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EPIDEMIOLOGICAL STUDY OF BORDER DISEASE VIRUS INFECTION IN SOUTHERN CHAMOIS (*RUPICAPRA PYRENAICA*) AFTER AN OUTBREAK OF DISEASE IN THE PYRENEES (NE SPAIN)

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Abstract

In 2001 and 2002, an outbreak of a previously unreported disease, associated with a border disease virus (BDV), caused high mortality in the Southern chamois (*Rupicapra pyrenaica*) population in the Alt Pallars-Aran National Hunting Reserve in the Catalan Pyrenees (NE Spain). Between 2002 and 2006, sera and/or tissue samples taken from 116 healthy chamois shot during the hunting season, plus 42 from chamois affected by different diseases, were studied. A blocking enzyme-immunosorbent assay (ELISA) was used to study pestivirus seroprevalence in 114 healthy hunted and 31 diseased chamois, yielding positive results in 73.7% and 22.6% of the chamois respectively. Comparative virus neutralization tests (VNT) performed on 42 seropositive samples with 6 pestivirus strains yielded statistically higher titres to BDV Spain 97, followed by BDV chamois, BDV 137/4, BDV Moredun, Bovine Diarrhoea virus-1 (BVDV-1) NADL and BVDV-2 atypical. Virological investigations for pestivirus detection were performed using an antigen ELISA test in 82 healthy and 18 diseased chamois, RT-PCR in 16 healthy and in all diseased chamois, and virus isolation in 14 diseased chamois. No viral antigen was detected in any of the healthy animals. A pestivirus, characterized as BDV by monoclonal antibodies, was detected in the 10 chamois showing clinical signs consistent with BDV infection. Sequence analysis in the 5' unstranslated region (5'-UTR) revealed that they were grouped into the BDV-4 genotype. In the remaining chamois, infectious keratoconjunctivitis, pneumonia, trauma and contagious ecthyma were diagnosed. The cause of death was unknown in 5 chamois. The results suggest that the infection has become endemic in the population and that it could have a significant impact on chamois population dynamics.

KEY WORDS: pestivirus, border disease virus, chamois, *rupicapra pyrenaica*

1. INTRODUCTION

Border disease virus (BDV) is one of the four main viral species recognized within the genus Pestivirus in the family Flaviviridae (Thiel et al., 2005). Although pestiviruses have been designated according to the species of isolation, extensive interspecies transmission has been demonstrated, particularly with bovine viral diarrhoea virus (BVDV) and BDV. Border Disease

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(BD) can therefore be caused by three of the four species currently recognized as members of the genus Pestivirus: BDV, BVDV-1 and BVDV-2 (Sullivan et al., 1995, 1997; Gianaspero et al., 2004; Olde Riekerink et al., 2005).

BD is a congenital disease affecting mainly sheep and goats, and has substantial loss-related economic implications world-wide. Clinical manifestations include barren animals, abortions, stillbirths and small and weak lambs, sometimes with tremor, abnormal body conformation, hairy fleece and abnormal pigmentation. After birth, it usually causes a transient, mild disease and stimulates the production of long-lasting virus-neutralising antibodies. Persistently infected (PI) animals, which eliminate the virus almost constantly through secretions and excretions, are the single most important source of infection for other animals (Nettleton et al., 1992, 1998; Nettleton, 2000).

In addition to cattle, goats, sheep and pigs, ruminant pestiviruses have been isolated from many other wild ruminant species, and serologic surveys have demonstrated prior infection with pestiviruses in more than 40 species worldwide, but there are few confirmed cases of isolation of a ruminant pestivirus and disease, and there is no evidence that they have significant population impacts on free-ranging animals (Van Campen et al., 2001; Vilcek and Nettleton, 2006). However, an outbreak of a previously unreported disease, associated with a new pestivirus belonging to the BDV virus cluster, was reported in the Catalan Pyrenees (NE Spain) in 2001 and 2002, and was supposedly responsible for a population decrease of 40-45 % in the most affected areas. It was the first time a BDV had been associated with an outbreak of disease in a wild species (Arnal et al., 2004; Hurtado et al., 2004; Marco et al., 2007). For the first time in French Pyrenean chamois, a BDV has been described in spleen samples from two animals (Frolich et al., 2005). More recently, it has been suggested that this infection has been widespread in the chamois population of the Orlu Reserve in the French Pyrenees since 1995, even though no mass mortality or chamois with clinical signs have been observed (Pioz et al., 2007).

