Accepted Manuscript

Title: Serological evidence of caprine herpesvirus 1 infection in Mediterranean France

Authors: Julien Thiry, Claude Saegerman, Christophe Chartier, Pascale Mercier, Véronique Keuser, Etienne Thiry

PII: S0378-1135(07)00518-4
DOI: doi:10.1016/j.vetmic.2007.10.009
Reference: VETMIC 3854

To appear in: *VETMIC*

Received date: 6-9-2007
Revised date: 11-10-2007
Accepted date: 12-10-2007

Please cite this article as: Thiry, J., Saegerman, C., Chartier, C., Mercier, P., Keuser, V., Thiry, E., Serological evidence of caprine herpesvirus 1 infection in Mediterranean France, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.10.009

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Serological evidence of caprine herpesvirus 1 infection in Mediterranean France

Julien Thiry^a, Claude Saegerman^b, Christophe Chartier^c, Pascale Mercier^c, Véronique Keuser^a,

Etienne Thiry^{a*}

^a Virology and Viral Diseases, ^b Epidemiology and Risk Analysis Applied to Veterinary Sciences,
Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège,
4000 Liège, Belgium

^c Laboratory of Caprine Study and Research, French Food Safety Agency, 79012 Niort, France

* Corresponding author: Dr E. Thiry, Department of Infectious and Parasitic Diseases, Virology and
Viral Diseases, Faculty of Veterinary Medicine, University of Liège, Boulevard de Colonster, 20,
B43b, B-4000 Liège, Belgium. Phone: (32) 4 366 42 50, fax: (32) 4 366 42 61, e-mail:
etienne.thiry@ulg.ac.be

Running title – Serological evidence of CpHV-1 in Corsica

Abstract

Caprine herpesvirus 1 (CpHV-1) is responsible of a systemic disease in kids and genital diseases inducing abortions in adult goats. In Europe, CpHV-1 is widespread in Mediterranean countries such as Greece, Italy and Spain. As France is geographically close to these countries, a survey was conducted to investigate the presence of CpHV-1 in goats in a Mediterranean department (Corse-du-Sud) and in continental departments (Dordogne and Vendée) of this country. Taking into account the close antigenic and genetic relationships between bovine herpesvirus 1 (BoHV-1) and CpHV-1, the serological detection was performed by using BoHV-1 glycoproteins B (gB) and E (gE) blocking ELISAs. The analysis of 2,548 serum samples in a BoHV-1 gB blocking ELISA revealed that a ruminant alphaherpesvirus infection related to BoHV-1 was widespread in Corse-du-Sud whereas no positive animals was detected in Dordogne and Vendée. Furthermore, the specificity and the sensitivity of the BoHV-1 gB blocking ELISA to detect a BoHV-1 related infection in goats were evaluated. A subsequent analysis by a BoHV-1 gE blocking ELISA demonstrated that 22.6% of gB-positive serum samples were also gE-positive. Cross-seroneutralisation assays afforded the unambiguous identification of antibodies against CpHV-1 in gB-positive goats. The likely presence of CpHV-1 in Corse-du-Sud supported by a high seroprevalence (61.9%) in all investigated flocks extends the number of countries infected with CpHV-1. Moreover, the difference observed between Corse-du-Sud and Dordogne and Vendée suggests that CpHV-1 is more prevalent in Mediterranean countries or regions than in central and northern Europe.

Keywords – herpesvirus; bovine; caprine; ELISA; prevalence; France

1. Introduction

Caprine herpesvirus 1 (CpHV-1) causes mainly a systemic infection in neonatal kids. Although most infections in adults are subclinical, CpHV-1 can provoke vulvovaginitis (Grewal and Wells, 1986), balanoposthitis (Tarigan et al., 1987), and abortion (Keuser et al., 2002). Although the economical impact of the infection has not been determined yet at the level of a country, it may be important in infected flocks because of abortions, returns to oestrus, and neonatal mortality (Thiry et al., 2007a). CpHV-1 was first isolated in 1975 in California from newborn kids showing enteritis (Saito et al., 1974). Five years later, it was recovered in the Bregaglia valley of Switzerland from kids affected with a similar disease (Mettler et al., 1979). The distribution of CpHV-1 infection has not been systematically studied in goats but has been worldwide identified (Thiry et al., 2007a) as in Europe (Buonavoglia et al., 1996; Keuser et al., 2004; Koptopoulos et al., 1988; Mettler et al., 1979; Muluneh and Liebermann, 1990), Australia (Grewal and Wells, 1986), Canada (Chenier et al., 2004), New Zealand (Horner et al., 1982) and USA (Saito et al., 1974). In many countries where goats play an economical role, a high prevalence is observed: more than 50% in Greece (Koptopoulos et al., 1988), between 30 and 60% in southern Italy (Guercio et al., 1998; Tempesta et al., 1994; Thiry et al., 2006c) and 21% in Spain (Keuser et al., 2004; Thiry et al., 2006c). The CpHV-1 infection was identified neither in Belgium nor in France (Thiry et al., 2006c). Therefore, in Europe, although the first isolation was performed in Switzerland, CpHV-1 infection seems to be more prevalent in Mediterranean than in Northern European countries.

