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Abortion in goats by Caprine alphaherpesvirus 1 in Spain

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Contents

An abortion outbreak occurred in a goat herd of Murciano-Granadina breed in Almeria Region in Spain where 80 pregnant females aborted. All bacteriological and parasitological examinations resulted negative, whereas virological investigations and real-time PCR assay showed the presence of Caprine alphaherpesvirus 1 DNA in the pathological specimens from aborted foetuses. Nucleotide sequence analysis revealed that the DNA was highly close related to the Swiss strain E-CH (99.7%) and a little less extent to the Italian BA.1 strain (99.4%). Histopathological examination revealed multifocal, well-circumscribed, 50- to 200-μm-diameter foci of coagulative necrosis in the liver, lungs and kidneys of three foetuses. In the periphery of the necrosis, there were frequently epithelial cells with the chromatin emarginated by large, round, amphophilic intranuclear viral inclusion bodies. The source of the infection in the herd could not clearly find out even some hypothesis were formulated. This seems to be the first report of an abortion outbreak due to Caprine alphaherpesvirus 1 in a goat herd in Spain.

1 | INTRODUCTION

Caprine alphaherpesvirus 1 (CpHV-1), a ruminant alphaherpesvirus closely related to Bovine alphaherpesvirus 1 (BoHV-1), was first isolated from goats in 1974 and further characterized in 1975 (Berrios, McKercher, & Knight, 1975; Saito, Gribble, Berrios, Knight, & McKercher, 1974). The pathogenesis of the infection is not yet completely understood, although the virus is believed to infect goats via the nasal or reproductive route, having high tropism for the genital tract (Tempesta et al., 2001, 2004). Viral infection can induce vulvovaginitis, balanoposthitis, respiratory disease and occasionally abortions in adult goats (Grewal & Wells, 1986; Horner, Hunter, & Day, 1982; Tarigan, Webb, & Kirkland, 1987; Williams, Vickers, Tramontin, Petrites-Murphy, & Allen, 1997), whereas enteritis and systemic infection occur more frequently in young (Roperto et al., 2000; Waldvogel, Engels, Wild, Stunzi, & Wyler, 1981). Similar to other alphaherpesvirus, CpHV-1 can induce subclinical disease and be latent for long periods of time (Tempesta, Pratelli, Greco, Martella, & Buonavoglia, 1999). However, reactivation of the virus under natural conditions has been shown to be rare and only occurring in animals as a consequence of stressing factors such as oestrus in the presence of low neutralizing antibody titres (Tempesta, Buonavoglia, Sagazio, Pratelli, & Buonavoglia, 1998). Reactivation of infection has been induced experimentally in latently infected goats after treatment with high doses of dexamethasone (Buonavoglia et al., 1996).

Although CpHV-1 infections have a worldwide distribution, outbreaks of clinical disease have rarely been observed, mainly consisting of systemic disease in young and genital lesions in adult animals (Belton, 1992; Grewal & Wells, 1986; Horner et al., 1982; Piper, Fitzgerald, Ficorilli, & Studdert, 2008; Roperto et al., 2000; Saito et al., 1974). CpHV-1 as a determinant of abortion in goats accounts for only a low percentage of the total causes (McCoy et al., 2007), and it has only been described sporadically (Chénier, Montpetit, & Hélie, 2004; McCoy et al., 2007; Uzal et al., 2004; Williams et al., 1997).

Caprine alphaherpesvirus 1 (CpHV-1) was isolated in the South of Spain (Cordoba) in two seropositive goats without clinical signs, and the identification of the virus was achieved after reactivation with corticosteroids (Keuser, Espejo-Serrano, Schynts, Georgin, & Thiry, 2004). In this report, we describe, for the first time in Spain, an outbreak of abortion in goats from which CpHV-1 was successfully identified as the causative agent by histopathology, real-time PCR and PCR assays.

TABLE 1 Oligonucleotides used in the CpHV-1 real-time PCR and gel-based PCR assays

Primer/probe	Sense	Position[a]	Amplicon size
CapIII[b]	+	632–653	414 bp
CapIV[b]	–	1,027–1,046	
CpHV-For[c]	+	897–918	82 bp
CpHV-Rev[c]	–	959–979	
CpHV-Pb[c]	+	926–951	

[a]Oligonucleotide position is referred to the sequence of the gC gene of CpHV-1 reference strain BA.1 (GenBank accession number: AY821804).
[b]Gel-based PCR.
[c]Real-time PCR.

