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Small Ruminant Research 32 (1999) 101–106

Small Ruminant Research

Caprine arthritis-encephalitis virus in semen of naturally infected bucks

C.E. Travassos[a,*], C. Benoît[a], S. Valas[b], A.G. da Silva[c], G. Perrin[a]

[a]*CNEVA Station Régionale de Pathologie Caprine- 60 rue de pied de fond 79012, NIORT, France BP: 3081*
[b]*Laboratoire de Retrovirus et Therapie; Université Bordeaux II, Bordeaux, France*
[c]*Instituto de Microbiologia Prof. Paulo de Goes, CCS – bloco I – Universidade Federal do Rio de Janeiro – Ilha do Fundão, Rio de Janeiro, Brazil*

Accepted 1 September 1998

Abstract

Semen and peripheral blood mononuclear cells (PBMC) samples of 15 infected and 3 non-infected bucks were evaluated for the presence of caprine arthritis–encephalitis virus (CAEV), by double-nested polymerase chain reaction (d-n-PCR). In order to locat the presence of vírus, semen samples were separated into cell-free seminal fluids (CFSF), enriched non-spermatozoal cells fraction (NSCF), and spermatozoa (SPZ). All PBMC samples from infected bucks were positive for CAEV-DNA but only 8 out of 15 semen samples were positive for CAEV-DNA. The presence of virus was not necessarily concordant in different fractions of semen from the same buck over time. In addition, the virus was isolated from CFSF in primary goat synovial membrane (GSM) cell culture, in which productive infection was demonstrated by syncytia formation. This is the first report describing the presence of CAEV-DNA and also of replication-competent virus in semen from CAEV seropositive bucks. Such results suggest the possibility of sexual transmission of CAEV.

Keywords: Caprine arthritis–encephalitis virus; Polymerase chain reaction; Semen; Transmission

1. Introduction

Caprine arthritis–encephalitis virus (CAEV), a lentivirus of goats, is the cause of leucoencephalomyelitis and progressive polyarthritis, pneumonia and mastitis in young and adult goats, respectively, (Cork et al., 1974; Crawford et al., 1980; Narayan et al., 1980). Worldwide in distribution, but located mostly in intensive dairy systems, CAEV is responsible for considerable economic losses in affected herds, through premature culling of goats due to the progressive and often debilitating arthritis, and decreased milk production associated with indurative mastitis (Adams et al., 1983; Ellis et al., 1987; Lerondelle et al., 1989). Despite strenuous efforts, eradication of CAEV from infected herds is based on prevention of colostrum or milk-borne transmission remains a difficult undertaking and has demonstrated limited success owing in part to delayed seroconversion in some infected animals, and poorly defined mechanisms for transmission of the virus (Adams et al., 1983; East et al., 1987; Mackenzie et al., 1987; Rower et al., 1991; Rimstad et al., 1993; East et al., 1993). The major route of

*Corresponding author. Tel.: +55-21-941-590; fax: +55-21-560-8344; e-mail: imadags@microbio.ufrj.br

0921-4488/99/$ – see front matter
PII: S0921-4488(98)00172-2

CAEV transmission is from an infected doe to kids through ingestion of contaminated colostrum or milk (Adams et al., 1983), but other routes could be important. Studies have documented that CAEV may be transmitted by other routes, such as maternal-fetal transmission and, intimate goat to goat contact with infectious secretions (Adams et al., 1983; East et al., 1987; Zink et al., 1990; East et al., 1993). However, as opposed to the other retroviruses (Krieger et al., 1991; Mermin et al., 1991; Nash et al., 1995; Jordan et al., 1995), the sexual transmission of CAEV is not well defined. The presence of virus in the semen gives new perspectives as to preventive measures.

The objective of this study was to determine if CAEV could be detected by PCR in seminal components of semen from seropositive bucks.

2. Materials and methods

2.1. Sample collections and processing

2.1.1. Semen samples

Fresh semen samples (1–2 ml) were obtained from 18 bucks (ages at 2–4 years), including samples from three breeds (nine Saanen, eight Alpine, and one Boer), 15 were naturally CAEV-infected and seropositive (Nos. 1–15) and three CAEV non-infected and seronegative (Nos. 16–18). The latter three were used as controls. Semen samples were collected with an artificial vagina and processed within 4 h of collection. The semen samples were separated into three distinct fractions: cell-free seminal fluid (CFSF), enriched non-spermatozoa cell fraction (NSCF), and spermatozoa (SPZ).

