, the first three after the first trypsinization. However, one of them (02) presented positivity before the loss.

Table 2
Result of nPCR of proviral DNA extracted from supernatant collected from culture of mesenchymal cells from Wharton's jelly of goat umbilical cord

Animals	Collections			
	1st	2nd	3rd	4th
C−	−	−	−	−
C+	+	+	+	+
01	−	−	PL	PL
02	−	+	PL	PL
03	−	−	PL	PL
04	−	−	−	−
05	−	−	−	−
06	+	+	+	+
07	−	−	+	−
08	−	−	−	−
09	−	PL	PL	PL
10	−	−	−	−
11	−	+	−	−
12	−	+	−	−
13	−	−	−	−
14	−	−	+	−
15	−	−	−	−

PL: plot loss; C−: negative control (sample with exonuclease-free ultrapure water specific for molecular biology); C+: positive control (DNA extracted from antigen produced from coculture of nictitating membrane cells infected with CAEV-Co strain of SRLVs); (−): sample negative for caprine lentivirus; (+): sample positive for caprine lentivirus.

The presence of syncytia containing more than four nuclei was observed in nine of the samples, with higher evidence in 04, 07, 12, and 15 in cells derived from Wharton's jelly from goat umbilical cord. In addition, the existence of more spaced areas was observed in the monolayers of all samples, possibly indicating a higher level of cell death compared to the negative control of the culture (Figure 1).

Figure 1. Culture of mesenchymal cells from Wharton's jelly from goat umbilical cord. A: negative control of the cell culture composed of goat nictating membrane cells negative for SRLVs (magnification 100X); B: sample 04 with presence of syncytium (magnification 160X); C: sample 05 with presence of syncytium (magnification 160X); D: sample 06 with cell destruction (magnification 160X); E: sample 07 with presence of syncytium (magnification 160X); F: sample 08 with cell destruction (magnification 160X); G: sample 10 with cell destruction (magnification 160X); H: sample 11 with cell destruction (magnification 160X); I: sample 12 with presence of syncytium (magnification 160X); J: sample 13 with cell destruction and syncytium (magnification 160X); K: sample 14 with mild cell destruction and syncytium (magnification 200X); L: sample 15 with cell destruction (magnification 200X); M: positive control of the culture with goat nictating membrane cells inoculated with standard strain CAEV-Co and SRLVs (magnification 160X).

The cell culture plates showed CPE when stained, such as the presence of syncytia and cell destruction, ranging from very mild to intense (Table 3). A lytic effect was also observed with destruction of cell monolayers, and the most intense result was observed in samples 06, 08, 10, 11, 13, and 15 (Table 3). All other samples presented lysis although with less intensity.

Table 3
Level of viral cytopathic effects in mesenchymal cells of Wharton's jelly from goat umbilical cord visualized over 63 days of culture

Animals	Viral cytopathic effects	
	1st	2nd
C–	–	–
C+	+++	+++
01	PL	PL
02	PL	PL
03	PL	PL
04	++	+++
05	++	++
06	+++	++
07	+	+++
08	+++	+
09	PL	PL
10	+++	–
11	++++	+
12	++	++
13	+++	–
14	+	+
15	++++	++

PL: plot loss; C–: negative control (goat nictating membrane cells negative for SRLVs); C+: positive control (goat nictating membrane cells inoculated with CAEV-Co strain of SRLVs); (–): no cytopathic effect; (+): very mild effect; (++): mild effect; (+++): moderate effect; (++++): intense effect.

Furthermore, CPE was confirmed with the visualization of a 187-bp product amplified from the target sequence of the proviral DNA, which occurred in six of the 15 samples, producing infectious viruses in the supernatant.

High identity was observed when defining and aligning the consensus sequences of each of the three isolated samples. This situation was also observed between them and the standard strains CAEV-Co and MVV K1514. Furthermore, the sequences also showed a high relationship with the Brazilian strains BR CNPC (GenBank accession numbers EU300976, EU300977, and EU300978) (Figure 2).