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In this study, we investigated the seroprevalence and viroprevalence of BDV infection in the Southern chamois population of the area affected by the previously reported outbreak of disease. The detected BDV were characterised at the genetic level.

2. MATERIALS AND METHODS

2.1. Animals and samples

All chamois were captured at the Alt Pallars-Aran National Hunting Reserve (42º 33'N, 1º 19'E) in the Central Pyrenees (Catalonia, NE Spain), bordered to the north by France and to the east by Andorra, covering approximately 100,000 ha (Fig. 1). The total census of the chamois population dropped from 3,880 in 2000, before the outbreak of a BDV-associated disease, to 2,670 at the end of 2002, shortly after the outbreak was supposedly over. While the population remained stable in 2003 and 2004, the number of chamois decreased slightly in 2005 and 2006 (2,380 and 2,280 chamois counted, respectively), even though no signs of epidemics or substantial mortality were observed.

Between October 2002 and December 2006, sera and/or spleen samples from 158 chamois were studied after the outbreak described above. Samples from 116 adult chamois (55 female, 31 male and 30 undetermined, age-range 4 to 21 years) were collected during the hunting season (September-December): 60 animals were studied in 2002, 22 in 2003 and 34 in 2004. Rangers accompanying hunters were asked to collect a cardiac blood sample and the spleen, immediately after shooting. All animals appeared healthy at time of cull. It was not possible to obtain samples in 2005 and 2006 due to a hunting ban imposed because of the decrease in chamois population numbers.

Another 42 chamois found diseased (16 female and 26 male, age range 2 months to 17 years) were collected in the Reserve. Four chamois were collected in 2002, four in 2003, ten in 2004, eight in 2005 and 16 in 2006. Necropsy and routine bacteriological and histological methods were performed on all diseased chamois. Spleen samples were collected from all animals, blood samples being available for only 31 chamois.

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On arrival at the laboratory, blood samples were centrifuged at 1200xG for 15 minutes, and sera obtained frozen at -20ºC, awaiting serological analyses. Spleen samples were also frozen at -20ºC.

2.2. Laboratory analyses

Serological analyses to detect pestivirus-specific antibodies were performed in 114 healthy hunted chamois and 31 diseased chamois, using a blocking enzyme linked immunosorbent assay (ELISA) (Synbiotics, Lyon, France), that detects specific antibodies targeted against the p80/125 protein common to all BVDV and BDV strains.

In order to confirm the ELISA test results, as well as determine antibody specificity, sera from 42 positive healthy chamois were tested with a comparative virus neutralization test (VNT) for neutralizing antibodies against BVDV-1 strain NADL, BVDV-2 strain atypical, BDV strain Spain 97 (Vega et al., 2002), BDV strain Moredun, BDV strain 137/4 and BDV strain chamois isolated from the affected chamois in 2002. Neutralizing antibody titres were expressed as the reciprocal of the highest dilution that neutralized 100 tissue culture infective doses (100 TCID 50%) in all cultures. Titres of 1:10 and higher were considered as positive. Viral replication was monitored by the Immuno-Peroxidase Monolayer Assay (IPMA) with polyclonal home-made pestivirus-specific serum.

Spleen homogenates were tested for pestivirus antigen by a sandwich ELISA antigen test (Synbiotics, Lyon, France) in 82 healthy hunted and 18 diseased chamois. In order to confirm the antigen ELISA test results, reverse transcription-polymerase chain reaction (RT-PCR) was performed in 16 of the healthy hunted chamois and in all 42 diseased chamois, using previously described panpestivirus primers (Pesti 3 and Pesti D) (Katz et al., 1993; Hofmann et al., 1994). Virus isolation was performed in 14 of the chamois (5 animals showing signs consistent with BDV infection and 9 with other diseases), in accordance with the OIE Manual of Diagnostic Tests (OIE, 2004). Briefly, the homogenates of spleen and kidney were inoculated on a

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confluent monolayer of permanent sheep-line pestivirus-free SFT-R cells obtained from the Federal Institute for Virus Diseases of Animals (Island of Riems, Germany). The presence of virus was detected by the IPMA test using a polyclonal home-made pestivirus-specific serum.

