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Short communication

Genetic and antigenic characterization of Brazilian SRLV strains: Natural small ruminant interspecies transmission from mixed herds.

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ABSTRACT

Cross-species transmission events and mixed infection of small ruminant lentiviruses (SRLVs) were studied in seven goats and two sheep from three small ruminant mixed flocks from Northeast and Southeast Brazil. Genetic and antigenic analyses with *gag/env* genes and ELISA multiepitope SU1/SU5 recombinant antigens were carried out, respectively. The genetic analysis of *gag* and *env* sequences showed high viral diversity in both species, MVV-like (subtype A1) and CAEV-like B1 in goats, and CAEV-like (subtype B1) in sheep, revealing SRLV interspecies transmission from sheep to goats and vice versa in Brazilian farms. Two Brazilian caprine lentiviruses were segregated in two new genetic clades based on *gag* analyses, which suggests a new classification into heterogenic genotype A. Furthermore, goat isolates were grouped into subtype A1 and B1 clusters. Cross-reactive antibodies were detected in goats using ELISA with a recombinant antigen carrying SU1 and SU5 immunodominant epitopes; the results showed anti-CAEV and MVV antibodies in goats and anti-CAEV antibodies in sheep. This result can be associated with the high divergence in the V4 region due to SRLV variability. All results confirm cross-species infection of SRLV in Brazilian mixed herds.

Small ruminant lentiviruses (SRLVs), which include arthritis-encephalitis virus (CAEV) and maedi-visna virus (MVV), are a genetic continuum of lentiviral species that were initially isolated from goats and sheep, respectively (Ramírez et al., 2013). Although the major route of transmission of these viruses is through the ingestion of virus-infected milk, less efficient routes have been associated with prolonged close contact of naïve animals with infected animals (Shah et al., 2004a).

SRLVs are phylogenetically divided into five groups (A–E), which include different subtypes, based on their *gag* and *pol* genes (Shah et al., 2004a). Genotype groups differ by 25% to 37%, and subtypes differ from 15% to 27%, in their nucleotide sequences (Shah et al., 2004a). Group A (MVV-like) has 22 recognized subtypes, A1–A22; group B (CAEV-like) has five subtypes, B1–B5; groups C and D include viruses isolated from goats and sheep in Norway and Swiss and Spanish sheep, respectively; and group E has only two subtypes, E1 and E2 (Colitti et al., 2019; Gjerset et al., 2006; Michiels et al., 2020; Molaee et al., 2020; Olech et al., 2012; Olech et al., 2018; Santry et al., 2013; Shah et al., 2004a; Shah et al., 2004b). However, based on *gag* gene phylogenetic analysis, group D is now reclassified as genotype A (Ramírez et al., 2013).

The majority of these subtypes are able to cross the species barrier between goats and sheep, and vice versa, under natural conditions (Gjerset et al., 2007; Pisoni et al., 2005; Santry et al., 2013; Shah et al., 2004b), and dual infection and/or recombination in both SRLVs has been reported (Fras et al., 2013; Olech et al., 2012; Olech et al., 2018; Pisoni et al., 2007; Santry et al., 2013) as a consequence of the high genetic variation in retroviruses (Gifford, 2012). High SRLV genetic

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variations could explain the lack of proofreading driven by the reverse transcriptase enzyme and the introduction of new mutations in the SRLV genome. Additionally, host interactions, the relationship with the APOBEC3 enzyme, an intrinsic protein known to incorporate deleterious mutations into the viral genome, and the occurrence of recombination between strains adds to their adaptability and genetic diversity; selection by the host immune system will limit the variation in genetic and phenotypic constraints (Larruskain and Jugo, 2013; Michiels et al., 2020; Ramírez et al., 2013).

Genotypes A and B have been identified in Brazilian sheep and goats, respectively (Feitosa et al., 2010; Lima et al., 2004; Ravazzolo et al., 2001). In addition, genotype A was reported in naturally infected goats (Castro et al., 1999), showing the possibility of interspecies transmission in Brazilian herds. Recombination occurs frequently in the envelope gene and contributes to a high incidence of antigenic variation in MVV (Andrésdóttir, 2003) and CAEV (Valas et al., 2000), principally in the V4 and V5 regions. The surface glycoprotein (SU) of lentiviruses contains determinants important for cellular host range, infectivity, cytopathogenicity, and disease progression, which elicit humoral immune responses during natural infection, and five variable regions (V1 to V5) and four conserved regions (C1 to C4) were identified (Valas et al., 2000). In the V4 region, there is a neutralizing domain that is immunogenic and very divergent, where MVV has six amino acid deletions (Saltarelli et al., 1990). Evaluating the variability profile of the SU from Brazilian SRLV is important for lentivirus control. The aims of the present study are to report the Brazilian high SRLV diversity by genetic and antigenic characterization, to highlight the natural transmission of SRLV between infected sheep and goats from Brazilian mixed flocks and to report the first occurrence of CAEV-like subtype B1 in Brazilian sheep.