2 | MATERIALS AND METHODS

All the investigations and procedures were made following international guidelines and ethics.

2.1 | Case report

The abortion outbreak occurred in a goat herd of Murciano-Granadina breed in Almeria (South-East of Spain). The herd consisted of a total of 1,050 goats kept in an intensive dairy productive system without any contact with other species. The herd had no previous history of abortion over the past few years, there were no recent factors of stress (feeding changes, management, etc.), and introduction of new animals had not occurred over the last 5 years. During the last 2 years, female replacement goats were vaccinated with an attenuated live vaccine against *Chlamydophila abortus*. A total of 80 abortions occurred over a period of 45 days during the autumn birth season (October–November). The abortion occurred at different gestation periods. Goats that had aborted did not show any overt clinical sign. Some of the young were stillbirth or born weak. In the case of twins, usually one of the young survived and the other died at birth. In three aborted foetuses, the necropsy was carried out, and the respective placentas were also observed for gross pathological changes. Samples from brain, lungs, liver, kidney, sera from foetuses and placenta and sera from does were collected for laboratory investigations.

2.2 | Laboratory investigations

Samples from placentas and organs were fixed in 10% non-buffered formalin, routinely embedded in paraffin and processed for histopathology. Slides were stained with haematoxylin–eosin, Gram and Stamp.

2.3 | Paraffin block samples showing necrotic foci were processed to detect CpHV-1 DNA

DNA samples were extracted from pools of tissue sections embedded in paraffin using the Speed tools tissue DNA extraction Kit (Biotools B & M labs S.A., Madrid, Spain) according to the manufacturer's instructions.

DNA extracts were tested by a CpHV-1 real-time PCR assay, following the method developed by Elia et al. 2008. Briefly, real-time PCR was carried out in a 25-µl reaction volume containing 15 µl of PCR buffer IQ™ Supermix, (Bio-Rad Laboratories Srl, Segrate, Italy), 600 nM of primers CpHV-For and CpHV-Rev, 200 nM of probe CpHV-Pb (Table 1) and 10 µl of DNA. Serial ten-fold dilutions (from 10^9 to 10^2 DNA copies/10 µl of standard DNA) of a plasmid PGEM®-3Z (Promega, Madison WI, USA), containing the nearly full-length glycoprotein C (gC) gene of CpHV-1, were used to generate a standard curve. Duplicates of the CpHV-1 standard dilutions and DNA templates were simultaneously subjected to real-time analysis. An exogenous internal control, consisting of 10,000 copies of canine parvovirus type 2 (CPV-2) DNA per ml of lysis buffer, was added to each sample prior to DNA preparation to control for PCR inhibition. Real-time PCR was carried out in a iCycler *iQ* real-time PCR detection system (Bio-Rad Laboratories S.r.l., Segrate [MI], Italy). The following thermal cycle protocol was used: activation of Taq DNA polymerase (Takara LA Taq™, Takara Bio Inc., Mountain View, CA, USA) at 95°C for 10 min and 45 cycles consisting of denaturation at 95°C for 1 min, primer annealing and extension at 70°C for 1 min.

To confirm the real-time PCR results and characterize at the molecular level the detected CpHV-1 strain, a conventional gel-based PCR was performed as described elsewhere (Tempesta et al., 1999). Briefly, forward and reverse primers able to amplify 414-bp portion of the gC gene of CpHV-1 strain BA.1 (GenBank accession number AY821804) and targeting 632–653 and 1,027–1,046 gene positions, respectively, were used. PCR was carried out in a total volume of 25 µl containing 5 µl of DNA, 2.5 µl of PCR buffer 10X, 1.5 mM $MgCl_2$, 1.25 mM of each oligonucleotide triphosphate, 200 µM of each primer, 1.5 U of Taq DNA polymerase (Takara LA Taq™, Takara Bio Inc., Mountain View, CA, USA), 2.5 µl of glycerol and diethyl pyrocarbonate (DEPC) H_2O up to 25 µl. The thermal profile consisted of a 1 min at 94°C, 40 cycles at 94°C for 1 min (denaturation), 70°C for 1 min (annealing) and 72°C for 1 min (polymerization) followed by a final extension at 72°C for 10 min. Ten microlitres of the PCR products was analysed by electrophoresis in 1.5% agarose gel and visualized by UV light after ethidium bromide staining.

