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SURVEILLANCE OF BORDER DISEASE IN WILD UNGULATES AND AN OUTBREAK IN PYRENEAN CHAMOIS (*RUPICAPRA PYRENAICA PYRENAICA*) IN ANDORRA

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ABSTRACT: The Principality of Andorra is surrounded by areas in which Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) populations were severely affected by infection with border disease virus (BDV) which caused disease outbreaks between 2001 and 2009. Nevertheless, the Andorran chamois populations were not affected during this period. In light of the severe impact of BDV on several of the neighboring Pyrenean chamois populations, we monitored local Andorran populations in an effort to detect pestivirus antibodies and BDV in wild ungulates. In addition, an episode of mortality between 2009 and 2010 in chamois was investigated. We analyzed samples (spleen or serum) from 175 Pyrenean chamois, 284 European mouflon (*Ovis orientalis musimon*), 13 roe deer (*Capreolus capreolus capreolus*), and five wild boars (*Sus scrofa castilianus*). With the exception of three dead chamois found between 2009 and 2010, all samples came from healthy animals hunted during the hunting season. A commercial blocking enzyme-linked immunosorbent assay (ELISA) was used to test sera for antibodies against pestivirus. Positive sera were tested with a comparative virus neutralization test (VNT) using three BDV strains and a bovine viral diarrhea virus strain. Reverse-transcription–polymerase chain reaction (RT-PCR) was performed on all sera and spleen homogenates. Antibodies against pestivirus were detected by ELISA in four of the 69 chamois (5%; 95% CI=1.29–13.11). The VNT confirmed three of these chamois were infected with a BDV. Viral RNA was detected by RT-PCR in three chamois—one apparently healthy animal hunted in 2009 and two dead animals. Viral sequences showed that the three chamois were infected with a BDV-4, the same genotype that was involved in previous episodes of mortality in the Pyrenees. Although Pyrenean chamois from Andorra had had little contact with the pestiviruses until 2009, in this year BDV was associated with a severe disease outbreak.

Key words: Andorra, border disease virus, European mouflon, *Ovis orientalis musimon*, pestivirus, Pyrenean chamois, *Rupicapra pyrenaica pyrenaica*.

INTRODUCTION

Pestiviruses (Family *Flaviviridae*) are enveloped spherical viruses approximately 40–60 nm in diameter. Four species of pestiviruses are accepted by the International Committee on Taxonomy of Viruses: Bovine viral diarrhea virus 1 (BVDV-1) and 2 (BVDV-2) affecting cattle, border disease virus (BDV) infecting sheep and goat, and classical swine fever virus (CSFV) affecting swine (Thiel et al., 2005).

Ruminant pestiviruses are not strictly host-specific, and antibodies against these viruses have been reported in several domestic and wild *Artiodactyla* species (Loken, 1995; Nettleton et al., 1998; Vilcek and Nettleton, 2006). However, no cases of disease caused by pestivirus in free-ranging wild ruminants other than Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) have been reported in Europe. In Pyrenean chamois, BDV genotype-4 (Arnal et al., 2004) has been shown to be the etiologic agent of the disease that appeared for the first time in 2001 in the Alt Pallars-Aran National Hunting Reserve (NHR). After this outbreak, the population was found to have decreased by about 41%, most probably due to the disease (Marco et al., 2007). After this first outbreak, several epizootics occurred in other chamois

populations in the Pyrenees. During 2005, an outbreak led to collapse of the chamois population in the Cerdanya-Alt Urgell NHR, with an estimated cumulative decrease of 85.6%. In June 2005, the disease spread to the nearby Cadí NHR and private hunting areas, causing an estimated cumulative decrease of 63% (Marco et al., 2009). A retrospective study by Marco et al. (2011) revealed that BDV infection had been present in chamois populations since at least 1990, 11 years before the first disease outbreak.

The Pyrenean chamois is a small mountain ruminant. It is one of the most characteristic animals of the Pyrenees and a highly important game species that generates substantial economic benefits for local communities. The disease associated to BDV-4 had great social and economic impact on the Pyrenees. The Pyrenean chamois affected by this disease were depressed and weak and experienced difficulties when moving. Some animals presented abnormal behavior and varying degrees of alopecia and skin hyperpigmentation as well as hematologic and biochemical alterations. At necropsy, cachexia and secondary infectious processes were described in all animals (Marco et al., 2007; Fernández-Sirera et al., 2011).