On the basis of its antigenic and genetic properties, CpHV-1 belongs to a cluster of ruminant alphaherpesviruses closely related to bovine herpesvirus 1 (BoHV-1) (Thiry et al., 2006a). BoHV-1 is responsible of diseases of major economic concern in many parts of the

77 world and especially in Europe. The infection causes a variety of clinical diseases in cattle
 78 including infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV)
 79 (Muylkens et al., 2007). In order to detect and control IBR, specific molecular and serological
 80 diagnostic tests were developed. Currently, the serological diagnosis is based on the use of
 81 commercial BoHV-1 glycoprotein B (gB) and glycoprotein E (gE) blocking enzyme-linked
 82 immunosorbent assays (ELISA) which are fast, inexpensive and simple methods for screening
 83 large numbers of test samples (Kramps et al., 2004). The existence of the consistent group of
 84 ruminant alphaherpesviruses closely related to BoHV-1 leads to a theoretical problem to
 85 distinguish serologically between these viruses (Thiry et al., 2006a). When they are analysed
 86 by ELISA or seroneutralisation, BoHV-1 and related ruminant alphaherpesviruses cross-react
 87 due to antigenic cross-relationships (Lyaku et al., 1992; Martin et al., 1990; Nixon et al.,
 88 1988). Thus, the use of a BoHV-1 gB blocking ELISA allows the identification of such a
 89 ruminant alphaherpesvirus infection but is not able to make a differentiation.

90 The current study aims to investigate the epidemiological situation in France, a
 91 country which includes both Mediterranean and northern areas, in order to study if CpHV-1
 92 prevalence is different in Mediterranean than in Northern Europe. Furthermore, the results of
 93 this survey were improved by the validation of the serological assay in order to detect a
 94 BoHV-1 related infection and especially a CpHV-1 infection in goats. The combination with a
 95 BoHV-1 gE blocking ELISA could lead to distinguish between CpHV-1 and BoHV-1 as
 96 demonstrated for bovine herpesvirus 5 (BoHV-5) (Wellenberg et al., 2001). Cattle
 97 experimentally infected with BoHV-5 are detected seropositive by a BoHV-1 gB blocking
 98 ELISA and seronegative by a BoHV-1 gE blocking ELISA (Wellenberg et al., 2001). BoHV-
 99 5 being the alphaherpesvirus the most closely related to BoHV-1 (Ros et Belák, 1999 ; 2002)
 100 and showing antigenic differences for gE (Kaashoek et al., 1995 ; Wellenberg et al., 2001), it
 101 could be hypothesised that CpHV-1 gE has more pronounced antigenic differences and may

allow the differentiation from BoHV-1 by using BoHV-1 gB and gE blocking ELISA. As BoHV-1 gB blocking ELISA is used as a screening tool to detect antibodies against a related BoHV-1 infection, the validation of this test in goats was evaluated under field and experimental conditions.

2. Materials and methods

2.1. Flocks, animals and serum samples

Field serum samples were collected in several French flocks in Corse-du-Sud (department 2A), in Dordogne (department 24) and in Vendée (department 85). Goat flock size ranged from 7 to 304 adult animals over than 6 months of age. All flocks were composed of dairy breeds and produced milk for dairies or for on-farm cheese making. The sampling size corresponded to the whole adult flock size. The experimental serum samples came from four Belgian goats intranasally inoculated with the CpHV-1 E/CH strain at a dose of 10^6 plaque forming units (PFU) per nostril (Keuser et al., 2002) and two Danish calves intranasally inoculated with the BoHV-1 Iowa strain at a dose of 5×10^7 PFU per nostril (Muylkens et al., 2006). Prior to inoculation, absence of antibodies against CpHV-1 in goats was confirmed by serum neutralisation assay (Keuser et al., 2002) and absence of antibodies against BoHV-1 in calves was confirmed by BoHV-1 gB blocking ELISA (Muylkens et al., 2006).