The PCR products were purified by Montage® PCR filter units (Millipore, Milan, Italy) and sequenced using BigDye 3.1 Ready reaction mix (Applied Biosystems, Foster City, CA, USA) and ABIPRISM 377 DNA Sequencer (Applied Biosystems).

The obtained nucleotide and amino acid sequences were analysed using the analytical tools by the National Center for Biotechnology Information (NCBI) and aligned by the program ClustalW (Larkin et al., 2007).

3 | RESULTS

3.1 | Post-mortem findings and histopathology

There were no macroscopic lesions in any of the foetuses or the placentas, and no evidence of a common causative agent of abortion in goats was found. Examination under the microscope revealed multifocal, well-circumscribed, 50- to 200-µm-diameter foci of

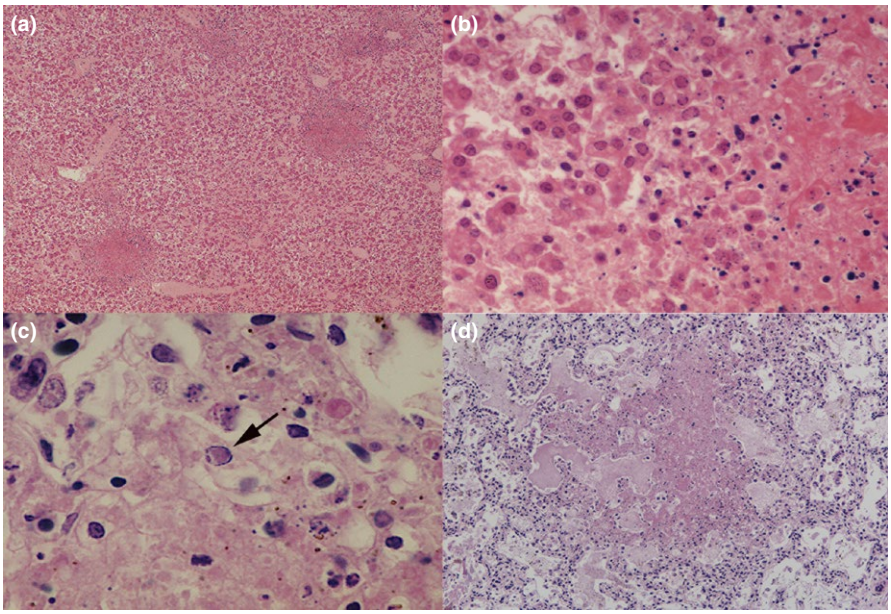

FIGURE 1 (a) Several foci of coagulative necrosis in liver parenchyma, up to 500 μm in diameter with a mild inflammatory cell infiltrate at the periphery. H&E. 4x; (b) Detail of the periphery of a necrotic focus showing karyorrhectic, karyolytic, nuclear pyknosis and parietal hyperchromatosis in hepatocytes. H&E. 40x; (c) Intranuclear inclusion body (arrow) in a hepatocyte with marginated nuclear chromatin. H&E. 50x; (d) Lung. Similar focus of coagulative necrosis to those observed in the liver. H&E. 10x

coagulative necrosis in the liver, lungs and kidneys of the three foetuses (Figure 1a/d). Foci of necrosis were characterized by a central necrotic tissue, degenerating neutrophils and abundant karyorrhectic debris rimmed by a mild inflammatory cell infiltrate mainly composed of lymphocytes and macrophages (Figure 1b). In the periphery of the necrosis, there were frequently hepatic cells with the chromatin emarginated by large, round, amphophilic intranuclear viral inclusion bodies (Figure 1c). In the brain, similar small scattered necrotic foci were observed, but no intranuclear viral inclusion bodies were observed in this location.