The viruses isolated from 4 chamois were antigenically characterised by the IPMA test using WS363 (BDV specific) and WV433 (BVDV-2 specific) monoclonal antibodies obtained from the Central Veterinary Laboratory of Weybridge (UK), as well as C-16 (pestivirus-specific), CA-3 and CT-6 (BVDV-1-specific), obtained from the Reference Laboratory of Classical Swine Fever (Hannover, Germany).

Sequence analyses of the 243 bp fragment of the 5'UTR region of 8 isolates were performed using primers 324 and 326 (Vilcek et al., 1994). Purified amplicons (Minelute Gel Extraction Kit, Qiagen) were analyzed with Big Dye Terminator v.3.1 Kit and the ABI 3130xl Genetic Analyzer (Applied Biosystems, Warrington, UK).

The phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) using automatic root location. A bootstrap analysis of 1000 replicates was performed by creating series of bootstrap samples to test tree branch reliability.

2.3. Statistical analysis

SAS 9.1 software was used for statistical evaluation. The proportion of affected animals by sex and year was evaluated by a Pearson's chi-square test. To analyze the antibody neutralizing titres to the different pestiviruses, data normality was checked using the Shapiro-Wilk statistic test. Since data was not normally distributed, the Friedman's rank test was used. Mean comparisons were made using the Fisher protected least significant difference test (LSD) with the rank sum multiple comparison test (Ipe, 1987).

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3. RESULTS

Of the 114 healthy chamois sera tested, 82 (71.9%) showed a positive ELISA result (Table 1). Sex information was available in only 84 chamois. Of the 55 females and 29 males, 40 (72.7%) and 22 (75.8%) respectively were positive. The proportion of seropositive males and females was not significantly different (p = 0.75). Seroprevalence varied throughout the 3 years of study. In 2002, shortly after the outbreak, 81.0% of the chamois were positive; the figures for 2003 and 2004 were 72.7% and 55.9% respectively. The proportion of positive samples during the three years was significantly different only between 2002 and 2004 (p=0.018). The comparative VNT confirmed the ELISA results in the 42 chamois tested (Table 2). The highest antibody titres observed were for the BDV strain Spain 97, followed by BDV chamois, BDV 137/4, BDV Moredun, BVDV-1 and BVDV-2 strains. Statistical differences were found in antibody titres between almost all strains tested, except between BDV Moredun and BVDV-1 NADL, and BVDV-1 and BVDV-2. Negative results were obtained, either with the ELISA antigen test or by RT-PCR, for pestivirus presence in all the healthy chamois tested (Table 1).

Of the 42 chamois studied with different diseases, 13 were diagnosed with infectious keratoconjunctivitis, six with bacterial pneumonia, seven with trauma (three due to dog attack, two due to illegal hunting and two with severe long bone fractures), one with contagious ecthyma and 10 had clinical signs consistent with the disease associated with a previously-reported BDV. In five chamois, including two very old animals, it was not possible to determine the cause of death. The ELISA antibody test yielded positive results in seven of the 31 sera (22.6%) available for testing: four of eight chamois with infectious keratoconjunctivitis, one of four chamois with trauma, the chamois with contagious ecthyma and in one of three chamois with unknown cause of death. Sera available from chamois with pneumonia (3) and BDV infection (8) were negative for this test (Table 1). The 10 chamois with clinical signs consistent with BDV infection were found in 2005 (4) and 2006 (6) (Table 3). RT-PCR results were positive in all cases, and a pestivirus was isolated from the spleen of 5 chamois (Table 1). Four isolates reacted positively with C-16 and WS363 monoclonal antibodies and were negative for WV433, CA-3 and CT-6 monoclonal antibodies. Of the 32 chamois with other diseases, 18 were tested

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with the ELISA-antigen test, four being positive; however the RT-PCR and virus isolation tests were negative. The remaining chamois (28) were also negative for the RT-PCR test.