2.2. Enzyme linked immunosorbent assay (ELISA)

To detect antibodies in serum samples, a BoHV-1 gB blocking ELISA (SERELISATM IBR/IPV gB Ab Mono Blocking, Synbiotics Europe, Lyon, France) and a BoHV-1 gE

blocking ELISA (Herdchek Anti-IBR gE, Idexx, Germany) were used following the instructions of the manufacturers. Serum samples were analysed in duplicate and the average was used to calculate the competition percentage. The cut off was fixed to 45% of competition. Serum samples identified as doubtful were analysed in a second round, when identified twice doubtful, serum samples were considered as positive.

2.3. Serum neutralisation assay (SN)

Serum samples were heated at 56°C for 30 min. Serial twofold dilutions of each serum sample were incubated at 37°C for 2 h. in the presence of 75 PFU of CpHV-1 Ba-1 strain (Buonavoglia et al., 1996) or 12 h. in the presence of 75 PFU of BoHV-1 Iowa strain (Miller and van der Maaten, 1984). Each virus-serum dilution was then dispensed into 96 wells microplates containing Madin Darby Bovine Kidney (MDBK, ATCC CCL-22) cells grown as previously described (Lemaire et al., 1999). Readings were made after incubation for 3 days at 37°C. The titres were expressed as the reciprocal of the highest dilution of serum sample inhibiting cytopathic effect.

2.4. Statistical analyses

Statistical analyses were carried out in STATA/SE 8 (StataCorp, 2003).

3. Results

Serums from flocks sampled in Corse-du-Sud (n=1,430), Dordogne (n=997) and Vendée (n=121) departments in France were tested in a BoHV-1 gB blocking ELISA. The apparent prevalences are given in Table 1. In Corse-du-Sud, all flocks tested were

seropositive. The within-herd prevalence ranged from 38.7% to 73.7% with an individual prevalence of 57.9% (95% confident interval (CI): 55.3%-60.5%). In Dordogne and Vendée, serum samples were consistently negative. In regards to these data, the specificity and the sensitivity of the BoHV-1 gB blocking ELISA to detect a BoHV-1 related infection in goats were evaluated with a cut-off of 45% of competition. On the basis of 1,118 serum samples from Dordogne and Vendée, regions presumably free of BoHV-1 related virus infection in goats, the measured specificity was 100% (95% CI: 99.67%-100%). The sensitivity of the ELISA was assessed by testing serum samples obtained from serial bleedings of four goats experimentally infected with CpHV-1 (n=31). All serum samples but two taken at day 7 post infection were detected positive. The measured sensitivity was 93.5% (95% CI: 78.6%-99.2%). The true prevalences were calculated for each region (Table 1) according the following equation: True Prevalence = (Apparent Prevalence + Specificity - 1) / (Sensitivity + Specificity - 1) (Toma et al., 2001). In Corse-du-Sud, the within-herd prevalence ranged from 41.36% to 78.86% with an individual prevalence of 61.93% (95% CI: 59.13%-64.68%). Dordogne and Vendée obviously kept their free status. The distribution of the data obtained from the investigated goat population clearly illustrates that a BoHV-1 gB blocking ELISA significantly detects a BoHV-1 related infection in goats (Fig. 1). The goat population in Corse-du-Sud presents a bimodal distribution (Fig. 1a) contrarily to the non-infected population in Dordogne and Vendée (Fig. 1b) and the experimental infected population (Fig. 1c).

A BoHV-1 gE blocking ELISA was applied on serum samples detected positive in the BoHV-1 gB blocking ELISA (gB-positive) to fully distinguish BoHV-1 from a related infection. The analysis revealed that 22.6% of gB-positive serum samples in Corse-du-Sud were positive in the BoHV-1 gE blocking ELISA (gE-positive) (Table 2). In this context, different categories of serum samples were tested by a serum neutralisation assay (Table 3).

Cross-seroneutralisation assays showed that serums from CpHV-1 experimentally infected goats neutralise CpHV-1 to a much greater extent than BoHV-1. Such one way cross-seroneutralisation was observed with gB-positive serum samples and affords evidences that the infection serologically detected in goats by the BoHV-1 gB blocking ELISA is a CpHV-1 infection (Table 3).

4. Discussion

The serological diagnosis of ruminant alphaherpesviruses related to BoHV-1 is made difficult by their antigenic cross-relationships. The existence of cross-reactions in serological assays as seroneutralisation and the ability of these viruses to cross, at least in some instances, the species barrier raise theoretical issues for differential diagnosis (Thiry et al., 2006a). The currently available serological tests are almost unable to discriminate related ruminant alphaherpesviruses. The current study reports the serological evidence of CpHV-1 infection occurring in Corse-du-Sud, a Mediterranean French department situated in Corsica, by applying a BoHV-1 gB blocking ELISA which has been evaluated in experimental conditions for sensitivity and under field conditions for specificity to detect CpHV-1 in goat species. Cross-seroneutralisation assays confirmed that antibodies detected were directed against CpHV-1 and not against BoHV-1.