After brief centrifugation at 800 *g* for 10 min, the supernatant (CFSF) was collected, clarified at 5000 *g* for 10 min, and then concentrated by centrifugation at 20 000 *g* for 2 h on a sucrose gradient at 20%. The supernatant was removed, and the pellet ressuspended in 4 ml of the culture medium (HMEM with 8% DMSO). Virus isolation was performed on primary goat synovial membrane cell (GSM) in culture media (HMEM with 4% of fetal calf serum). When syncytia were evident, or at the end of 8 weeks, infected and uninfected GSM cells were harvested, washed twice with PBS, pH 7.2, pelleted at 700 *g* for 10 min and freeze-dried at −80°C.

The remaining pellet containing NSCF and SPZ was re-suspended in 5 ml of PBS, pH 7.2. After dilution, a NSCF was obtained by centrifugation on a 5 ml Percol gradient 1077 at 400 *g* for 30 min. The interface was collected, the cells washed twice with PBS, pH 7.2 and freeze-dried at −80°C. The pellet containing the SPZ fraction, was collected, washed twice by centrifugation in PBS, pH 7.2 and processed using a differential lysis technique previously described to separate SPZ from residual contamination cells (Von Beroldingen et al., 1990).

2.1.2. Blood samples

Blood samples (10 ml in EDTA) were obtained by jugular venipuncture from all bucks. Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation on a Ficol gradient (Histopaque-Sigma 1077) at 400 *g* for 30 min, washed twice with PBS, pH 7.2 and freeze-dried at −80°C.

2.1.3. Antibodies detection

Serum antibodies were analyzed by ELISA (Chekit CAEV/MVV® Dr. BOMMELI AG, Bern, Switzerland; Behring Diagnostic) according to the manufacturers protocol.

2.2. DNA extraction and proviral DNA amplification

DNA extraction was performed using the nucleic acid extraction kit «Isoquick» (Microprobe) according to the manufacturer's protocol. Briefly, cells were lysed using guanidine thiocyanate and a nuclease-binding matrix was added. After centrifugation the extracted DNA was precipitated by ethanol, dissolved in TE 0.1X (Tris–HCl, pH 8, 1 mM; EDTA 0.1 mM) and quantified spectrophotometrically. DNA fragments of 175 bp and 350 bp corresponding to the *pol* and *env* regions, respectively, were co-amplified by nested-PCR.

The outer primers used were 5374 (5′-ACAGAAGAGAAATTAAAAGG-3′, bp 2236–2255) and 5376 (5′-ATCATCCATATATATGCCAAATTG-3′, bp 2712–2689) for *pol* region, and 99001 (5′-AGGTAAGTATAAACCCCAGGTAAG-3′, bp 6122–6145) and 5086 (5′-ATTCTTATCTTTTATTCCCCCATA-3′, bp 6962–6939) for *env* region. The inner primers were 5375 (5′-CAGGGAAGTGGAGAATGTTAAT-3′, bp 2357–2378) and 5377 (5′-

GGTATAGTAAAATATGCATCTCCT-3′, bp 2510–2487) for *pol* region, and 99006 (5′-CATTGGCCTT-TATGGCAAATGG-3′, bp 6471–6492) and 99008 (5′-GATGTTCATAAAGGCCATGCTCTC-3′, bp 6812–6789) for *env* region. Positions of the PCR primers used to amplify CAEV subgenomic fragments are numbered according to the published sequence of CAEV-Cork (Saltarelli et al., 1990). PCR reactions were performed in a GeneAmp PCR system 9600 thermocycler (Perkin–Elmer). One μg of genomic DNA was added to a first round reaction of 50 μl containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin, with 200 μM (each) deoxynucleoside triphosphate, 100 ng (each) outer primer, and 1U of Taq DNA polymerase (AmpliTaq, Perkin–Elmer). A 5 μl aliquot was again amplified in a second round in the same reaction mixture containing 100 ng (each) inner primer. After a denaturation at 95°C for 5 min, samples were submitted to 35 cycles of denaturation at 92°C for 40 s, annealing at 56°C for 50 s, and extension at 72°C for 1 min during the first round. The annealing and extension conditions were 60°C for 40 s and 72°C for 50 s, respectively, during the second round. Each round was followed by a final extension at 72°C for 4 min. A 10 μl aliquot from the final inner nested amplification was resolved by electrophoresis on a 3.0% agarose gel and appropriate sized PCR products were visualized after ethidium bromide staining by transillumination with UV light.