The Principality of Andorra is surrounded by areas in which chamois populations were severely affected by the disease between 2001 and 2009. Unexpectedly, however, Andorran chamois populations were not affected by BDV during this period. Given that BDV infection had been related to severe declines in chamois populations in several areas of the Pyrenees, we surveyed for pestivirus antibodies and BDV in wild ungulates in Andorra. An episode of mortality between 2009 and 2010 was also investigated.

MATERIALS AND METHODS

Study area

The study area consisted of the Principality of Andorra, a 468-km^2 country situated in the Pyrenees bordering on France and Spain (Fig. 1). Andorra is a mountainous country with 65 peaks over 2,500 m. The country's hunting areas are divided into three types: there are two small reserves in which hunting is banned and the Enclar Hunting Reserve (HR; 42°53′N, 1°46′E) where hunting is managed by the Andorran Government; chamois hunting is allowed in the rest of the country and hunters apply great pressure to chamois. Consequently, chamois in the hunting reserves are relatively isolated and reach moderate to high densities (18 chamois/100 ha in the Enclar HR) while in the rest of the territory densities are low (2 chamois/100 ha).

Animals

We analyzed samples from 175 Pyrenean chamois, 284 European mouflon (*Ovis orientalis musimon*), 13 roe deer (*Capreolus capreolus capreolus*), and five wild boars (*Sus scrofa castilianus*). All samples came from healthy animals hunted during the hunting season (Table 1). At the beginning of 2010, chamois hunting was banned in Andorra due to the decline in its population, and no more samples were obtained from this source.

Samples were also obtained from three chamois carcasses found between 2009 and 2010: spleen samples were taken from a 1-yr-old male chamois found dead with cachexia and pneumonia and also from a very scavenged and decomposed chamois carcass; the third sample was taken from the brain of a decomposed and scavenged chamois carcass.

Blood samples were obtained by cardiac puncture from hunted animals. Spleen was obtained after hunting and at necropsy from two of the three carcasses. In one case only was the head (brain) available. Blood samples were placed in sterile serum separator tubes (Eurotubo, Rubí, Spain) and centrifuged at 1,200 × G for 15 min. Sera, spleen samples, and the brain sample were stored at −20 C until analyzed.

Serologic tests

Sera were tested for antibodies against pestivirus with a commercial blocking enzyme-linked immunosorbent assay (ELISA; BVD/MD/BD P80, Antibody Screening, Pourquier, Montpellier, France). This test has a minimum specificity of 99.2% and an observed sensitivity of 100% for testing BDV-positive sheep sera. In order to confirm the results of the ELISA and to determine the specificity of the antibodies, ELISA-positive sera were tested with a comparative virus neutralization test (VNT) using BVDV-1 strain NADL

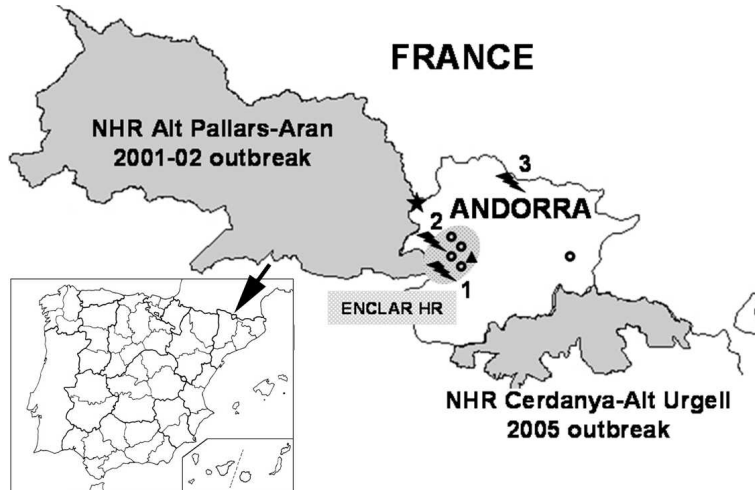

FIGURE 1. The Principality of Andorra (inset; arrow), and its borders with two national hunting reserves (NHR) in Spain, where important outbreaks of border disease have recently occurred (main image) in Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*); and the most relevant findings regarding the outbreak. Enclair Hunting Reserve (HR) is the oval, shaded area within Andorra. ↘ Border disease virus RT-PCR-positive cases (chamois 1, 2, and 3). Chamois 1 was hunted and was an apparently healthy animal; chamois 2 and 3 were carcasses with clinical signs consistent with pestivirus infection. ▲ Carcass with lesions consistent with pestivirus infection unconfirmed by RT-PCR. ○ Carcasses with lesions consistent with pestivirus infection not analyzed by RT-PCR. ☆ RT–PCR-positive chamois found in Andorra in 2002 (Arnal et al., 2004).