The analysis of goat serum samples in a BoHV-1 gB blocking ELISA revealed that BoHV-1 related infection did not occur in Dordogne and Vendée, or only occurred at a low prevalence, not detected by our survey. In contrast, a high seroprevalence was observed in Corse-du-Sud. The gB-positive serum samples most likely come from animals infected with

CpHV-1. Indeed, several studies showed that gB is relatively well conserved within related ruminant alphaherpesviruses (Ros and Belák, 2002; Thiry et al., 2007b). Thus, BoHV-1 gB may include epitopes which most of them are shared with CpHV-1; antibodies directed against CpHV-1 may cross react in an ELISA using BoHV-1 gB as antigen as demonstrated for BoHV-5 (Wellenberg et al., 2001). The analysis of serum samples from CpHV-1 experimentally infected goats in a BoHV-1 gB blocking ELISA demonstrated a good sensitivity. Furthermore, the serum neutralisation assay demonstrated that CpHV-1 was indeed neutralised with both field and experimental goat serum samples.

The use of a BoHV-1 gE blocking ELISA could lead to the discrimination between BoHV-1 and other ruminant alphaherpesvirus infections as demonstrated for BoHV-5 (Wellenberg et al., 2001). BoHV-5 is one of the ruminant alphaherpesviruses the most related to BoHV-1 whereas CpHV-1 seems to be the most distantly related to BoHV-1 (Ros and Belák, 1999; 2002). Recent partial sequencing of US8 gene encoding gE confirmed the genetic divergence within the cluster of BoHV-1 related ruminant alphaherpesviruses (Thiry et al., 2007b). At the same level, BoHV-5 shows differences in the gE antigenicity (Kaashoek et al., 1995; Wellenberg et al., 2001). Therefore, serum samples from goats seropositive to BoHV-1 gB were expected to be negative in a BoHV-1 gE blocking ELISA. Surprisingly, 22.6% of gB-positive serum samples were also positive in BoHV-1 gE blocking ELISA. A first explanation would be that gE-positive goats could have been infected with BoHV-1. Nevertheless, natural infections of goats with BoHV-1 have been only seldomly reported (Tolari et al., 1990 ; Whetstone et Evermann, 1988), whereas goats have been successfully infected experimentally with BoHV-1 inducing a serologic response and establishment of a latent infection (Six et al., 2001). In Corsica, the direct contact between cattle and goats is incidental because flocks and herds are not mixed although sharing the same rangeland pastures in mountains and plains. Therefore, the transmission of BoHV-1 from cattle to goats

would be only exceptional, such cross-infection being very rare, and a BoHV-1 infection in goats is not likely to occur. The very high seroprevalence observed in the current study strongly supports a large circulation of CpHV-1. Furthermore, the differentiation between BoHV-1 and CpHV-1 can be made taking profit of a one way cross-neutralisation (Engels et al., 1983). In the current study, serum samples neutralised CpHV-1 to a much greater extent than BoHV-1 and afforded the ultimate identification of a CpHV-1 infection. On the other hand, goat population in Corsica is almost exclusively composed by the local breed underlining the quasi-absence of introduction of animals from the continental France or from Italy for ages (Hugot and Bouche, 1999). The only and limited importations are from southern continental departments as Var or Bouches-du-Rhône in particular with Rove breed animals (Gauthier, personal communication).

As a BoHV-1 infection is not a likely explanation, the detection of gE-positive and gE-negative results could be explained by large differences in the antigenicity of gE epitopes as described for Pseudorabies virus (Jacobs and Kimman, 1994). Furthermore, BoHV-1 strains that do not express a particular gE-epitope in cell culture can induce antibodies that are detected in a BoHV-1 gE blocking ELISA (van Oirschot et al., 1999). In contrast, the gE-positivity observed could reflect the level of immunisation induced after a CpHV-1 infection. As demonstrated for a BoHV-1 infection, a primary infection leads to a high production of antibody (Muyllkens et al., 2007). Therefore, when a serum is found highly positive by both BoHV-1 gB and gE blocking ELISA, goats could be highly immunised after a recent CpHV-1 infection or reactivation. Contrarily, a lower antibody response could be the result of a previous infection leading to latent persistence. Indeed, latency is closely associated with long-term maintenance of BoHV-1 neutralising antibodies during two to three years after infection (Kaashoek et al., 1996). CpHV-1 could follow the same pattern of evolution and

latently infected goats could have lower antibody levels associated with gB-positive and gE-negative results.