The genetic typing of the eigth pestivirus analyzed revealed that chamois were infected with BDV-4 genotype (Fig. 2). The sequences involved this study are deposited in GenBank under name and accession number: ARAN-1 (AM765800), ARAN-2 (AM765801), ARAN-3 (AM765802), ARAN-4 (AM765803), ARAN-5 (AM765804), ARAN-6 (AM765805), ARAN-7 (AM765806), ARAN-8 (AM765807).

4. DISCUSSION

The outbreak of disease associated to a previously-reported BDV in Southern chamois (Marco et al., 2007), was a novel event, since in the literature there is no report of disease associated with this virus in a wild species (Van Campen et al., 2001; Vilcek and Nettleton, 2006). After this severe die-off episode in 2001 and 2002, no more epidemics or significant mortality have been observed in the Reserve. Thus, it was conceivable that the chamois population had acquired a certain level of immunity. This hypothesis was confirmed by the overall high seroprevalence (71.9%) observed in this study. However, seroprevalence decreased significantly between 2002 and 2004. It was not possible to study the population's immune status in 2005 and 2006 because of a hunting ban due to the slight population decrease over these two years. An overall similar high seroprevalence (70.3%) has been found in Southern chamois from the Orlu Reserve in the French Pyrenees, about 40 km in a straight line from our study area (Figure 1) (Pioz et al., 2007). However, no mortality or disease outbreak has ever been reported. In other areas of the Pyrenees, where no pestivirus or associated disease have been detected, seroprevalence varied from 7.5% to 27.3% (Arnal et al., 2004).

In other geographical areas where chamois are found (e.g. the Alps) serological and virological pestivirus studies have shown different results. A pestivirus antibody prevalence of 5.6% and 25.5% was found in France and Italy respectively (Baradel et al., 1988; Olde Riekerink et al., 2005), whereas negative results were obtained in Austria and elsewhere in the Italian Alps

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(Citterio et al., 2003; Krametter et al., 2004). None of these studies has detected a pestivirus. However, recently in the French Alps a pestivirus was identified by RT-PCR in several healthy chamois (Gibert and Gauthier, unpublished data).

In the comparative VNT, the chamois sera showed high titres of neutralizing antibodies not only for the BDV chamois strain, but also for the other BDV strains, specially the BDV Spain 97 (Table 2), which has a high homology with the unusually virulent BDV strain Aveyron (Vega et al., 2002). These results concur with a previous study, in which antisera raised against different BDV strains neutralized heterologous BDV strains to a similar extent (Becher et al., 2003). Interestingly, in our study the highest titres of neutralizing antibodies were observed against the BDV Spain 97 strain. Neutralizing antibody titres against members of heterologous pestivirus species have been reported to be at least eightfold lower than against members of the homologous species (Avalos-Ramirez et al., 2001). However, in our study, we have found these differences between homologous species. Titres for the BDV strain Spain 97 were more than tenfold higher than for the chamois strain in 4 animals (numbers 14, 17, 38 and 41), and eightfold higher in 5 (chamois 8, 12, 25, 29, 31) (Table 2). This may indicate the existence of an additional independent infection process due to another BDV strain (most probably of ovine origin) in the chamois population. Sheep and cattle graze from May to September in alpine meadows and frequently share grazing areas and water sources with chamois. Thus it is feasible that sheep could have played a key role in the spread of BDV among chamois (Olde Riekerink et al., 2005). However, this possibility needs to be further investigated, since clustering strains by neutralization test is only correct if antisera for each strain are included (true cross-neutralization studies) (Dekker et al., 1995). Chamois number 10 was a 6-year-old female with very high antibody titres for the BDV chamois strain, and was the only case in which titres were clearly higher (more than tenfold) than those to BDV Spain 97 (Table 2). This would most probably indicate a recent natural infection in this animal with the chamois strain.