3.2 | Molecular detection and characterization of CpHV-1

Real-time PCR showed the presence of CpHV-1 DNA in the pools of tissue sections from liver, lung and kidney of the foetuses, with viral DNA titres of 3.39 and 5.16 × 10^{-7} DNA copies/ml^{-1} of template, respectively. Gel-based PCR confirmed the results obtained by real-time PCR and allowed to obtain a 414-bp gC fragment subsequently sequenced. Sequences were analysed and submitted to GenBank with the accession numbers: MF039649 and MF039650. Sequence analysis showed that the Spanish CpHV-1 strains detected in aborted foetuses were identical (100% nt identity) each other. The closest genetic relatedness was found with the Swiss prototype strain E-CH (99.7% of nt identity), whereas the Spanish strains were slightly less related to the Italian isolate BA. 1 (99.4% of nucleotide identity). The relatedness with the Swiss and the Italian strains was also evident when amino acid sequences were analysed, with an identity of 99.2% and 98.4%, respectively.

3.3 | Testing for other pathogens

ELISA test was performed on sera from does and foetuses to test for *Toxoplasma gondii* antibodies, giving negative results. Gram and Stamp stainings on direct impression smear from foetal tissues did not detect any specific bacteria. Standard bacteriological cultures were performed with tissues from the foetus; they all failed to grow any organism. No bacteria (*Campylobacter* spp., *Coxiella* spp., *Chlamydia* spp.) and parasites (*T. gondii*) were detected. The evidence of histopathological lesions, the presence of intranuclear viral inclusion bodies and CpHV-1 sequence results confirmed causative diagnosis of abortion and excluded the need for investigation of other viral pathogens.

4 | DISCUSSION

Infectious causes of abortion in goats are widespread and predominantly of bacterial origin (Moeller, 2001). Differential diagnoses based on the gross lesions observed in the placenta and the foetus could be tentatively performed. In fact, the histopathological areas of necrosis observed in several tissues, with limited inflammatory response around the necrotic foci and the presence of characteristic intranuclear viral inclusion bodies pointed directly towards a herpesvirus infection. Other common causative agents of abortion in goats were all ruled out after negative bacteriological culture, ELISA and absence of characteristic pathological changes of these aetiological agents. CpHV-1 infection in goats is responsible for typical clinical signs not necessarily occurring at the same time and in the same animal. Indeed, outbreaks of vulvovaginitis usually occur without being associated with signs in the foetus or abortion (Belton, 1992; Grewal & Wells, 1986; Horner et al., 1982). On the other hand, outbreaks of abortion usually occur without genital pathologic changes (vesiculo-ulcerative vulvovaginitis) in adult goats, (McCoy et al., 2007; Tempesta et al., 2004; Williams et al., 1997) as reported in the present study.

The source of infection in this herd remains uncertain; the farmer had not introduced new animals in the herd over the previous years. As observed in natural and experimental infections and similar to other herpesvirus infections, CpHV-1 could remain latent in some animals

for long time periods (Tempesta et al., 1999). Even though no particular treatments or stressful situations were reported in the herd involved in the described outbreak, reactivation of a latent infection could not be ruled out (Buonavoglia et al., 1996; Tempesta et al., 1998).

CpHV-1 had been previously isolated from two seropositive goats in the South of Spain; however, there were no clinical signs of genital pathology or abortion reported in either of the animals from which the virus was isolated (Keuser et al., 2004). The presence of CpHV-1 infection in two goat herds in the South of Spain that were geographically separated (350 km far from each other), along with the occurrence of overt disease in one case, suggests a high CpHV-1 prevalence in that region having a large goat population, as it occurs in other Mediterranean countries (Keuser et al., 2004; Koptopoulos, Papanastosopoulou, Papadopoulos, & Ludwig, 1988; Thiry et al., 2008). Although CpHV-1 has been previously identified in Spain, to our knowledge, this is the first description of abortions caused by this agent in the Iberian Peninsula. The detection of CpHV-1 described in this report represents a further confirmation of the spreading of the infection and the ability of the virus to cause reproductive failures and abortion in does.

In our opinion, this infection should be included in the diagnostic panels for common causes of abortion in goats in Spain, and serological studies would be necessary to know the widespread of the infection, especially in a region with a high goat population.

CONFLICT OF INTEREST

The authors declared no potential conflict of interests with respect to the research, authorship and/or publication of this article.

AUTHOR CONTRIBUTIONS

All the authors contributed equally.

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