BoHV-1 marker vaccines made of a mutant with a deletion in one of the non-essential genes have been developed to be used in eradication campaigns of IBR initiated in several European countries. The commonest used vaccines are either attenuated or killed viruses carrying a deletion in the gene encoding the glycoprotein E (Bosch et al., 1996; Kaashoek et al., 1994). The control of the infection relies on the use of BoHV-1 gB and gE blocking ELISA in order to differentiate infected and gE-negative vaccinated animals (EFSA, 2005). In the context of the IBR certification in France, some cattle have been identified seropositive to BoHV-1 from herds qualified as free of IBR. An attempted explanation would be the cross-infection of cattle with a related ruminant alphaherpesvirus as for example CpHV-1 (Thiry et al., 2006b). The obtained results do not support this hypothesis. Indeed, CpHV-1 infection is absent in continental France or the seroprevalence is at least very low. Nevertheless, the current sampling is restricted to Dordogne and Vendée, and therefore cannot rule out the presence of CpHV-1 infection in other French regions especially in the Mediterranean part of the country as CpHV-1 infection has been identified in Corsica in the current study.

As demonstrated for BoHV-1 (Kaashoek et al., 1996), CpHV-1-specific humoral response is an indicator of a CpHV-1 infection that has taken place. In addition, specific antibody titres could persist in goats for many years, detection of latently infected animals and flocks by serological tests is therefore reliable and of great diagnostic relevance. Moreover, a gB-positive/gE-negative result could not be attributed to a BoHV-5 cross-infection because BoHV-5 infection has never been described in Europe so far. In regards to the specificity and the sensitivity measured, a BoHV-1 gB blocking ELISA is a useful tool to detect CpHV-1 infection in a flock. A negative result in BoHV-1 gE blocking ELISA could differentiate BoHV-1 from CpHV-1 infection in goats. In the case of a positive result, a cross-

seroneutralisation assay will identify the infection. A continuous monitoring of the epidemiology of ruminant infection with CpHV-1 is essential in European countries involved in IBR control and eradication in order to identify potential BoHV-1 related virus reservoirs which could be implicated in IBR misdiagnosis.

Acknowledgements

The authors are grateful to Lorène Dams and Dominique Ziant for their excellent technical assistance. We also thank the French Departmental Veterinary Analysis Laboratories of La Roche-sur-Yon, Coulounieix-Chamiers and Ajaccio for providing goat serum samples. This work was financed by the Walloon Region of Belgium, Synbiotics Europe and Idexx.

References

- Buonavoglia, C., Tempesta, M., Cavalli, A., Voigt, V., Buonavoglia, D., Conserva, A., Corrente, M., 1996. Reactivation of caprine herpesvirus 1 in latently infected goats. *Comp. Immunol. Microbiol. Infect. Dis.* 19, 275-281.
- Bosch, J.C., Kaashoek, M.J., Kroese, A.H., van Oirschot, J.T., 1996. An attenuated bovine herpesvirus 1 marker vaccine induces a better protection than two inactivated marker vaccines. *Vet. Microbiol.* 52, 223-34.
- Chenier, S., Montpetit, C., Helie, P., 2004. Caprine herpesvirus-1 abortion storm in a goat herd in Quebec. *Can. Vet. J.* 45, 241-243.
- Engels, M., Gelderblom, H., Darai, G., Ludwig, H., 1983. Goat herpesviruses: biological and physicochemical properties. *J. Gen. Virol.* 64, 2237-2247.
- European Food Safety Authority, 2005. Definition of a BoHV-1-free animal and a BoHV-1-free holding, and the procedures to verify and maintain this status. *EFSA Journal* 311, 1-65.
- Grewal, A.S., Wells, R., 1986. Vulvovaginitis of goats due to a herpesvirus. *Aust. Vet. J.* 63, 79-82.