Animals for this study were selected from forty Brazilian small ruminant flocks from the Northeast, Southeast and South regions that contained a total of 1882 goats and 67 sheep between 2009 and 2011. Seven goats aged over six months (Saanen, Alpine, Angle Nubian and British Alpine breeds) and two sheep (Santa Inês breed) were chosen for the study based on the diagnosis of SRLV serum antibodies using AGID tests (Biovetech, Pernambuco, Brazil) and two ELISAs with multiepitope SU1/SU5 recombinant antigens (SS-B1 and SS-0016), which detected the B1 and A13 SRLV subtypes, respectively (Olech et al., 2012), for antigenic characterization of Brazilian SRLV strains. These 17-kDa antigens (SSB1 and SS-0016) containing only the immunodominant regions of the SU1 and SU5 epitopes fused together were generated by removing the Gag domain; this method was performed with sequences of the B1 French viral 680 SU1 (Valas et al., 2000) plus the SU5 sequence of 3056 strains (Germain and Valas, 2006), and SU1/SU5 from the A13 Polish s0016 SRLV strain (Olech et al., 2012). These animals were selected for their relatively great divergence from each other and were from three SRLV seropositive mixed flocks (G, H and U) located in the Northeast (Paraíba and Maranhão states) and Southeast (Minas Gerais state) of Brazil (Table 1).

SRLV viruses were obtained from primary culture with three goat synovial membranes (GSMs) (Br295 (20G-MG/10), Br195 (21G-MG/10), Br628 (43U-MA/11)) and one choroid plexus goat (CP) (Br329 (24H-PB/10)) based on the Castro et al. (1999) protocol with modifications. Briefly, GSM and CP tissues were minced in Petri dishes with 0.5 mL of culture medium (MEM) containing 20% fetal bovine serum (FBS); then, the explants were transferred to 25 cm^2 flasks with tissue culture medium (10% FBS, 1% penicillin G (200 U mL^{-1}), streptomycin (200 μg mL^{-1}), and 0.5% amphotericin B (50 μg mL^{-1})) and incubated for up to two months at 37 °C in a 5% CO2 atmosphere. When the cytopathic effect (CPE) was observed, cells and medium were harvested biweekly and stored at -−80 °C for DNA extraction with a QIAamp mini DNA kit (Qiagen, Courtaboeuf, France) following the manufacturer's instructions. Peripheral blood leukocyte (PBL) cells were used as a viral isolation substitution source when animal tissues were not successful (3 goats; Br1163, Br7003, and Br9007; and 2 sheep; Br198 and Br200).

We attempted to sequence all proviral DNA of SRLV strains and/or PBL for the *env* and *gag* genes. Either the V1-V2 (394 bp) or V4-V5 (608 bp) *env* region was amplified by nested PCR. PCR was performed as previously described Germain et al. (2008), except that the cycling conditions for amplification were as follows: first cycle (95 °C, 30 s, 56 °C, 40 s, 72 °C, 90 min) and second cycle (95 °C 30 s, 58 °C 40 s, 72 °C 50 s). The primer pairs Ptat (5'-ACAAAGATGGCTWGCWATGCTTA-3′, nt 5807) and Penv (5'-ATGCCAGCAATCCAATTCWTGGT-3'′, nt 8179) were designed by aligning several *env* sequences from GenBank and were used in the first round of PCR. Primer pairs A51/B31 and 567/564 were used in the second round for the amplification of the V1-V2 and V4-V5 fragments, respectively (Germain and Valas, 2006; Mordasini et al., 2006). For region V1-V2, PCR products were obtained from six animals (Br295 (20G-MG/10), Br329 (24H-PB/10), Br628 (43U-MA/11), Br9007, Br7003, and Br198), and for V4-V5, seven animals were used (Br295 (20G-MG/10), Br195 (21G-MG-10), Br329 (24H-PB/10), Br628 (43 U-MA/11), Br1163, Br9007, and Br7003).

Additionally, the *gag* fragment (990 bp), containing the whole capsid (CA) and a nearly complete matrix (*MA*) sequence, was amplified using

Table 1
Genetic and antigenic characterization of Brazilian SRLV strain from Brazilian goats and sheep.

				Flock	Herd	Brazilian		ELISA[b] (SU1/SU5)	Sequence-Genotype		
									ENV	ENV	GAG
Species	Animal/no.	Breed	Specimen	Goat/Sheep	Origin	State/Region	AGID	SSB1/ SS0016	V1V2	V4V5[c]	MA/CA
Goat	20G-MG/10 (Br295)	Saanen	GSM cultivar	Yes	1 (G)	MG/SE	+	+/−	B1	B1	WS
	21G-MG/10 (Br195)	Saanen	GSM cultivar	Yes	1 (G)	MG/SE	+	+/−	WS	**B1**[e]	**A**
	24H-PB/10 (Br329)	Brith alpine	CP cultivar	Yes	2 (H)	PB/SE	+	+/−	B1	B1	WS
	43U-MA/11 (Br628)	A. nubiane	GSM cultivar	Yes	3 (U)	MA/NE	+	NT	B1	B1	WS
	Br1163	A. nubiane	PBL[a]	Yes	2 (H)	PB/NE	+	+/+	WS	**mutation**[d]	**A**
	Br9007	A. nubiane	PBL[a]	Yes	3 (U)	MA/NE	IN	+/−	B1	B1	WS
	Br7003	A. nubiane	PBL[a]	Yes	3 (U)	MA/NE	+	+/−	B1	B1	WS
Sheep	Br198	Santa inês	PBL[a]	Yes	3 (U)	MA/NE	NT	+/−	B1	WS	**B1**
	Br200	Santa inês	PBL[a]	Yes	3 (U)	MA/NE	NT	+/−	WS	WS	**B1**