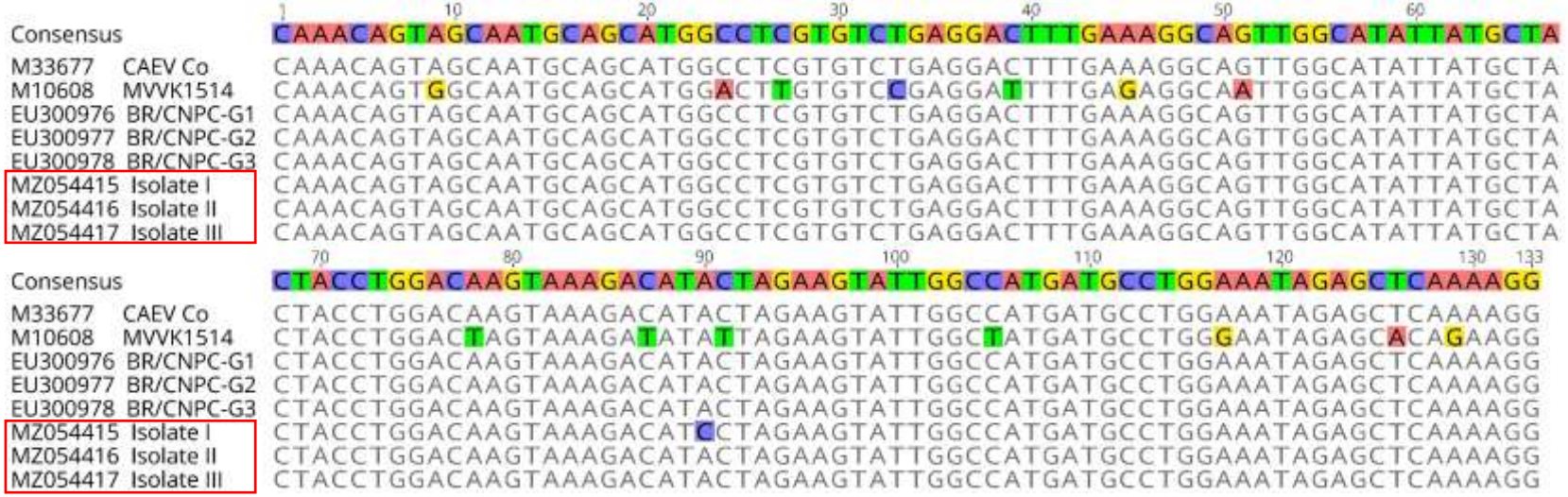

Figure 2. Alignment of 129-base pair (bp) fragments of the gag gene from the proviral DNA consensus sequences of caprine lentivirus (highlighted in the image) with standard strains CAEV-Co, MVV K1514, and Brazilian strains described in GenBank.

Discussion

The literature reports the detection of proviral DNA of SRLVs in the blood of ovine fetuses (2/32), but without amplification in tissue samples from the spleen, thymus, umbilical cord, and cotyledons (Sanchez et al., 2016). To our knowledge, this is the first study to detect natural infection by caprine lentivirus in mesenchymal cells from Wharton's jelly of the umbilical cord of goats. Detection and isolation in cultures of umbilical cord cells may indicate infection by caprine lentivirus in the uterus, as it is a structure that maintains the connection between the fetus and the mother (E. Araújo et al., 2006). Also, the infection of approximately 10–35% of neonate goats by SRLVs not naturally breastfed (J. F. Araújo et al., 2020; Hasegawa et al., 2017), as well as evidence of infection in lambs (Brodie et al., 1994; Sánchez et al., 2016), suggests intrauterine infection although the timing and sources of infection are not completely clear. Additionally, the detection of HIV in human newborns either by cell culture or through PCR from blood collected in the first days of life suggests infection in the uterus (Biggar et al., 1997). These findings corroborate the data of the present study, which detected animals positive for caprine lentivirus in the collection on day zero in the first minutes of life of the neonates.

This finding increasingly strengthens the potential of the intrauterine route as a source of infection for SRLVs in goats and sheep. Initially considered specific pathogens, caprine and ovine lentiviruses, formerly called CAEV and MVV, are described as a genetic continuum because they share high phylogenetic similarity (Minguijón et al., 2015; Wolf, 2021). However, the few differences between these two etiological agents are related to failures during the replication process of caprine lentivirus in sheep choroid plexus cells (Chebloune et al., 1996). In this sense, phylogenetic research and cases of interspecies transmission reaffirm the need for these viruses to be generically called SRLVs (Souza et al., 2015).

The birth of a positive animal from a mother negative for caprine lentivirus (negative goat, offspring 02) is explained by the virus capacity to compartmentalize, one of its escape mechanisms (Ramírez et al., 2012). This characteristic has made diagnosis increasingly difficult, as the virus migration from the bloodstream to other organs, tissues, or fluids becomes an important risk factor for the disease (Olech & Kuźmak, 2019; Peixoto et al., 2021, 2023; Ramírez et al., 2012). This mechanism allows an animal that tests negative in one type of biological sample to still have the potential to transmit the disease, as the virus may be present in another system, including the reproductive one. Some studies in the literature have already isolated SRLVs in samples from the female reproductive tract (Cavalcante et al., 2013; Fieni et al., 2003; Lamara et al., 2002). Thus, several studies have recommended the association of diagnostic techniques applied to different biological samples (J. F. Araújo et al., 2020; Azevedo et al., 2019; Hasegawa et al., 2017; Panneum & Rukkwamsuk, 2017).