- Guercio, A., Greco, G., Lanizzoto, G., Di Marco, V., Todaro, M., 1998. Valutazione della diffusione di anticorpi anti Herpes Virus della capra in allevamenti caprini della Sicilia. Atti. SIPAOC 12, 138-142.
- Horner, G.W., Hunter, R., Day, A.M., 1982. An outbreak of vulvovaginitis in goats caused by a caprine herpesvirus. N. Z. Vet. J. 30, 152.
- Hugot, S., Bouche, R., 1999. Regard sur les pratiques de l'élevage extensif de la chèvre corse : préalable à la mise en place d'un schéma de sélection. Options Méditerranéennes 38, 137-143.
- Jacobs, L., Kimman, T.G., 1994. Epitope-specific antibody response against glycoprotein E of pseudorabies virus. Clin. Diagn. Lab. Immunol. 1, 500-505.
- Kaashoek, M.J., Moerman, A., Madic, J., Rijsewijk, F.A., Quak, J., Gielkens, A.L., van Oirschot, J.T., 1994. A conventionally attenuated glycoprotein E-negative strain of bovine herpesvirus type 1 is an efficacious and safe vaccine. Vaccine 12, 439-444.
- Kaashoek, M.J., Moerman, A., Madic, J., Weerdmeester, K., Maris-Veldhuis, M., Rijsewijk, F.A., van Oirschot, J.T., 1995. An inactivated vaccine based on a glycoprotein E-negative strain of bovine herpesvirus 1 induces protective immunity and allows serological differentiation. Vaccine 13, 342-346.
- Kaashoek, M.J., Rijsewijk, F.A., Oirschot, J.T., 1996. Persistence of antibodies against bovine herpesvirus 1 and virus reactivation two to three years after infection. Vet. Microbiol. 53, 103-110.
- Keuser, V., Gogev, S., Schynts, F., Thiry, E., 2002. Demonstration of generalized infection with caprine herpesvirus 1 diagnosed in an aborted caprine fetus by PCR. Vet. Res. Commun. 26, 221-226.
- Keuser, V., Espejo-Serrano, J., Schynts, F., Georgin, J.P., Thiry, E., 2004. Isolation of caprine herpesvirus type 1 in Spain. Vet. Rec. 154, 395-399.
- Koptopoulos, G., Papanastasopoulou, M., Papadopoulos, O., Ludwig, H., 1988. The epizootiology of caprine herpesvirus (BHV-6) infections in goat populations in Greece. Comp. Immunol. Microbiol. Infect. Dis. 11, 199-205.
- Kramps, J.A., Banks, M., Beer, M., Kerkhofs, P., Perrin, M., Wellenberg, G.J., Oirschot, J.T., 2004. Evaluation of tests for antibodies against bovine herpesvirus 1 performed in national reference laboratories in Europe. Vet. Microbiol. 102, 169-181.
- Lemaire, M., Schynts, F., Meyer, G., Thiry, E., 1999. Antibody response to glycoprotein E after bovine herpesvirus type 1 infection in passively immunised, glycoprotein E-negative calves. Vet. Rec. 144, 172-176.
- Lyaku, J.R., Nettleton, P.F., Mardsen, H., 1992. A comparison of serological relationships among five ruminant alphaherpesviruses by ELISA. Arch. Virol. 124, 333-341.