(GenBank accession number M31182; Collett et al., 1988), BDV-1 strain 137/4 (GenBank accession number U65052; Vilcek et al,. 1997), BDV-4 strain Esp97 (GenBank accession number FR714860), and BDV-4 strain Aran 1 (GenBank accession number AM765800; Marco et al., 2008). This final strain was isolated from a diseased chamois found in 2005 in the Spanish NHR of Alt Pallars-Aran, which borders Andorra to the west.

VNT was performed as previously described (OIE, 2008) using Madin–Darby bovine kidney (MDBK) cells. Neutralizing antibody titers were expressed as the reciprocal of the highest dilution that neutralized 100 tissue culture infective doses ($TCID_{50}$) in all cultures, calculated according to Reed and Muench (1938). Titers of 1:10 and higher were considered positive. Viral replication was monitored by the immuno-peroxidase monolayer assay (IPMA)

TABLE 1. Samples from hunted ungulates in Andorra by species, hunting season, quantity, and quantity and type of samples.

Species[a]	2004–2005	2005–2006	2006–2007	2007–2008	2008–2009	2009–2010	Total *n*
Chamois	*n*=13 13 sera	*n*=15 15 sera	*n*=1 2 sera	*n*=45 1 serum 44 spleen	*n*=48 19 sera 44 spleen	*n*=53 19 sera 48 spleen	175
Mouflon	*n*=46 46 sera	*n*=110 110 sera	*n*=68 68 sera	*n*=60 60 sera	–[b] –	– –	284
Wild boar	– –	*n*=2 2 sera	*n*=3 3 sera	– –	– –	– –	5
Roe deer	*n*=1 1 sera	*n*=6 6 sera	*n*=3 3 sera	*n*=3 3 sera	– –	– –	13

[a] Chamois = *Rupicapra pyrenaica pyrenaica* ; mouflon = *Ovis orientalis musimon*, wild boar = *Sus scrofa castilianus*; roe deer = *Capreolus capreolus capreolus*.

[b] Dashes indicate no sample.

with home-made polyclonal pestivirus-specific serum.

Virus detection

Reverse-transcription–polymerase chain reaction (RT-PCR) was performed on all sera, spleen homogenates, and one brain homogenate using described panpestivirus primers 324 and 326 (Vilcek et al., 1994) and a commercial kit (One-Step PCR kit, Qiagen, Hilden, Germany). Before the RT-PCR, viral RNA was extracted using a commercial kit (Nucleospin Viral RNA Isolation, Macherey Nagel, Düren, Germany).

Sequence analysis

Sequence analyses of the 243-base pair fragment of the 5′UTR region of the RT-PCR positive samples 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 1,000 replicates was performed by creating series of bootstrap samples to test tree branch reliability (Fig. 2).

We calculated true prevalence and its 95% confidence interval using the functions for analyzing epidemiologic data in the library epiR (Stevenson, 2011).

RESULTS

Surveillance program

Antibodies against pestivirus were detected by ELISA in four (one animal hunted in 2005, another in 2006, and two in 2009) of the 69 chamois (5%; 95% CI=1.29–13.11). No antibodies were found in other species. The neutralizing antibody titers of the positive chamois (named *a, b, c*, and *d*) are shown in Table 2. The World Organisation for Animal Health (OIE) has established that a 3-fold difference or more between endpoints of two titrations should be considered decisive for an infection by the virus species yielding the highest titer (OIE, 2008). However, no rule has been established for different subgroups of the same pestivirus species. Three chamois (*a*, *c*, and *d*) had BDV-specific antibodies, but the differences between titers against the different BDV strains were too small to determine the BDV infective strain. The fourth chamois (*b*) had less than 3-fold differences between the titers against BVDV and BDV.

Viral RNA was detected by RT-PCR in one apparently healthy chamois hunted in December of 2009 (hereafter chamois 1), and genetic typing revealed that the genotype in this case was BDV-4. The virus appears in the phylogenetic tree as ANDORRA-1 (Fig. 2). Information about this virus RNA-positive chamois appears in Table 3.

Description of outbreak

In October 2009 during the monitoring program, a chamois carcass was found in the west of the Enclar HR, near the border with the Spanish Alt Pallars-Aran NHR. In the same area, two more chamois carcasses were detected in November and December; subsequently, an unusually high number of carcasses was found there in January 2010. In all, between October 2009 and March 2010, 79 carcasses were found. All were found in the Enclar HR except for one carcass found in the north of the country in January 2010 and another in the east in March 2010 (Fig. 1). All but six carcasses consisted of only skin and bones, and necropsy could only be performed on three. Viral RNA was detected by RT-PCR in two (hereafter chamois 2 and 3; Table 3). Genetic typing of these viruses confirmed that these chamois were infected with BDV-4 and were named as ANDORRA-2 and ANDORRA-3 (Fig. 2).