3. Results

CAEV-DNA was not detected in non-infected bucks but detected in eight of the 15 semen samples of infected bucks. The presence of virus was not necessarily concordant in different fractions of semen from the same buck. Of the eight positive semen samples, three were positive in the NSCF only (bucks No. 4, 12 and 13), three were positive in the CFSF fraction only (bucks No. 1, 6, and 10), and two were positive in both the fractions (bucks No. 5 and 15). Amongst these five PCR-positive samples in CFSF fraction (bucks No. 1, 5, 6, 10 and 15), only three (bucks No. 5, 6 and 15) demonstrated productive infection by syncytium formation within 10, 14 and 22 days after the inoculation. All the PBMC samples and none of the SPZ fractions were positive. Surprisingly, in all the samples tested, the *env* sequence appeared to be preferentially amplified over *pol*. Of the five positive NSCF samples, two (bucks No. 4 and 5) were positive in both sequences, and three (bucks No. 12, 13 and 15) were positive in the *env* sequence only. Of the five positives CFSF samples, 2 (bucks No. 5 and 6) were positive in both the sequences, two (bucks No. 1 and 15) were positive only in *env* sequence, and one (buck No. 10) was positive in the *pol* sequence only. Of the 15 PBMC positive samples, eight (bucks No. 4, 5, 7, 9, 10, 11, 13, 15) were positive in both the sequences, six (bucks No. 1, 2, 3, 6, 8, 12) were positive in *env* sequence only, and one (buck No. 14) was positive in the *pol* sequence only. Concurrent with other studies, these results probably reflect a lower efficiency of amplification of CAEV *pol* (Zanoni et al., 1992; Barlough et al., 1994).

The results for all the samples are presented in Table 1.

4. Discussion

The PCR amplification of proviral DNA has proven to be the most sensitive method available for lentivirus detection in blood and semen from human and animals (Mermin et al., 1991; Van Voorhis et al., 1991; Rimstad and Ueland, 1992; Hamed et al., 1993; Barlough et al., 1994; Jordan et al., 1995; Nash et al., 1995). We sought to apply this technique to investigate whether CAEV was present in semen from seropositive bucks. In this study the semen samples were separated into cellular (NSCF and SPZ) and acellular fractions (CFSF). Potential cellular reservoirs of CAEV in ejaculates include spermatozoa and nonspermatozoal cells, such as monocytes/macrophages, immature germ cells, and epithelial cells. The cellular fraction was separated into NSCF and a purified SPZ fraction, using a differential cell lysis procedure previously described, to release purified SPZ–DNA as described by previous studies using sperm in the PCR (Von Beroldingen et al., 1990). We detected CAEV-DNA in five of the 15 semen samples in NSCF population (monocytes/macrophages, epithelial cells, and immature germ cell), but all 15 SPZ purified fractions were negative. These results demonstrated that non-spermatozoal cells, probably monocytes/macrophages or others cells present in NSCF, e.g.: epithelial cells, but

Table 1
Analysis of fractionated semen and peripheral blood mononuclear cells from 18 bucks (15 CAEV seropositive, 1–15, and 3 seronegative, 16–18) for the presence of CAEV-DNA *pol* and *env* sequence by double nested PCR

Buck	Syncytia (dpi)	CFSF		NSCF		SPZ		PBMC	
		Pol	*env*	*pol*	*env*	*pol*	*env*	*pol*	*env*
1		−	+	−	−	−	−	−	+
2		−	−	−	−	−	−	−	+
3		−	−	−	−	−	−	−	+
4		−	−	+	+	−	−	+	+
5	10	+	+	+	+	−	−	+	+
6	14	+	+	−	−	−	−	−	+
7		−	−	−	−	−	−	+	+
8		−	−	−	−	−	−	−	+
9		−	−	−	−	−	−	+	+
10		+	−	−	−	−	−	+	+
11		−	−	−	−	−	−	+	+
12		−	−	−	+	−	−	−	+
13		−	−	−	+	−	−	+	+
14		−	−	−	−	−	−	+	−
15	22	−	+	−	+	−	−	+	+
16		−	−	−	−	−	−	−	−
17		−	−	−	−	−	−	−	−
18		−	−	−	−	−	−	−	−

PBMC – Peripheral blood mononuclear cells; CFSF – Cell-free seminal fluids was inoculated in GSM cells as described in the text; NSCF – Non spermatozoal cells fraction; SPZ – Spermatozoa; + – positive sample; - – negative sample; dpi – days post infection.

not in the SPZ purified fraction, carry the proviral form of CAEV in semen. CAEV infection has been demonstrated in other cell-types, such as epithelial cells of the intestinal crypts and renal tubules by in situ hybridization (Zink et al., 1990). In relation to related viruses, such as HIV-1, the role of SPZ as target cells is controversial (Mermin et al., 1991; Pudney and Anderson, 1991; Hamed et al., 1993; Nuovo et al., 1994). In addition to HIV-1, feline immunodeficiency virus (FIV) was detected in swim-up sperm preparation from infected cats (Jordan et al., 1995). In our study, although the possibility that SPZ may be infected with CAEV was not excluded, our results suggest that this possibility is low.