- 357 Martin, W.B., Castrucci, G., Frigeri, F., Ferrari, M., 1990. A serological comparison of some
358 animal herpesviruses. *Comp. Immunol. Microbiol. Infect. Dis.* 13, 75-84.
- 359
- 360 Mettler, F., Engels, M., Wild, P., Bivetti, A., 1979. Herpesvirus-infektion bei Ziecklein in der
361 Schweiz. *Schweiz. Arch. Tierheilkd.* 121, 655-662.
- 362
- 363 Miller, J.M., Van der Maaten, M.J., 1984. Reproductive tract lesions in heifers after
364 intrauterine inoculation with infectious bovine rhinotracheitis virus. *Am. J. Vet. Res.* 45, 790-
365 794.
- 366
- 367 Muluneh, A., Liebermann, H., 1990. Occurrence of caprine herpesvirus (BHV-6) infection in
368 goat population of the GDR. *Arch. Exp. Veterinarmed.* 44, 254-257.
- 369
- 370 Muylkens, B., Meurens, F., Schynts, F., Farnir, F., Pourchet, A., Bardiau, M., Gogev, S.,
371 Thiry, J., Cuisenaire, A., Vanderplasschen, A., Thiry, E., 2006. Intraspecific bovine
372 herpesvirus 1 recombinants carrying glycoprotein E deletion as a vaccine marker are virulent
373 in cattle. *J. Gen. Virol.* 87, 2149-2154.
- 374
- 375 Muylkens, B., Thiry, J., Kirten, P., Schynts, F., Thiry, E., 2007. Bovine herpesvirus 1
376 infection and infectious bovine rhinotracheitis. *Vet. Res.* 38, 181-209.
- 377
- 378 Nixon, P., Edwards, S., White, H., 1988. Serological comparisons of antigenically related
379 herpesviruses in cattle, red deer and goats. *Vet. Res. Commun.* 12, 355-362.
- 380
- 381 Ros, C., Belák, S., 1999. Studies of genetic relationships between bovine, caprine, cervine,
382 and rangiferine alphaherpesviruses and improved molecular methods for virus detection and
383 identification. *J. Clin. Microbiol.* 37, 1247-1253.
- 384
- 385 Ros, C., Belák, S., 2002. Characterization of the glycoprotein B gene from ruminant
386 alphaherpesviruses. *Virus Genes* 24, 99-105.
- 387
- 388 Saito, J.K., Gribble, D.H., Berrios, P.E., Knight, H.D., Mc Kercher, D.G., 1974. A new
389 herpesvirus isolate from goats: Preliminary report. *Am. J. Vet. Res.* 35, 847-848.
- 390
- 391 Six, A., Banks, M., Engels, M., Bascunana, C.R., Ackermann, M., 2001. Latency and
392 reactivation of bovine herpesvirus 1 (BHV-1) in goats and of caprine herpesvirus 1 (CapHV-
393 1) in calves. *Arch. Virol.* 146, 1325-1335.
- 394
- 395 StataCorp, 2003. Stata Statistical Software: Release 8, Stata Corporation, College Station,
396 Texas.
- 397
- 398 Tarigan, S., Webb, R.F., Kirkl, D., 1987. Caprine herpesvirus from balanoposthitis. *Aust. Vet.*
399 *J.* 64, 321.
- 400
- 401 Tempesta, M., Cavalli, A., Voigt, V., Buonavoglia, D., 1994. Presenza di anticorpi per
402 caprine herpesvirus 1 (CapHV.1) in allevamenti caprini dell'Italia meridionale. *Atti. SIPAOC*
403 11, 121-122.
- 404
- 405 Thiry, J., Keuser, V., Muylkens, B., Meurens, F., Gogev, S., Vanderplasschen, A., Thiry, E.,
406 2006a. Ruminant alphaherpesviruses related to bovine herpesvirus 1. *Vet. Res.* 37, 169-190.

- Thiry, J., Tempesta, M., Camero, M., Tarsitano, E., Bellacicco, A.L., Thiry, E., Buonavoglia, C., 2006b. A live attenuated glycoprotein E negative bovine herpesvirus 1 vaccine induces a partial cross-protection against caprine herpesvirus 1 infection in goats. *Vet. Microbiol.* 113, 303-308.
- Thiry, J., Keuser, V., Schynts, F., Chartier, C., Tempesta, M., Espejo-Serrano, J., Saegerman, C., Thiry, E., 2006c. Evaluation de la prévalence sérologique de l'infection à herpesvirus caprin 1 dans le sud-ouest de l'Europe. *Epidémiol. et Santé Anim.* 49, 55-58.
- Thiry, J., Engels, M., Tempesta, M., Thiry, E., 2007a. Caprine herpesvirus 1 infection. In: Lefèvre, P.C., Blancou, J., Chermette, R. (Eds), *Infectious and Parasitic Diseases of Livestock*, Lavoisier Editions, Provigny, France, in press.
- Thiry, J., Widén, F., Grégoire, F., Linden, A., Belák, S., Thiry, E., 2007b. Isolation and characterisation of a ruminant alphaherpesvirus closely related to bovine herpesvirus 1 in a free-ranging red deer. *BMC Vet. Res.*, 3:26.
- Tolari, F., White, H., Nixon, P., 1990. Isolation and reactivation of bovid herpesvirus 1 in goats. *Microbiologica* 13, 67-71.
- Toma, B., Dufour, B., Sanaa, M., Bénét, J.J., Shaw, A., Moutou, F., Louza, A., 2001. *Epidémiologie appliquée à la lutte collective contre les maladies transmissibles majeures*, Association pour l'étude de l'épidémiologie des maladies animales, Maisons-Alfort, pp. 49-78.
- van Oirschot, J.T., Kaashoek, M.J., Maris-Veldhuis, M.A., Rijsewijk, F.A., 1999. Strains of bovine herpesvirus 1 that do not express an epitope on glycoprotein E in cell culture still induce antibodies that can be detected in a gE-blocking ELISA. *Vet. Microbiol.* 65, 103-113.
- Wellenberg, G.J., Mars, M.H., van Oirschot, J.T., 2001. Antibodies against bovine herpesvirus (BHV) 5 may be differentiated from antibodies against BHV1 in a BHV1 glycoprotein E blocking ELISA. *Vet. Microbiol.* 78, 79-84.
- Whetstone, C.A., Evermann, J.F., 1988. Characterization of bovine herpesviruses isolated from six sheep and four goats by restriction endonuclease analysis and radioimmunoprecipitation. *Am. J. Vet. Res.* 49, 781-785.