In bold, there are the gene sequences and SRLV genotype results of the Br195 (21G-MG/10) and Br1163 goats, and Br198 and Br200 sheep.
Abbreviations: AGID, Agar Gel Immunodiffusion; PBL, Peripheral blood leukocyte; GSM, Goat sinovial membrane; CP, Choroid plexus; IN, Inconclusive; WS, Weak signal, NT, Not tested; Br, Brazil; MA, Maranhão, MG, Minas Gerais; PB, Paraíba; Flocks from herd origins, identified by letter: G, H, U; SE, Southeast; NE, Northeast; MA, Matrix; CA, Capsid.
[a] DNA was extracted from PBL.
[b] + = Positive; − = Negative.
[c] Alignment of the aa sequences from SRLV SU (V4V5) region.
[d] Mutation: insertion GKCQANETC.
[e] Alignment of the aa deduced sequence only V4 region.

nested PCR, according to Olech et al. (2012) with modifications in the cycling conditions, first cycle (95 °C 30 s, 55 °C 40 s, 72 °C 90 s) and second cycle (95 °C 30 s, 51 °C 40 s, 72 °C 60 s).

The partial *env* (V1-V2 and V4-V5) and *gag* amplicons were purified from agarose gels using a QIAquick Gel Extraction Kit (Qiagen, Courtaboeuf, France) and sequenced in both directions using a standard ABI BigDye terminator reaction. Nucleotide and amino acid (aa) sequences were aligned to perform tree construction and aa analysis, respectively, using Clustal W in MEGA version X and Bioedit version 7.2.6 (Hall, 1999; Kumar et al., 2018). Manual rearrangements of the alignments, including gap exclusion and length adjustment, were applied to achieve optimal results. Pairwise genetic distances were calculated using MEGA version X with Kimura 2-parameter mode (Kimura, 1980). Phylogenetic construction was carried out using the neighbor-joining method implemented in MEGA with the Kimura 2-parameter gamma distance (Saitou and Nei, 1987). The statistical confidence limits of the phylogram topologies were assessed with 1000 bootstrap replicates. The SRLV Brazilian sequences in this study are available under accession number in the GenBank database KF861573 (Br195 (21G-MG/Br)), KF861572 (Ovine Br198), KF861555 (Ovine Br200), and KF861574 (Br1163) for *gag* sequences; MZ367335 (Br9007), MZ367338 (ovine Br198), MZ367339 (Br7003), MZ367331 (Br628/43 U-MA/Br), MZ367322 (Br295/20G-MG/Br), and MZ367323 (Br329/24H-PB/Br) for V1V2 *env* sequences.

Among the goats, six were AGID positive, and one was indeterminate (Br9007). All goats and sheep tested by SSB1 r-ELISA were positive, among which one goat (Br1163) was positive for both r-ELISAs, which detected the B and A SRLV genotypes.

Among the SRLV seropositive goats, we obtained four viral isolates in which *env* and *gag* genes were sequenced and analyzed. Analysis of the VI-V2 *env* phylogenetic tree results (isolates and PBLs) revealed that SRLV sequences from goats and sheep clustered together with the B1 subtype (Table 1, Fig. 1-A). Ovine and caprine lentivirus *gag* sequences were aligned with SRLV genotype B (subtype B1) and A sequences, respectively (Table 1, Fig. 1-B). Because of the more representative genotype, divergent sequences were only shown for the *gag* gene. The nucleotide divergence between CA Brazilian sequences ranged from 8.7 to 34.5, and the diversity compared to all group A strains ranged from 12.9 to 37.8. Specified nucleotide divergences involving Brazilian strains are shown in Table 2. Due to weak *gag* PCR signals, only sequences from four samples were obtained.

To improve genetic characterization and verify Brazilian SRLV aa conservation, alignment of the deduced *env* (V4-V5) and *gag* (CA/MA) aa sequences together with A1 (strain K1514) and B1 (strain cork) reference sequences (Figs. 2 and 3) from four and six animals (Br195 only *env*-V4), respectively, was performed. The ovine sequences Br198 and Br200 showed seven aa deletions in the C-terminus of MA, which have already been described in all CAEV sequences (Fig. 3) (Olech et al., 2012). The caprine strains Br195 (21G-MG-10) and Br1163 had two aa deletions (GG) in the middle of CA (position 100) (L'Homme et al., 2011) and seven aa insertions (C-terminal MA), which have been reported in all MVV sequences (position 198) (Fig. 3) (Saltarelli et al., 1990). The aa sequence analysis of V4-V5 of Br1163 has an insertion of several aa (GKCQANETC) in the V4 region (position 52), which is in a similar location as an aa deletion observed in MVV-like virus (Fig. 2-A). Furthermore, analyses of V4 aa from the goat isolate Br195 (21G-MG-10) showed a CAEV-like sequence because there was no aa deletion characteristic of MVV (Fig. 2-B).

