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Profile of the Caprine arthritis-encephalitis virus (CAEV) in blood, semen from bucks naturally and experimentally infected in the semi-arid region of Brazil

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ABSTRACT

The aim of this study was to report the chronology of Caprine arthritis-encephalitis virus elimination and compare the blood and semen viral profiles of animals naturally and experimentally infected by SRLV raised in the semi-arid region of Brazil. The experiment was carried out at the Brazilian Center for Goat Research (Embrapa). Nine bucks were selected, four naturally infected by CAEV and five animals proven negative that were inoculated with the goat lentivirus (CAEV-Cork strain). Every week the animals were submitted to semen collection using an artificial vagina. The blood was collected by puncturing the jugular vein with tubes containing EDTA, 7 days after inoculation (experimentally infected group) or at the start of the experiment (naturally infected group) and then at every 30 days. The genomic viral DNA was extracted from semen and blood and then Nested-PCR was applied. An agar gel microimmunodiffusion was performed to detect anti-CAEV antibodies. The results were described in percentage and analyzed by the Chi square test ($P < 0.05$). The presence of anti-CAEV antibodies was detected in the 16th week after inoculation that characterized the seroconversion from four of the five naturally infected goat bucks (80%). The fifth reproducer presented late seroconversion, totaling 32 weeks post-inoculation. A quantity was observed in the total of samples collected of 12.50 and 17.14% positive results in the blood and 10.98 and 11.25% positive results in the semen of the naturally and experimentally infected animals, respectively, and there was no statistical difference. No statistically significant differences were observed regarding the presence of proviral DNA in the blood and semen of the naturally and experimentally infected animals. A viral elimination pattern was not identified during the assessment period, but the presence of proviral DNA was shown at shorter intervals after the 18th week and the 22nd week, for the experimentally

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and naturally infected bucks, respectively. Therefore, recently infected goats in the period prior to seroconversion eliminated small ruminant lentivirus proviral DNA in the semen and are important sources of infection that should be considered in a control program of this lentivirus, and the Nested-PCR technique is a relevant tool to select virus-free ejaculates.

1. Introduction

Caprine arthritis-encephalitis (CAE) causes economic losses in the affected herds. It is believed that mastitis may reduce milk production by 10%. These losses indirectly affect offspring weight gain because of the decrease in milk production. In addition to the economic consequences, CAE also interferes in the well-being and quality of life of the animals affected by the disease (Peterhans et al., 2004).

Contaminated colostrum and milk ingestion is considered to be the main source of infection for kids so that the virus is disseminated down the generations (Pisoni et al., 2007) and the infected animals become permanent carriers of the virus (Franke, 1998).

Sexual transmission is the main transmission path for HIV (human immunodeficiency virus) that is present in the genital secretions in the form of free viral particles (viral RNA) in the seminal fluid and in the form of proviral DNA integrated in leukocytes and macrophages present in the semen. However, the virus does not penetrate the spermatozoa because these cells do not have the CD4 receptor and the CXCR4 and CCR5 co-receptors. HIV RNA is present in the seminal fluid of 90% untreated men and the proviral DNA is present in the semen of 60–80% untreated men (Leruez-Ville et al., 2006).

Theorically, small ruminant lentivirus (SRLV) may be present in the sex organs and semen of infected animals in three forms: incorporated in the cells (proviral DNA), as virions (complete virus particles released by budding from plasma membrane) and as free virus released by cell lysis (Peterson et al., 2008).

Andrioli et al. (1999) used the Nested polymerase chain reaction (Nested-PCR) and detected the presence of CAE proviral DNA in semen of naturally infected goats that did not present in any other pathology. Ali Al Ahmad et al. (2008) observed similar results to those of Andrioli et al. (1999) in naturally infected animals and further verified the presence of DNA in the testis, epididymis, vas deferens and vesicular glands.

Animals positive for SRLV in the PCR blood test presented positive result in one or more tissues and fluids of the reproductive tract. Specifically in goats, SRLV DNA was present only in the testis, vesicular glands, ejaculated semen and epididymis (Peterson et al., 2008).

