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Small ruminant lentivirus variants and related clinical features in goats from southeastern Brazil

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ABSTRACT

Caprine arthritis encephalitis (CAE) is a multisystem infectious disease caused by a small ruminant lentivirus that is widely disseminated in the goat flock of southeastern Brazil. Little information about its variants is available in Brazil. Phylogenetic analysis was performed on the sequences of 14 clinical isolates of CAEV that obtained from mammary glands, lung, brain, milk and blood of goats with any clinical form of the disease in Sao Paulo State, Brazil. Part of the *pol* gene of CAEV was amplified by nested-PCR and sequenced. These sequences were compared with 42 reference strains previously described and commonly used in phylogenies. The sequences derived from this study were more related to caprine strains than to ovine strains. Additionally, subtype B of the CAEV group was detected in different clinical samples with distinct clinical symptoms. Subgroup B1 was the predominant genotype. A subgroup C-like was isolated from animals that presented interstitial indurative mastitis. Animals from which the sequences belonged to this subgroup presented interstitial mastitis induration, chronic arthritis and chronic interstitial pneumonia. Subgroup C sequences have not been reported in Brazil previously, while circulating subgroup B1 variants were already known to be present in the country. This study provides new information about CAEV variants in Brazil, since we isolated and reported the subgroup C-like. A variant from B1 subgroup was the predominant genotype. More studies are necessary in order to confirm the presence of CAEV genotype C or even a new genotype. CAEV is an important disease of goat production and all knowledge is necessary to understand its epidemiology and, prevent this illness.

1. Introduction

Small ruminant lentiviruses (SRLV) are distributed worldwide among sheep and goats. Different clinical forms of the disease have been described in Brazil among SRLV-infected small ruminants. CAEV and VISNA are considered to be genetically distinct, but antigenically related pathogens of goats and sheep. Caprine arthritis encephalitis (CAE) is characterized as an infectious viral disease caused by the Caprine arthritis encephalitis virus (CAEV), family Retroviridae, order Orthoretrovirinae, genus Lentivirus. The virus affects goats at all ages, no matter the gender, breed or production type. The infection is considered to be a life-long state, as the lentivirus persists in the organism throughout the animal's life, being generally persistent and asymptomatic. It may cause multisystem diseases that are generally chronic in their development, with a progressive worsening of the lesions, weight loss, weakness and eventual death (Callado et al., 2001). CAEV can induce four clinical forms of the disease: articular (chronic arthritis), mammary

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(indurative mastitis in adult goats), pulmonary (acute progressive pneumonia) and nervous (leukoencephalomyelitis).

A recent nomenclature system was proposed based on the 1.8 kb *gag–pol* and 1.2 kb *pol* gene sequences evaluated on 104 isolates from Switzerland and six sequences that were available in the Genbank database. SRLV are classified into four equidistantly related groups, named A–D, with subdivisions within each group. Group A can be further divided into at least seven subtypes, A1–A7, where the subtype A1 is identified by the genetically and geographically heterogeneous VMV and group B refers to the CAEV type and comprises two distinct subtypes, B1 and B2. The entire genome of groups C had already been sequence in Norway (Gjerset et al., 2006). The subgroup B1 contains isolates from France, Brazil, USA and Switzerland (Shah et al., 2004).

In Brazil, Castro et al. (1999) sequenced the first caprine isolates in the States of Minas Gerais (MG) and Pernambuco (PE), Brazil. One of sequences had more similarity to Maedi Visna virus than CAEV, suggesting a possible interspecies transmission. Ravazzolo et al. (2001) analyzed the phylogeny of the first isolated lentivirus from sheep in the State of Rio Grande do Sul (RS), Brazil and suggested the presence of a deletion in the *gag* gene in the Maedi Visna virus as a possible marker to differentiate the lentivirus transmission in sheep and goats. Feitosa et al. (2010) compared four *gag* gene sequences of small ruminant lentivirus from goats in Northern Brazil and reported that they belonged to subtype B1.

Based on the informations above, the objective of this study was to identify the CAEV variants isolated from goats reared in the State of São Paulo, Brazil, and link these with clinical features.

2. Material and methods

2.1. Animals and samples

Nine naturally CAE- infected adult caprine reared in the State of São Paulo, Southeastern region of Brazil, were used for sample collection. The animals were submitted to a clinical examination according to Dirksen et al. (1993). Animals with arthritis were submitted to arthroscopy according to the technique described by McIlwraith et al. (2005) modified for caprine. All data was compiled in a clinical report.

Milk, total blood, synovial fluid, and organ samples (mammary gland, lung, and brain) were screened for CAEV detection by nested-PCR. Organs samples were collected whenever seropositive animals died. Serum antibody detection against CAEV was performed using Agar Gel Immunodifusion Test (AGID), according to Crawford and Adams (1981), using the CAE diagnosis kit (Biovetech, Brazil). Milk samples were collected in sterile tubes during the milking. All samples were processed in the Clinical and Molecular Virology Laboratory at the Biomedical Sciences Institute, University of São Paulo (USP).