To assess the immunological relatedness of subtypes, serum samples were tested against ELISA multiepitope SU1/SU5 using recombinant antigens, which detected B1 SRLV-specific antibody subtypes in both species. Furthermore, one goat (Br1163) was positive in both r-ELISAs, showing an immune response against both the B1 and A13 subtypes (Table 1); this goat was from a mixed flock on farm number 3.

In this work, we described the genetic and antigenic composition of SRLVs from naturally infected Brazilian sheep and goats. Genetic analysis carried out on partial *gag* and *env* sequences demonstrated high viral diversity in both animal species, such as subtypes A1 and B1 in goats and subtype B1 in sheep, displaying SRLV interspecies transmission from sheep to goats and vice versa in Brazil, as previously reported (Castro et al., 1999; Fras et al., 2013; Lamara et al., 2013; Olech et al., 2018; Santry et al., 2013; Shah et al., 2004b).

In addition, two Brazilian caprine lentiviruses (Br195 (21G-MG-10) and Br1163) segregated into distinct subtypes based on the *env* (V1/V2) and *gag* gene analysis, evidencing high viral diversity at the population level in the heterogenic genotype A (Fig. 1). Based on the CA sequence, Br1163 clustered near the K1514 strain and exhibited a pairwise genetic distance of 19.1% (K1514), which was higher than the 17.3% of all other MVV-like strains. Goat isolate Br195 (21G-MG-10) clustered with the V27 Brazilian strain and exhibited a divergence of 12.9 nucleotides (homology bootstrap 91%), which was above the 17.5% of all other MVV-like. These results suggest that both strains could belong to a new subtype within heterogeneous genotype A, taking into account the SRLV genotype classification (subtypes differing by 15% to 27%) (Shah et al., 2004a). However, further sequencing and analysis must be performed to validate the new SRLV subtype classification.

Analysis of the deduced aa from SU sequences exhibited the highest variation, in accordance with the variability described previously (Valas et al., 2000). Intersubtype divergence was particularly evident in the C-terminal domain in the V4 and V5 regions for both genotypes A and B. The V4 region from the Br1163 sequence exhibited several amino acid insertions that changed the motif (a turn) shown in most of the strains, formed by a proline residue, to a new one (loop), formed by the bond between two cysteine residues. According to Skraban et al. (1999), antigenic variants with cysteines mutated in the V4 region showed impaired growth in macrophages. This can be explained because V4 is a recognition site for coreceptors and a determinant of viral cell tropism (Gendelman et al., 1986). Furthermore, amino acid mutations in this region of the *env* protein allow the virus to escape neutralization (Kinsey et al., 1996). Therefore, the insertion was located in the previously identified neutralizing epitope (Hotzel et al., 2002; Skraban et al., 1999), which is a highly conformational and well-exposed immunogenic domain that could be involved in the emergence of neutralization escape variants (Valas et al., 2000), suggesting immunological selection pressures (Gjerset et al., 2007). Additionally, the analyses of the V4 region from Brazilian isolate Br195 (21G-MG/10) suggested that a possible dual infection with genotypes A and B occurred in flock 1, as reported previously (Fras et al., 2013; Olech et al., 2012; Olech et al., 2018; Pisoni et al., 2007).

In this work, we demonstrated the first evidence of natural horizontal infection of SRLV subtype B1 in Brazilian adult goats and sheep, showing that direct contact between these animals represents an important risk factor in viral transmission (Pisoni et al., 2005; Santry et al., 2013; Shah et al., 2004b). Viral transmission does not result from vertical transmission because although sheep and goat flocks grazed together in herd 3, kid and lamb feeding occurred with their respective dams in separate places. The immune response was detected against recombinant antigens carrying SU1 and SU5 immunodominant epitopes, showing their suitability for detecting SRLV-specific antibodies in Brazilian flocks (Olech et al., 2012). Using multiepitope recombinant antigens (SU1/SU5), goats and sheep were both classified as SRLV subtype B1 (Table 1) (Olech et al., 2012). These results are similar to the *gag* and *env* phylogenetic analyses found for sheep. However, cross-reactive antibodies were detected in goat Br1163, which was positive in both r-ELISAs detecting the B and A SRLV genotypes.

The high variability of the SU5 region for the type-specific immune response, as observed in this sequence, can be associated with the high divergence in the V4 domain (Andrésdóttir, 2003; Valas et al., 2000), a variable and conformational neutralizing epitope (Hotzel et al., 2002; Skraban et al., 1999). The results strongly indicate that mutations in this region are important to SRLV survival. These findings could interfere with SRLV serologic diagnostics and justify new control