PCR has been shown to be highly specific for CAEV proviral DNA detection in infected animals because crossed reaction has not been identified with other ruminant retroviruses such as Maedi Visna Virus (MVV), bovine immunodeficiency virus or bovine leukemia virus. This specificity is important because goats may be infected with other retroviruses (Clavijo and Thorsen, 1996).

The objective of this study was to report the chronology of CAEV elimination and compare the blood and semen viral profiles of naturally and experimentally infected by SRLV animals raised in the semi-arid region of Brazil.

2. Materials and methods

2.1. Animals

The experiment was carried out at the Brazilian Center for Goat Research (Embrapa/CNPC) located in the municipality of Sobral, northern region of the state Ceará (latitude 3°45′0.5″ South, longitude 40°20′45.8″ West, 111 m altitude). Nine bucks were selected, four naturally infected with SRLV after proving positive in at least two agar gel microimmunodiffusion (MAGID) tests at 60 day intervals and five animals proven negative that were inoculated intravenously with 10^6 $TCID_{50}$/mL caprine lentivirus (CAEV-Cork strain). These animals belong to the experimental herd at Embrapa Goats.

The naturally infected males belonged to the Saanen ($n = 1$) and Anglo-Nubian ($n = 3$) breeds and were aged 3–4 years. The experimentally infected group consisted of the breeds Canindé ($n = 2$) and Moxotó ($n = 3$) average age 2 years. The experimental groups were housed separately in stalls and kept in a semi-intensive grazing system, in an area isolated by double fencing, receiving balanced concetrate, elephant grass, mineral salt and water *ad libitum*.

2.2. Semen collection viral DNA extraction

Semen was collected from the animals every week using a short model artificial vagina, for a period of 6 consecutive months. The model was an ovariectomized female, induced to oestrus by applying 1.0 mg estradiol benzoate (Estrogin® – Farmavet). The ejaculates collected for the Nested-PCR test totaled 162 samples.

The semen samples were diluted in a solution based on skim milk to assess the sperm quality by the heat resistance test, and were later placed in criotubes and kept at −80 °C until DNA extraction.

To extract the genomic viral DNA, the semen samples were filtered individually in a Sephacryl S-400 column, as reported by Santurde et al. (1996) and then incubated at 56 °C for 60 min in a solution containing 200 μL Chelex 100 at 5%, 2 μL proteinase K (10 mg/mL) and 7 μL dithiothreitol 1 M. After centrifuging for 10 s at 13,000 × *g*, the tubes were placed in a boiling water bath for 8 min and then centrifuged for 3 min at 13,000 × *g* (Walsh et al., 1991). The material was stored at 5 °C until use in the Nested-PCR test with 3 μL used as template.

2.3. Blood collection and viral DNA extraction

Blood was collected by puncturing the jugular vein with tubes containing EDTA, 7 days after inoculation (experimentally infected group) or at the start of the experiment (naturally infected group) and then at every 30 days, in a total of seven collections from each experimentally infected animal and a monthly collection from those animals naturally infected that responded to semen collection, in a total of 52 samples. The blood collected was stored at −20 °C until DNA extraction.

To extract the viral DNA, the blood that was first thawed at room temperature (23 °C), centrifuged at 4000 × *g* for 6 min and 90% of the blood was discarded after centrifuging. Then 500 μL Tris–EDTA (TE) solution was added to the precipitate and centrifuged for 2–3 min at 10,000–15,000 × *g*, the supernatant was discarded and re-suspended in 500 μL TE solution and centrifuged again. Then 500 μL buffer K (10 mM Tris–HCl pH 8.0; 50 mM KCl; 2 mM $MgCl_2$; 0.5% Tween 20; 100 μg/mL proteinase K; ultrapure water without DNAse qsp 100 mL) was added to the pellet and incubated at 56 °C for 45 min followed by incubation at 95 °C for 10 min to inactivate the proteinase. The solution resulting from the processing was stored at 5 °C and 5 μL of this solution was used as template.