The detection of CAEV cell-free virus, was also demonstrated. In 15 CFSF samples that were inoculated in primary goat synovial membrane cells (GSM), five expressed CAEV-provirus detected by PCR after 8 weeks. Amongst these PCR-positive samples, three (No. 5, 6 and 15) demonstrated productive infection by syncytium formation within 10, 14 and 22 days after the inoculation. Samples No. 1 and 10 expressed CAEV provirus without syncytia, indicating that actively replicating virus were absent or at levels too low to be observed. The source and significance of cell-free CAEV in semen is uncertain as it may be shed by sources other than infected seminal cells induced by general systemic or local virus infection. Viral shedding may originate from various sites in the genital tract of the male. In addition to CAEV, HIV-1 and FIV have been detected in the semen as both the cell-infected and cell-free virus (Mermin et al., 1991; Hamed et al., 1993; Jordan et al., 1995).

Other animal lentiviruses, such as, bovine immunodeficiency virus (Nash et al., 1995), and equine infectious anemia virus (Gardner, 1990) have also been detected in semen, though their sexual transmission appears to be inefficient. Although the sexual transmission has not been demonstrated in our study, the detection of CAEV in semen both as cell-free virus and infected cells suggests the possibility of sexual transmission.

5. Conclusions

In summary, this is the first report describing detection of CAEV both as cell-associated and cell-free

virus in semen from seropositive bucks. CAEV was detected in 53.3% of the semen samples tested and was not necessarily consistent with different fractions of semen from the same buck over time. While 53.3% of semen samples had detectable CAEV nucleic acid in their semen, no CAEV-DNA was detected in SPZ. Infectious virus was demonstrated in vitro in CFSF by cultivation in primary GSM cell line. These observations support the current recommendation that natural or assisted reproduction with semen from seropositive bucks, should be avoided pending additional study.

Acknowledgements

This work was realized and supported by CNEVA-Station Regionale de Pathologie Caprine-Niort-France. C. Travassos, was recipient of fellowship from the Brazilian Research Council (CNPq and CAPES). The authors thank CAPRI-A France for providing access and delivery of free-infected samples, and all the personnel of SRPC for technical assistance.

References

Adams, D.S., Klevjer-Anderson, P., Carlson, J.L., McGuire, T.C., Gorham, J.R., 1983. Transmission and control of caprine arthritis–encephalitis. Am. J. Vet. Res. 44, 1670–1675.

Barlough, J., East, N., Rowe, J.D., Van Hoosear, K., DeRock, E., Bigornia, L., Rimstad, E., 1994. Double-nested polymerase chain reaction for detection of caprine arthritis–encephalitis virus proviral DNA in blood, milk and tissues of infected goats. J. Virol. Methods 50, 101–114.

Cork, L.C., Hadlow, W.J., Crawford, T.B., Gorham, J.R., Piper, R.C., 1974. Infectious leukoencephalomyelitis of young goats. J. Infect. Dis. 129, 134–141.

Crawford, T.B., Adams, D.S., Cheevers, W.P., Cork, L.C., 1980. Chronic arthritis in goats caused by a retrovirus. Science 207, 997–999.

East, N.E., Rower, J.D., Madewell, B.R., Floyd, K., 1987. Serologic prevalence of caprine arthritis–encephalitis virus in California goat dairies. J. Am. Vet. Assoc. 190, 182–186.

East, N.E., Rower, J.D., Dahlberg, J.E., Theilen, G.H., Pedersen, N.C., 1993. Modes of transmission of caprine arthritis–encephalitis virus infection. Small Rumin. Res. 10, 251–262.

Ellis, T.M., Robinson, W., Wilcox, G., 1987. Effect of colostrum deprivation of goat kids on the natural transmission of caprine retrovirus infection. Aust. Vet. J. 60, 326–329.

Gardner, M.B. 1990. Natural history of animal retroviruses: an overview. In: Alexander, N., Galemick, J., Spieler, J. (Eds.), Heterosexual Transmission of AIDS. Alan R. Liss, New York, pp. 93–10.