In Spain, the presence of BDV among extensively kept sheep has been described recently (Hurtado et al., 2003; Valdazo-Gonzalez et al., 2006, 2007). The authors of the reports suggest

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that the BDV circulating in Spanish sheep belongs to group BDV-4 (formerly named BDV-C), the same one as suggested for the chamois strain in the Pyrenees (Arnal et al., 2004; Hurtado et al., 2004) and in the present study. This widespread distribution of BDV in sheep in some Spanish regions highlights the potential risk of sheep as a pestivirus reservoir for other species (Berriatua et al., 2006; Valdazo-Gonzalez et al., 2006). It is also known that sharing alpine pasture is instrumental in the transmission of pestivirus in cattle (Braun et al., 1998).

In the current study, neither the antigen ELISA nor the RT-PCR tests detected any pestivirus in the healthy hunted chamois, even though it is feasible that the virus is still circulating in the Reserve's chamois population. This notwithstanding, four of the diseased chamois (one with pneumonia, two with trauma and one with contagious ecthyma) gave positive results to the ELISA antigen test, but negative to RT-PCR, suggesting that they must have been false positive animals. In 10 of the diseased chamois, clinical signs consistent with BDV infection previously described were observed (Marco et al., 2007). These included depression, weakness, as well as varying degrees of alopecia and skin hyperpigmentation of the head, neck and trunk. A pestivirus was detected by RT-PCR in all cases, characterised by monoclonal antibodies as BDV; sequence and phylogenetic analysis revealed BDV-4 genotype grouping and the same isolate as was found previously (Arnal et al., 2004; Hurtado et al., 2004). Moreover, in these same animals, the ELISA antibody test was negative. After the outbreak of a BDV-associated disease in 2001 and 2002, a single clinical case was detected in 2003 (Marco et al., 2007). Since then, no further clinical cases had been observed until June 2005 (4 cases) and 2006 (6 cases) (Table 3). These cases could be related to a decrease in seroprevalence in 2004 and virus persistence in the population. A possibly age-structure-dependent pestivirus infection dynamic in chamois has been proposed in animals from the French Pyrenees: a high number in population juveniles was related to low seroprevalence and high viroprevalence, mostly in animals under 2 years of age (Pioz et al., 2007). This concurs with the clinical course of the infection in domestic ruminants, where young animals are most susceptible to acute infections (Loken et al., 1991; Houe, 1999) but not with the infection's clinical course in this study's mainly adult chamois. However, we cannot rule out the hypothesis that more young animals have also

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been infected because in free-ranging wildlife, kids and young corpses are more difficult to find since they are smaller and quickly disposed of by scavengers.

It is not known for certain whether PI animals exist in wild ruminants, and very few cases have been reported (Anderson and Rowe, 1998, Grondahl et al., 2003), including one chamois during the 2002 disease outbreak (Hurtado et al., 2004). In sheep, moderate and high seroprevalence is associated with the presence of PI animals (Berriatua et al., 2004). Thus, the same could hold for the chamois population in this study, since we have detected high seroprevalence; the chamois with clinical BDV-associated disease were all seronegative, and most probably PI animals. However, it is difficult to conclude that these chamois were PI since most of them were adult or old animals (one was 11 years old), and survival of PI animals in domestic BDV or BVDV-infected ruminants is supposed to be poor (Nettleton et al., 1992; Radostits et al., 2000).

BD in sheep is mainly a congenital disease leading to fetal death or low survival rates in kids (Nettleton et al., 1992). We do not know whether this is the case in the chamois population, since abortions and dead kids are difficult to find in nature, being quickly disposed of by scavengers, as mentioned previously. The lack of recovery in Reserve chamois numbers after the outbreak of disease, and even the slight population decrease in recent years, as indicated by the annual census, could be related to these disorders. In the nearby French Reserve of Orlu, it has been suggested that BDV infection in chamois is detrimental to reproduction and for juveniles (Pioz et al., 2007). This idea would also be supported by initial testing showing that inoculation of the virus in a pregnant chamois female can lead to the birth of a PI offspring that dies prematurely (Gibert, Schelcher and Vautrain, unpublished observation).