2.2. DNA extraction

Two grams of brain, mammary gland, and lung samples were weighed, macerated separately, and suspended in PBS. The suspensions were kept at −70 °C until DNA extraction.

Milk, synovial fluid and macerated organ suspension (20 μL), 150 μL TNE (Tris HCl 10 mM, EDTA 10 mM and NaCl 10 mM; pH 8.0), 20 μL 10% SDS (sodium dodecyl sulfate), and 10 μL pK (Proteinase K) were incubated at 56 °C for 30 min. The phenol/chloroform/isoamyl alcohol extraction method was performed for milk, organs, and synovial fluid samples as described by Gregory et al. (2009). The extracted material (40 μL) was stored at −70 °C until amplification. DEPC water, and the standard CAEV Cork strain were used as negative and positive controls. Genetic material were extracted from total blood samples using the commercial GE Healthcare illustra™ blood genomicPrep Mini Spin Kit protocol (code 28-9042-64), according to manufacturer instructions.

Table 1
Oligonucleotide primers used in the PCR and nested-PCR for amplification of part of the *pol* gene.

Name	Oligonucleotide Sequence (5′ → 3′)	Position
P1[1]	DSAAGARAAATTARARGG	2198-2215
P2[1]	ATCATCCATRTATATBCCAAATTG	2672–2650
PN1[2]	GGAAADGCACCYCCACATTG	2299-2318
PN2[2]	CATGGTCCRAYATTATTAGG	2576–2556

a B = C, G or T; D = A, G or T; R = A or G; S = C or G.
[1] Leroux et al., 1995.
[2] Gregory et al., 2009.

2.3. Nested-PCR procedure

The DNA, storage buffer (20 mM Tris-HCl/pH 8.0, 0.1 mM EDTA, 1 mM DTT, stabilizers, 50% (v/v) glycerol), 10X PCR buffer (200 mM Tris-HCl/pH 8.4, 500 mM KCl; 50 mM $MgCl_2$); 10 μM of each primer (Gregory et al., 2009); 10 mM dNTP mixture and 1U Platinum®*Taq* DNA polymerase (Invitrogen Cat. No.10966-020) were used in a final volume of 50 μL. Both oligonucleotides PN1 and PN2 were designed for a conserved region of the *pol* gene (Leroux et al., 1995) using degenerate initiators situated between positions 2299 and 2576 (Gregory et al., 2009). The resulted PCR products were detected by electrophoresis through 1.2% agarose gel in the presence of TBE buffer (0.09 M Tris Borate and 0.002 M EDTA; pH 8.0), and ethidium bromide (1 μg/mL). We expected a fragment of 238 bp (Table 1)

2.4. DNA sequencing and sequence analysis

For the phylogenetic analysis, 14 partial CAEV pol sequences from clinical samples were used. We used a conserved region of *pol* gene (Leroux et al., 1995) that was sequenced using the *Dye Terminator Cycle Sequencing ABI PRISM® 3100 Genetic Analyzer* Kit (Applied Biosystems, USA). After the sequencing, samples were precipitated using 100% and 75% isopropanol (Merck) and resuspended in *HI DI* formamide (Applied Biosystems). The samples were denatured and placed in an ABI PRISM 3100 sequencer (Applied Biosystems), following the manufacturer's instructions.

Sequences were edited and aligned with BioEdit Sequence Alignment Editor software. Nucleotide sequences were analyzed with the Sequence Navigator version 1.0 program (Applied Biosystems) for Power Macintosh, aligned using the MegAlign v. 4.05-*DNAstar* program, and their variations were observed with the MegAlign v. 1.02: Molecular Evolutionary Genetics Analysis program. The genetic variability found in the virus was investigated in order to evaluate the association with the presented clinical signs. The 42 GenBank accession numbers used were VVU35679.1, VVU35680.1 and VVU35676.1, (Leroux et al., 1995); L78448.1 (Storset and Rimstad, 1996); AY900630.1 (Qo et al., 2005) M33677.1 (Saltarelli et al., 1990); M31646.1-SA-OMVV (Querat et al., 1990); AJ305050.1, AJ305051, AJ305052 and AJ305053 (Ravazzolo et al., 2001); AF108048.1, AF108049.1, AF108051.1, AF108053.1, AF108056.1 and AF108059.1 (Castro et al., 1999); AY454178.1, AY454197.1, AY454218.1, AY454175.1, AY454190.1, AY454210.1, AY454161.1, AY454231.1, AY454189.1 and AY454208.1 (Shah et al., 2004); FJ195346.1 (Glaria et al., 2009); DQ013218.1, DQ013215.1, DQ013231.1, DQ013225.1, DQ013236.1 and DQ013242.1 (Pisoni et al., 2005); AY101611.1 (Crawford et al., 1980); K03327.1 (Chiu et al., 1985); AF015182.1 (Valas et al., 1997); AF322109.1 (Gjerset et al., 2006); M10608.1 (Sonigo et al., 1985); S51392.1 (Sargan et al., 1991) and AM419950.2 (Laamanen et al.,