The inconsistency in nPCR results in the collected supernatants may be due to the lack of sufficient cell suspension at the time of collection (J. F. Araújo et al., 2020) or the low viral load of the samples (Ravazzolo et al., 2006), thus resulting in a negative

nPCR result. According to J. F. Araújo et al. (2020), the strong adhesion of the cell mat and the low number of dead cells in the supernatant prevents consistent detection of positive samples during collections and also causes more suspended cells in certain samples, thus leading to higher detection of proviral DNA. This greater or lesser number of suspended cells can be explained by the viral load of the sample (Ravazzolo et al., 2006). This explains why animal 06 presented constant positivity in all collections probably due to its higher viral load.

Regarding the results of cell culture, the period of cell migration out of the explants corroborates those in the literature (Dias et al., 2016; Martins et al., 2017). Hendijani et al. (2014) reported that the explant technique applied to human Wharton's jelly tissue allowed cells to begin migrating to the surface of the plate 13 days after the start of culture. According to Comar et al. (2014), cord blood matrix cells show some advantages when cultured, including faster proliferation, higher expansion capacities, broad multipotency (stem cells) and, therefore, less susceptible to latent viral infections. However, the permissibility of these cells to in vitro infections by SRLVs has already been confirmed (Dias et al., 2016; Martins et al., 2017), and the results of the present study demonstrated that these cells undergo in vivo infections by caprine lentiviruses, including the presence of intense cytopathic effects.

The cells presented a fusiform shape in the initial culture period. However, these cells differentiated into other shapes characteristic of viral infection as the days went by and, consequently, with viral replication. These data corroborate the study by Martins et al. (2017), who used cells from the matrix of the goat umbilical cord to test the permissiveness of SRLVs in vitro and detected this same differentiation process in the cell shape after viral inoculation. Moreover, another study revealed the same shape differentiation using Wharton's jelly cells to test the permissiveness of these SRLV infection in vitro (Dias et al., 2016).

Passeri et al. (2009) used an enzymatic technique in mare umbilical cord tissue with collagenase and reported the formation of individual spindle cells after three to four days of culture and colony formation in five days.

Importantly, Comar et al. (2014) demonstrated the in vivo capacity of polyomavirus infection in Wharton's jelly cells in studies with humans, but the percentages achieved were lower than those of the present study.

The observation of syncytia and cell destruction in cell cultures are some of the main CPEs characteristic of SRLVs evidenced in the literature (Dias et al., 2016; Martins et al., 2017; Oguma et al., 2014; Sousa et al., 2018; J. F. Araújo et al., 2020). Syncytium formation caused by in vitro inoculation with SRLVs has been previously described in umbilical cord cells of small ruminants (Dias et al., 2016; Martins et al., 2017). Cells fuse for syncytium formation because of direct interaction with the virus and this behavior is called fusion, occurring before viral replication (Ellis et al., 1985). Syncytium formation with higher intensity in certain samples can be due to the viral load and strain in the cell culture.

Furthermore, the formation of syncytia in cell culture only occurs when intact viral particles are present, excluding the possibility that this CPE occurs due to intracellular precursors of a single viral fusion protein (Ellis et al., 1985). Thus, the CPE observed in some samples of this study demonstrates the presence of intact viral particles in the naturally infected cell supernatant.

The data on cytopathic effects were reinforced with the sequencing of proviral DNA, which observed a strong similarity of the isolated genetic sequences with the standard strains CAEV-Co and MVVK1514 and the Brazilian sequences BR CNPC (EU300976, EU300977, and EU300978), thus proving that the isolated strains were from SRLVs. According to Souza et al. (2015) and Lima et al. (2017), sequencing is a valuable technique for proving strain similarities and has been used to confirm interspecific transmission of the virus among small ruminants. Furthermore, J. F. Araújo et al. (2020) used DNA sequencing to prove the strain of SRLVs.

Dias et al. (2016) studied the in vitro permissiveness of ovine Wharton's jelly cells to SRLVs and observed the need to elucidate the mechanism of vertical transmission of these lentiviruses to the fetus. In this sense, several viral types have already been detected in blood collected from the umbilical cord of mammals, such as herpesvirus (Abedi et al., 2014), cytomegalovirus (Albano et al., 2006; Endo et al., 2009), HIV (Biggar et al., 1997), hepatitis B virus (Liu et al., 2019), and papillomavirus (Sarkola et al., 2008).

Conclusion

Caprine lentivirus infective particles are present in mesenchymal cells of Wharton's jelly from the umbilical cord of naturally infected goats in the form of proviral DNA. Thus, the intrauterine route becomes a possible and important route of infection of SRLV in small ruminants.

Acknowledgments

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