Concomitant diseases should be investigated because they could also be responsible for the chamois population decrease. Since 2003 a wide-ranging outbreak of infectious keratoconjunctivitis has been spreading over an even larger area than that affected by the pestivirus. We were able to study 13 cases, and in none of them was a pestivirus detected by RT-PCR, even though three chamois had anti-pestivirus antibodies. Thus, it would seem that

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both infectious agents circulate independently in the population, both most probably having some impact on host population dynamics.

After the first outbreak of BDV-associated disease in 2001 and 2002, the high seroprevalence found in the population, together with the sporadic cases of BDV infection detected in recent years, suggest that the infection has become endemic in this area. However, the infection could still be responsible for the continued slight decrease in the chamois population over the past two years, even though other diseases, such as infectious keratoconjunctivitis, have been detected. Infection of the chamois population and sheep flocks with different BDV strains deserves further attention, since it is feasible that different pestiviruses are circulating in both directions. Recently, severe new outbreaks of pestivirus-associated disease in chamois have been detected in other parts of the Pyrenees, such as the Cerdanya-Alt Urgell and Cadi National Hunting Reserves (Figure 1) (Marco et al., 2006), which may suggest that the infection is becoming an important emerging disease for chamois.

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Figure 1. Map of the study area showing the National Hunting Reserves in Catalonia, NE Spain (1 Pallars-Aran, 2 Cerdanya-Alt Urgell, 3 Cadí) and the Réserve Nationale d'Orlu (4) in France.

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Table 1. Chamois tested for Pestivirus seropositivity and viropositivity at the Pallars-Aran National Hunting Reserve.

	Healthy chamois (positive/tested)	Chamois with suspected BDV infection (positive/tested)	Chamois with other diseases (positive/tested)
ELISA Ac	82/114	0/8	7/23
ELISA Ag	0/82	-	4/18
RT-PCR	0/16	10/10	0/32
Virus isolation	-	5/5	0/9

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Table 2. Virus neutralization titres of 42 Southern chamois sera against six different pestivirus strains.

Chamois	BDV Spain 97	BDV chamois	BDV 137/4	BDV Moredun	BVDV-1 NADL	BVDV-2 atypical
1	2560	1280	1280	80	20	80
2	640	1280	160	80	80	10
3	320	1280	160	5	5	0
4	80	160	40	5	5	0
5	320	160	80	0	0	0
6	20	5	0	0	0	0
7	160	320	640	20	40	20
8	640	80	10	0	0	0
9	160	40	20	0	0	5
10	1280	20480	1280	640	320	160
11	2560	2560	1280	40	20	5
12	640	80	20	5	0	0
13	1280	320	1280	20	40	40
14	2560	160	320	0	5	10
15	80	20	40	0	0	0
16	640	640	320	10	5	5
17	1280	40	80	20	0	20
18	320	320	320	20	10	10
19	5120	2560	320	20	40	80
20	640	1280	320	0	10	10
21	320	320	160	5	5	40
22	80	80	20	0	0	0
23	640	640	160	1280	80	20
24	320	160	160	10	20	0
25	320	40	40	40	10	0
26	20	0	0	0	0	0
27	2560	5120	640	2560	640	80
28	10	80	10	0	0	0
29	2560	320	640	640	320	10
30	320	80	80	10	10	20
31	1280	160	160	40	40	10
32	160	80	20	0	0	0
33	160	320	80	40	40	20
34	640	320	320	40	160	10
35	320	160	160	40	80	20
36	640	160	320	40	80	10
37	40	10	0	0	0	0
38	1280	40	160	320	40	0
39	160	40	20	0	0	0
40	160	80	20	10	40	10
41	640	40	80	20	20	20
42	320	80	40	80	160	10

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Table 3. Sampling date, sex and age of Southern chamois studied with BDV infection.