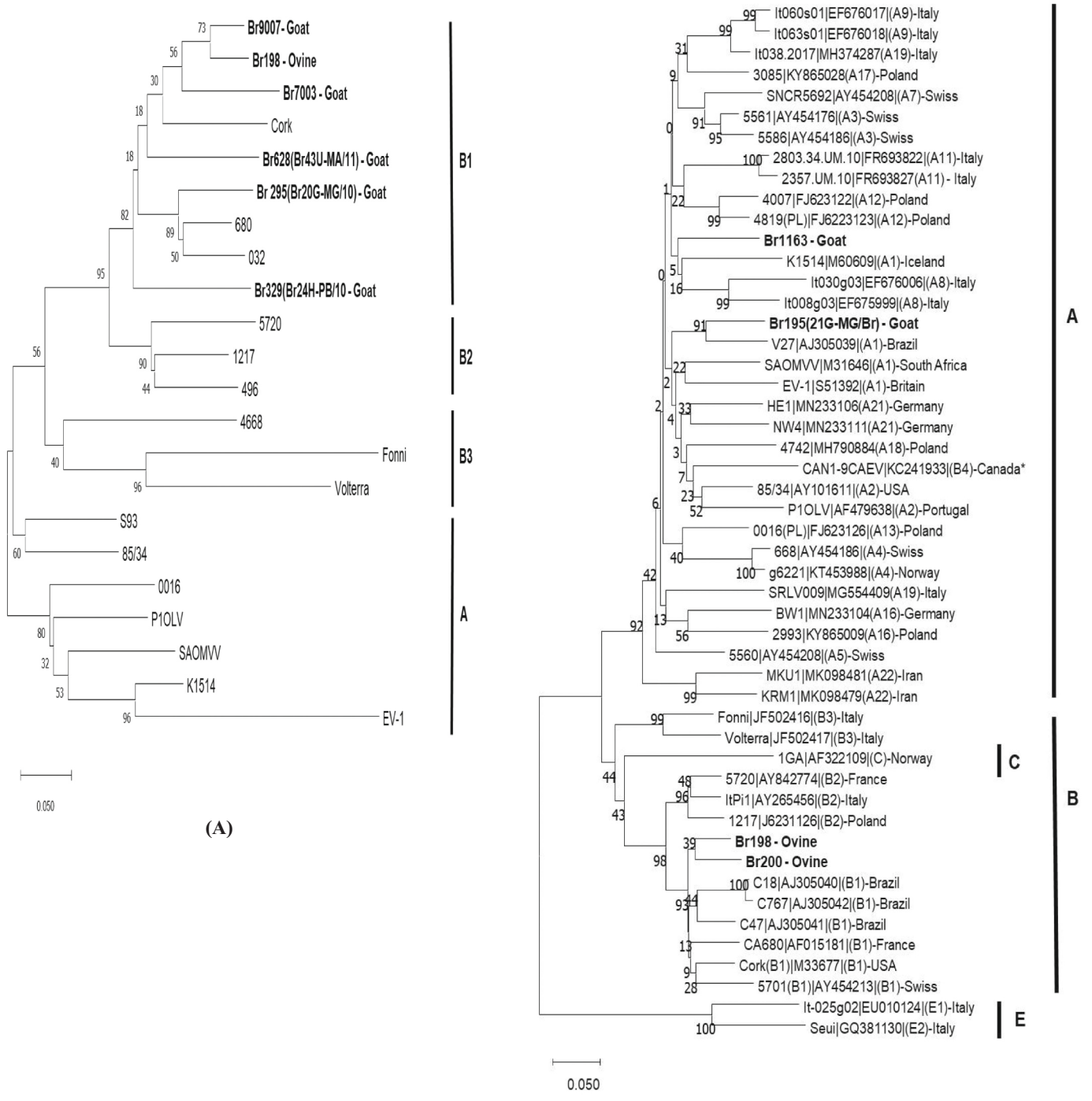

Fig. 1. Phylogenetic trees inferred from *env* and *gag* gene regions. Trees were produced by neighbor-joining using a 394 nt SU fragment (panel A) and a 467 nt CA fragment (panel B). The Brazilian strains described in this study are shown in bold (Br9007, Br7003, Br628/43U-MA/Br, Br295/20G-MG/Br, Br329/24H-PB/Br, Br195/21G-MG/Br, Ovine Br198, Ovine Br200, Br1163); the flock origins are in Table 1. Circulation of genotype A in goats (Br195/21G-MG/10) and Br1163) and B1 subtype in sheep (Br198 and Br200) was evident in Brazilian mixed flocks. GenBank accession numbers of the previously published SRLV sequences: Cork (M33677), 496 (FJ195346), SAOMVV (M31646), K1514 (M60609), EV-1 (S51392), P1OLV (AF479638), 4668 (AY445885), Fonni (JF502416), Volterra (JF502417), Seui (GQ381130), 1GA (AF322109), and g6221(KT453988) for complete genomes; 1217(FJ623113), 0016 (FJ623111), 680 (AJ400718), 032 (AJ400720), S93 (AF338226), 85/34 (U64439), and 5720 (AY842774) for *env* sequences; 85/34 (AY101611), 5720 (AY454218), 1217 (FJ623121), CAN 1-9CAEV (KC241933), 5560 (AY454175), 5561 (AY454176), SNCR5692 (AY454208), 5586 (AY454186), 4668 (AY445885), g6221 (KT453988), ItPi1 (AY265456), It008g03 (EF675999), It030g03 (EF676006), It060s01 (EF676017), It063s01 (EF676018), 2803.34.UM.10 (FR693822), 2357.UM.10 (FR693827), 0016 (FJ623120), 4007 (FJ623122), 4819(FJ623123), It-025g02 (EU010124), BW1|MN233104(A16)-Germany,2993 (KY865009), 3085 (KY865028), 009 (MH790878), It038.201 (|MH374287), SRLV009 (MG554409), HE (MN23310), NW4 (MN233111), MKU1(MK098481), KRM1(MK098479) and Brazilian strains (Br/UFRGS-4/C18 (AJ305040), Br/UFRGS-5/C47 (AJ305041), Br/UFRGS-2/C767 (AJ305042) e Br/UFRGS-2/V27 (AJ305039) for *gag* sequences. Brazilian sequences published in this paper: KF861573 (Br195/21G-MG/Br), KF861572 (Ovine Br198), KF861555 (Ovine Br200), and KF861574 (Br1163) for *gag* sequences MZ367335 (Br9007), MZ367338 (ovine Br198), MZ367339 (Br7003), MZ3673/31 (Br62843 U-MA/Br), MZ367322 (Br295/20G-MG/Br), and MZ367323 (Br329/24H-PB/Br) for *env* sequences (V1V2).