Hamed, K.A., Winters, M.A., Holodniy, M., Katzenstein, D.A., Merigan, T.C., 1993. Detection of human immunodeficiency virus type 1 in semen: Effects of disease stage and nucleoside therapy. J. Infect. Dis. 167, 798–802.

Jordan, H.L., Howard, J., Tompkins, W.A., Stoskopf, S.K., 1995. Detection of feline immunodeficiency virus in semen from seropositive domestic cats. J. Virol. 69, 7328–7333.

Krieger, J.N., Coombs, R.W., Collier, A.C., Ross, S.O., Chaloupa, K., Cummings, D.K., Murphy, V.L., Corey, L., 1991. Recovery of human immunodeficiency virus type 1 from semen: Minimal impact of stage of infection and current antiviral chemotherapy. J. Infect. Dis. 163, 386–388.

Lerondelle, C., Fleury, C., Vialard, J., 1989. La glande mammaire: organe cible de l'infection par le virus de l'arthrite-encephalite caprine (The mammary gland: A target organ for infection with caprine arthritis–encephalitis virus). Ann. Rech. Vet. 20, 57–64.

Mackenzie, R.W., Olivier, R.E., Rooney, J.P., 1987. A successful attempt to raise goat kids free of infection with caprine arthritis–encephalitis virus in an endemically infected goat herd. NZ Vet. J. 35, 184–186.

Mermin, J.H., Holodniy, M., Katzenstein, D.A., Merigan, T.C., 1991. Detection of human immunodeficiency virus DNA and RNA in semen by the polymerase chain reaction. J. Infect. Dis. 164, 769–772.

Narayan, O., Clements, J.E., Stradberg, J.D., Cork, L.C., Griffen, D.E., 1980. Biological characterization of the virus causing leukoencephalomyelitis and arthritis in goats. J. Gen. Virol. 41, 343–352.

Nash, J.W., Hanson, L.A., St. Cyr Coats, 1995. Bovine immunodeficiency virus in stud bull semen. Am. J. Vet. Res., vol. 56, pp. 760–763.

Nuovo, G.J., Becker, J., Sinsir, A., Margiotta, M., Khalife, G., Shevcuk, M., 1994. HIV-1 nucleic acid localize to the spermatogonia and their progeny. Am. J. Pathol. 44, 1142–1148.

Pudney, J., Anderson, D., 1991. Orchitis and human immunodeficiency virus type-1 infected cells in reproductive tissues from men with acquired immunodeficiency syndrome. Am. J. Pathol. 139, 149–160.

Rimstad, E., East, N.E., Torten, M., Higgins, J., De Rock, E., Pedersen, N.C., 1993. Delayed seroconversion following naturally acquired caprine arthritis-encephalitis virus infection in goats. Am. J. Vet. Res. 54, 1858–1862.

Rimstad, E., Ueland, K., 1992. Detection of feline immunodeficiency virus by a nested polymerase chain reaction. J. Virol. Methods 36, 239–248.

Rower, J.D., East, N.E., Thurmond, M.C., Franti, C.E., 1991. Risk factors associated with caprine arthritis–encephalitis virus infection in goats on California dairies. Am. J. Vet. Res. 52, 510–514.

Saltarelli, M., Quérat, G., Konings, D.A.M., Vigne, R., Clements, J.E., 1990. Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus. Virology 179, 347–364.

Van Voorhis, B.J., Martinez, A., Mayer, K., Anderson, D.J., 1991. Detection of human immunodeficiency virus type 1 in semen from seropositive men using culture and polymerase chain

reaction deoxyribonucleic acid amplification technique. Fertil. Steril. 55, 588–594.

Von Beroldingen, C.H., Blake, E.T., Higuchi, R., Sensabaugh, G.F., Erlich, H.A., 1990. Applications of PCR to the analysis of biological evidence. In: Erlich, H.A. (Ed.), PCR Technology: Principles and Application for DNA Amplification. Stockton Press, New York, pp. 209–223.

Zanoni, R.G., Nanta, I.M., Kuhnert, P., Pauli, U., Pohl, B., Peterhans, E., 1992. Genomic heterogeneity of small ruminant lentiviruses detected by PCR. Vet. Microbiol. 33, 341–351.

Zink, M.C., Yager, J.A., Myers, J.D., 1990. Pathogenesis of caprine arthritis-encephalitis virus. Cellular localization of viral transcripts in tissues of infected goats. Am. J. Pathol. 136, 843–854.