Chamois	Sampling date	Sex	Age (yrs)
A	June 2005	Female	5
B	September 2005	Male	7
C	November 2005	Male	6
D	December 2005	Male	7
E	July 2006	Male	2
F	July 2006	Male	11
G	July 2006	Female	1
H	September 2006	Male	3
I	September 2006	Female	4
J	November 2006	Female	5

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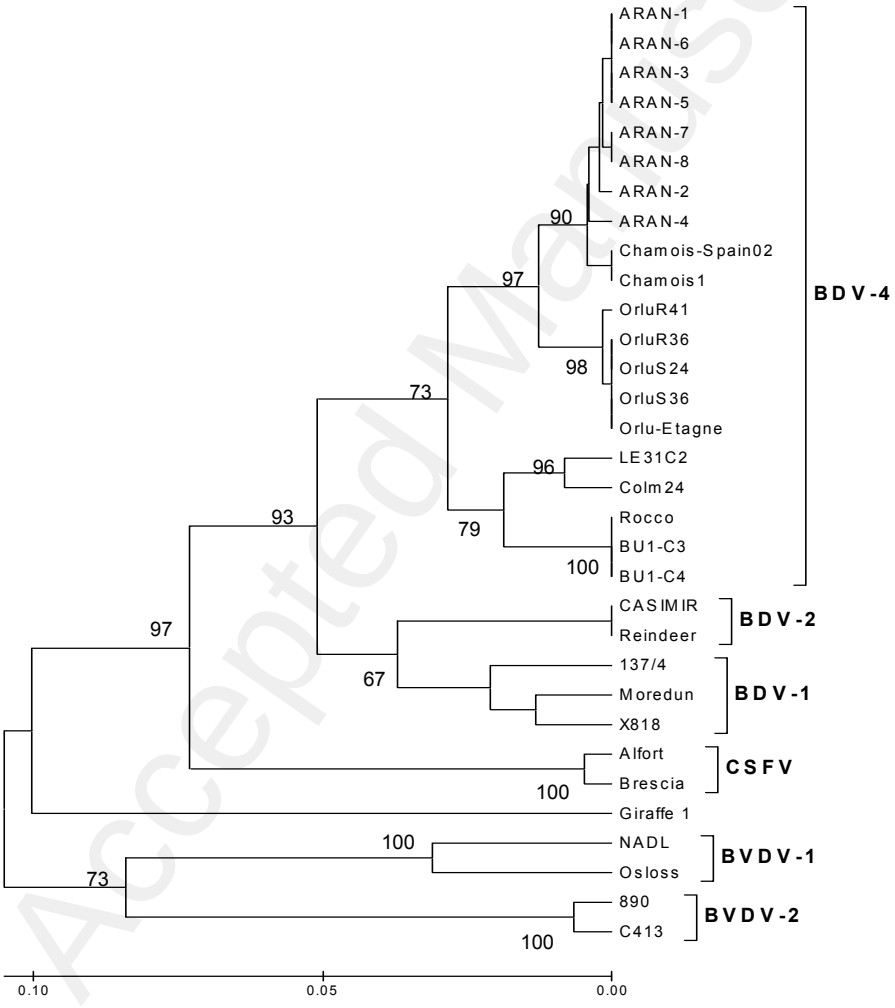
Fig. 2. Unrooted neighbour-joining phylogenetic tree based on the 5'UTR sequence among pestiviruses. Chamois strains grouped separately in BDV-4. Branches of chamois isolates show two subgroups: first includes all isolates from Vall d'Aran-Andorra (current study isolates, Chamois Spain-02 (Hurtado et al., 2004) and Chamois-1(Arnal et al., 2004)); second includes isolates from Orlu Reserve, France (Pioz et all, 2006). Branch numbers indicate bootstrap values (in percent; 1000 replicates). Sequences of strains taken from GenBank with following accession numbers: Chamois-Spain02 (AY641529), Chamois-1 (AY738080), OrluR41 (DQ898295), OrluR36 (DQ898294), OrluS24 (DQ898292), OrluS36 (DQ898293), Orlu-Etagne (DQ898291), LE31C2 (DQ361072), Colm24 (DQ361073), Rocco (DQ361067), BU1-C3 (DQ361068), BU1-C4 (DQ361069), CASIMIR (AB122085), Reindeer (AF144618), 137/4 (U65052), Moredun (U65023), X818 (AF037405), Alfort (X87939), Brescia (M31768), Giraffe1 (AF144617), NADL (M31182), Osloss (M96687), 890 (U18059), C413 (AF002227).