Table 2
Evolutionary Divergence (%) between CA (*gag*) nucleotide sequences of Brazilian viruses among SRLV subtypes*.

	Br195(21G – MG/Br)	BR1163	Br198(ov)	Br200(ov)	B1(Brazil)	B1(Cork)	B2	B3	A1(Brazil)	A1	A2	A3	A4	A5	A7	A8	A9	A11	A12	A13	A16	A17	A18	A19	A21	A22	C	E1	E2
Br195(21G-MG/Br)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Br1163	**18.4**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Br198(ov)	**32.2**	**33.7**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Br200 (ov)	**34.5**	**34.5**	**8.7**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B1 (Brazil)	34.2	34.3	**11.2**	**11.3**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B1 (Cork)	**35**	**33.9**	**8.9**	**8.9**	**10.1**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B2	31.7	31.4	11.8	12.2	14.7	11.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B3	27.7	24.8	23.7	24.6	25.2	25.4	20.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A1 (Brazil)	**12.9**	**20.3**	**29.8**	**33**	34.1	31.7	30.5	33.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A1 (K1514)	**23.7**	**19.1**	**33.1**	**35.8**	37.5	35.4	31.6	32.7	24.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A2	19.7	17.3	28.2	27.5	30.6	27.9	27.0	28.6	20.3	21.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A3	17.5	17.7	27	30.3	30.7	25.6	26.1	25.8	18.7	18,6	15.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A4	20.3	24.8	30.7	35.4	35.6	31.6	31.9	30.4	24.2	20.7	21.5	21.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A5	18.1	19.1	26.7	30	28.9	31.1	27.9	31.7	17.9	19.3	19.1	13.4	17.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A7	21.1	18.9	32.3	32.8	35.6	30.3	33.1	27.1	22.0	23.7	18.6	10.4	22.5	18.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A8	28.4	23.4	32.9	35.6	34.2	32.9	29.6	30.5	28.8	24.8	25.6	22.2	22	26	22.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A9	23.8	19.9	29.5	35.2	29.7	30.3	29.4	26.6	21.7	20.1	19.2	15.3	19.2	18.1	19.4	18.7	–	–	–	–	–	–	–	–	–	–	–	–	–
A11	24.7	20.2	31.3	31.7	29.6	29.6	29.6	27.4	21.5	21.8	20.2	18.5	26	21.2	21	23.1	18.9	–	–	–	–	–	–	–	–	–	–	–	–
A12	22.2	20.7	34.8	31.3	33.6	30.6	30.6	28.8	22.2	20,6	16	16	20.4	19.9	15.6	21.3	19.1	16.7	–	–	–	–	–	–	–	–	–	–	–
A13	19.5	19.2	30.5	34.4	32.7	32.0	30	29.6	21.1	21.7	16	15.8	16.4	19.4	18.8	21.9	21.5	20.2	17.8	–	–	–	–	–	–	–	–	–	–
A16	19.9	21.5	32.5	28.4	35.7	33.6	30.5	27.5	24.7	25.3	21.7	21.4	21.6	21.3	23.3	28	29.7	24.1	21.7	20.5	–	–	–	–	–	–	–	–	–
A17	19.6	20.1	29.2	30.4	32.1	30.1	30.6	30.7	20.3	17.6	15.8	14	18.8	14.6	16.2	23.7	15	23.3	16.8	19.8	19.5	–	–	–	–	–	–	–	–
A18	21.3	19.7	32.9	34	36	33.4	32.8	30.3	20.1	24.9	18.4	19.3	24.6	23.6	21	26.5	23.5	25.7	21.2	16.6	23	19.2	–	–	–	–	–	–	–
A19	21.3	18.1	26.8	32.1	28.3	28.0	26.7	23.6	20.9	20.4	17.3	13	17.1	14.5	17.9	18.6	5.6	18.7	18.2	18.7	24.7	13.3	20.4	–	–	–	–	–	–
A21	20.5	18.6	30.6	31.8	35.1	31.7	31.7	30.5	21.3	21.1	18.8	17.1	23.4	17.3	20.8	23.7	21	23	20.1	21.6	21	16.8	17.7	18.7	–	–	–	–	–
A22	28.7	24.6	32	37.8	31.2	35.9	32.1	30.4	23.6	29.2	28.2	23.1	24.4	20	28	29.4	25.2	25.5	24.6	24.5	27.9	23.9	24.5	24	28.3	–	–	–	–
C	30.6	35.8	27.7	26.6	27.3	29.6	26.3	25.6	36.2	46.3	38.3	34	34.3	31.2	33.9	38.4	44.3	36	36	35.6	29.9	36.1	37.6	36.4	35.3	36.5	–	–	–
E1	49.2	56.1	46.7	50.3	45.3	44.3	47.1	53.1	50.6	55.5	52.1	54.4	57.1	48.2	49.6	57.1	53.7	44	50.6	51.4	46.7	52.3	50,6	49.3	55.4	47.6	47.4	–	–
E2	49.5	53.1	44.8	45.7	47.9	47.9	47.4	47.3	47	56.9	55.9	56.3	48.8	46.1	50.3	52.9	47.7	54	55.9	50	48.6	45.9	51.7	47.4	54	49.7	50.8	19.2	–