Figure captions**Figure 1**

Distribution of goat population in Corse-du-Sud (a), Dordogne and Vendée (b), and in experimental conditions (c), in regards to the percentage of inhibition from a BoHV-1 gB blocking ELISA. The experimental conditions are explained in the section materials and methods.

Figure 1

(a)

(b)

(c)

Table 1

Serological prevalence of CpHV-1 infection in Corse-du-Sud, Dordogne and Vendée.

Department	Flocks	Animals ^a	Apparent prevalence ^b	True prevalence ^{b, c}
Corse-du-Sud	A	126	72.22 (63.54-79.83)	77.24 (67.96-85.38)
	B	143	49.65 (41.19-58.13)	53.10 (44.05-62.17)
	C	114	54.39 (44.79-63.74)	58.17 (47.90-68.17)
	D	118	73.73 (64.83-81.40)	78.86 (69.34-87.06)
	E	75	54.67 (42.75-66.21)	58.47 (45.72-70.81)
	F	144	55.56 (47.05-63.83)	59.42 (50.32-68.27)
	G	91	41.76 (31.50-52.57)	44.66 (33.69-56.22)
	H	195	66.67 (59.58-73.24)	71.30 (63.72-78.33)
	I	181	38.67 (31.54-46.18)	41.36 (33.73-49.39)
	J	102	63.72 (53.61-73.02)	68.15 (57.34-78.10)
	K	141	65.96 (57.51-73.72)	70.55 (61.51-78.84)
	Total	1430	57.90 (55.29-60.48)	61.93 (59.13-64.68)
Dordogne	L	22	0 (0-12.7)	0 (0-13.58)
	M	304	0 (0-0.99)	0 (0-1.05)
	N	115	0 (0-2.57)	0 (0-2.75)
	O	288	0 (0-0.99)	0 (0-1.06)
	P	15	0 (0-18.1)	0 (0-19.36)
	Q	56	0 (0-5.2)	0 (0-5.56)
	R	7	0 (0-35)	0 (0-37.43)
	S	82	0 (0-3.6)	0 (0-3.85)
	T	51	0 (0-5.7)	0 (0-6.10)
	U	30	0 (0-9.5)	0 (0-10.16)
	V	27	0 (0-10.5)	0 (0-11.23)
	Total	997	0 (0-0.30)	0 (0-0.32)
Vendée	W	121	0 (0-2.44)	0 (0-2.61)

^a Number of goats analysed in BoHV-1 gB blocking ELISA.

^b Seroprevalence expressed in percent with 95% confidence interval in brackets (exact binomial).

^c The true prevalence was measured according to a specificity of 100% and a sensitivity of 93.5%.

Table 2

Serological analysis in BoHV-1 gE blocking ELISA of serum samples detected positive in BoHV-1 gB blocking ELISA.

Flock	Animals ^a	gE-positive	gE-doubtful
A	91	15	4
B	71	11	9
C	62	14	7
D	87	18	6
E	41	7	4
F	80	8	8
G	38	7	0
H	130	10	10
I	70	11	9
J	65	4	2
K	93	14	9
Total	828	119	68

^a Number of goats detected positive in BoHV-1 gB blocking ELISA.

Table 3

Cross serum neutralisation assay of different categories of serum samples against BoHV-1 and CpHV-1.

Categories of serum samples	Neutralising antibody titre against	
	CpHV-1	BoHV-1
Goats experimentally infected with CpHV-1	1:32	1:8
	1:64	1:8
Calves experimentally infected with BoHV-1	<1:4	1:32
	<1:4	1:32
gB-negative/gE-negative ^a	<1:4	<1:4
	<1:4	<1:4
gB-positive/gE-negative ^a	1:8	<1:4
	1:16	<1:4
	1:16	<1:4
	1:32	1:4
	1:32	1:8
	1:64	1:4
	1:64	1:8
	1:64	1:16
gB-positive/gE-doubtful ^a	1:4	<1:4
	1:8	<1:4
	1:8	<1:4
	1:16	<1:4
	1:16	1:4
	1:16	1:4
	1:32	<1:4
	1:64	1:16
	1:64	1:32
	1:128	1:16
gB-positive/gE-positive ^a	1:8	<1:4
	1:8	<1:4
	1:16	<1:4
	1:16	1:4
	1:32	<1:4
	1:64	1:4
	1:64	1:8
	1:64	1:8
	1:64	1:16
	1:64	1:16

^a serum samples detected negative, doubtful or positive in BoHV-1 gB and gE blocking ELISA.