In bold, nucleotide pairwise distance of Br195(21G-MG/10), Br1163, Br198 and Br200 Brazilian viruses from this paper among Cork and K1514 reference sequences, and Brazilian SRLV.

* Analyses were conducted using the Kimura 2-parameter model. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). ov = ovine.

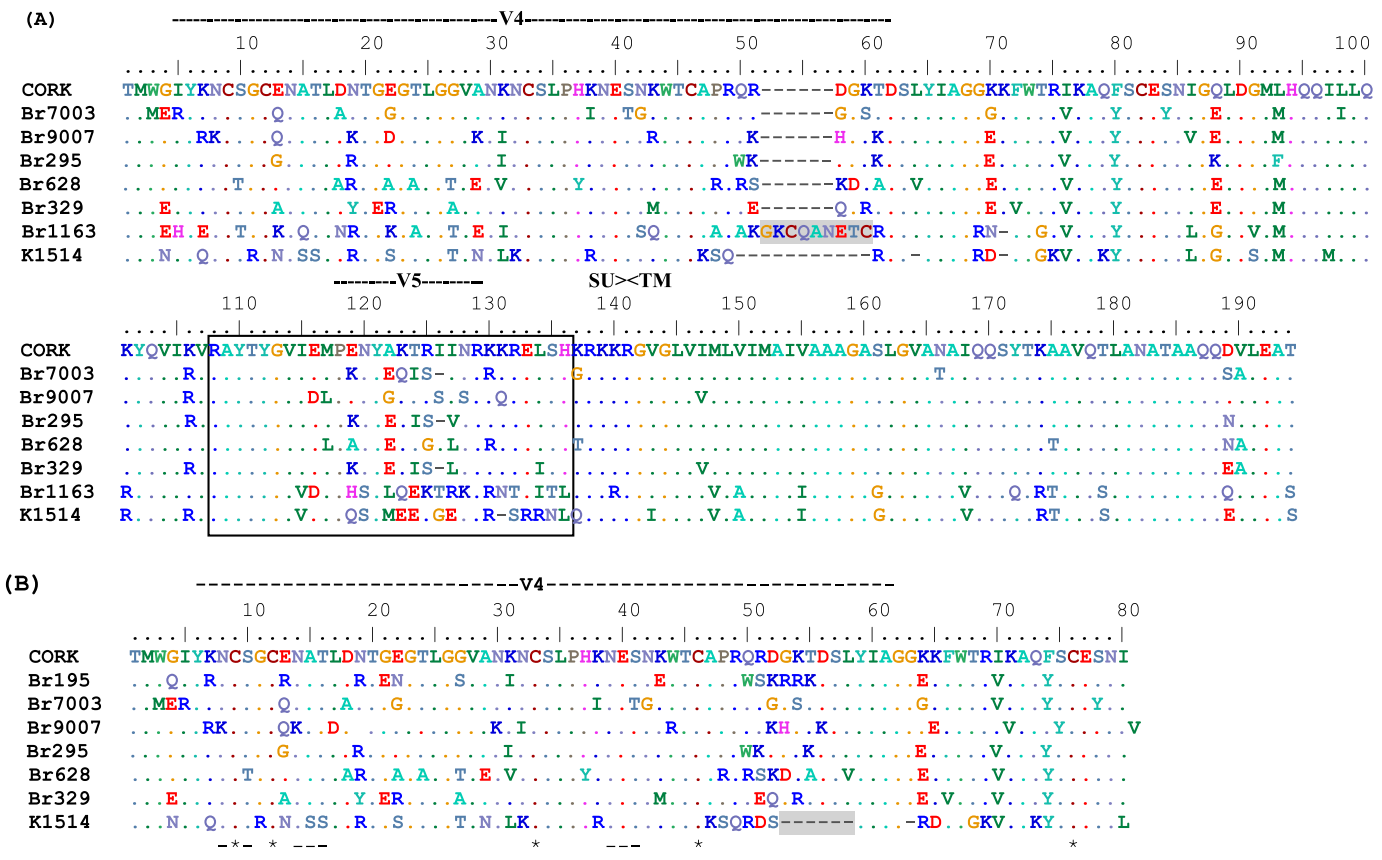

Fig. 2. Alignment of the deduced amino acid sequences of SRLV SU regions V4V5 (A) and V4 (B). The Brazilian SRLV amino acid sequences belonging to subtypes B1 (Br7003, Br9007, Br295 (20G-MG/Br), Br628 (43U-MA/Br), Br329 (24H-PB/Br), Br195 (21G-MG/Br) and A (Br1163) were aligned with genotype A (K1514) and B (Cork) reference strains. In the Br1163 sequence, there is an insertion (GKCQANETC) in the V4 region (position 52) formed by a duplication of the two cysteine residues (panel A). In the Br195 isolate, there is no aa deletion characteristic of MVV (gray) (panel B). Dots represent conserved residues, while dashes indicate gaps.

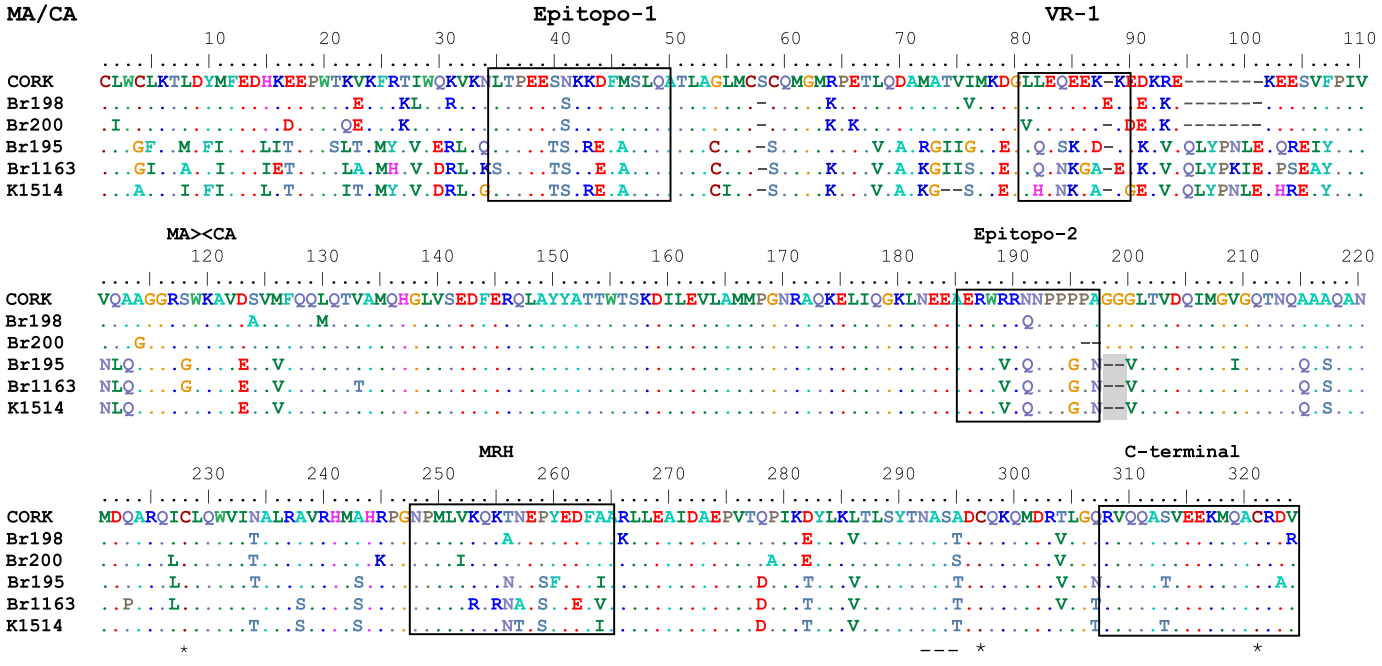

Fig. 3. Alignment of the deduced amino acid sequences of the SRLV MA/CA region. The Brazilian SRLV amino acid sequences belonging to subtypes B1 (Br198 and Br200) and A (Br1163 and Br195 (21G-MG/Br) were aligned with genotype A (K1514) and B (Cork) reference strains. The Br198 and Br200 ovine sequences showed a seven aa deletion in the C-terminal of MA. In Br1163 and Br195 (21G-MG/Br) goat sequences, there are aa deletions (GG) in the middle CA capsid, characteristic of MVV. Dots represent conserved residues, while dashes indicate gaps.

implementations of Brazilian lentiviruses considering each small ruminant species.

In conclusion, SRLV with high diversity is widespread in Brazilian goats, sheep, and mixed flocks, with subtypes A1 and B1 in goats and subtype B1 in sheep. Dual infection of CAEV and MVV in goats and the first appearance of SRLV subtype B1 in Brazilian sheep were reported by genetic and antigenic characterization. The presence of the B1 subtype in goats and sheep revealed SRLV interspecies transmission in Brazil with a large impact on infection dissemination. The high variability intersubtype divergence and the provable emergence of new subtypes in heterogeneous group A could interfere with serologic diagnosis in Brazilian flocks and the control of lentiviruses.

CRediT authorship contribution statement

G.F. Braz: Writing – original draft, Resources, Investigation, Formal analysis. **M.B. Heinemann:** Methodology, Writing – original draft. **J.K. P. Reis:** Visualization. **B.M. Teixeira:** Writing – original draft, Visualization. **J.C.M. Cruz:** Resources. **D.S. Rajão:** Resources. **F.G. Oliveira:** Resources. **R.S. Castro:** Resources, Conceptualization. **R.C. Leite:** Project administration, Funding acquisition. **S. Valas:** Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

None of the authors has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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