Although the total chamois mortality could not be assessed accurately due to the remoteness of the area involved, the census undertaken by the Andorran Government provides an indirect indication of mortality by determining population decrease. In the 2009 census, just before the

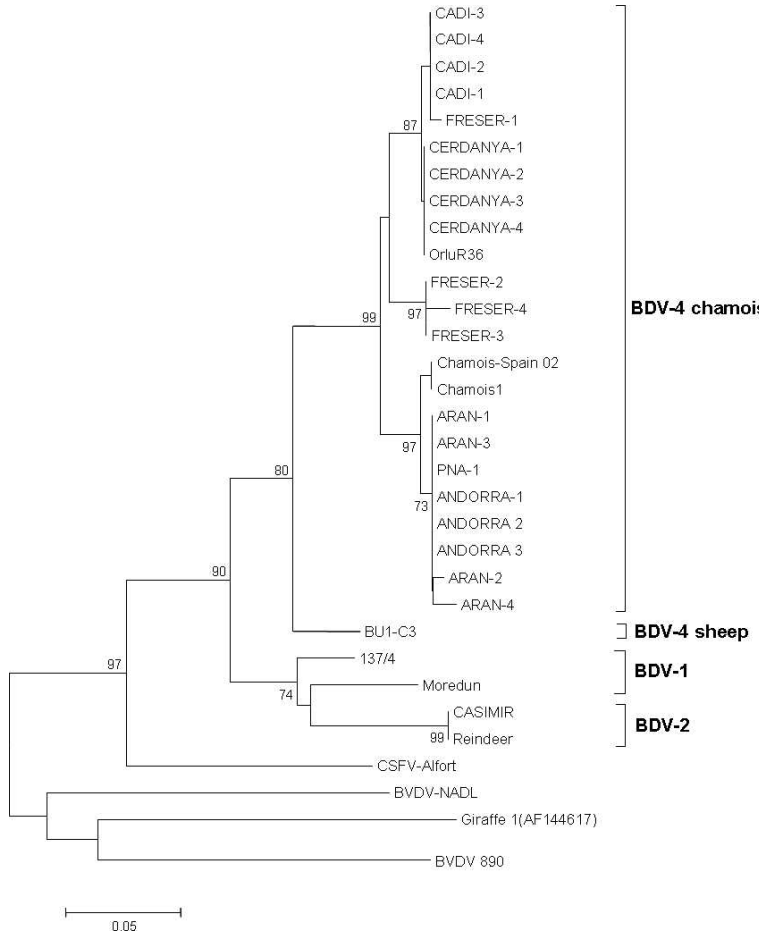

FIGURE 2. Unrooted neighbor-joining phylogenetic tree based on the 5′UTR sequence among pestiviruses. Chamois (*Rupicapra pyrenaica pyrenaica*) strains appear enclosed in a differentiated group into border disease virus 4 (BDV-4). The strains detected in chamois no. 1, 2, and 3 appear named as ANDORRA-1, -2, and -3, respectively. These strains cluster with other chamois viruses isolated in the bordering NHR of Alt Pallars-Aran (ARAN-1, -2, -3, and -4). The numbers on the branches indicate the bootstrap values (percentage of 1,000 replicates). Sequences of strains were taken from GenBank with the following accession numbers: Chamois-Spain02 (AY641529), ARAN-1 (AM765800), CADI-1 (AM905918), CADI-2 (AM905919), CADI-3 (AM905920), CADI-4 (AM905921), CERDANYA-1 (AM905930), CERDANYA-2 (AM905931), CERDANYA-3 (AM905932), CERDANYA-4 (AM905933), ORLUR36 (DQ898294), FRESER 2 (FN691777), FRESER 3 (FN691778), ARAN-1 (AM765800), ARAN-2 (AM765801), ARAN-3 (AM765802), ARAN-4 (AM765803), AND-1 (HE615083), AND-2 (HE615084), AND-3 (HE615085), BU1-C3 (DQ361068), 137/4 (U65052), Moredun (U65023), CASIMIR (AB122085), Reindeer (AF144618), Alfort (X87939), BVDV 890 (U18059), and NADL (M31182). Sequences of strains FRESER-1 and PNA-1 are not deposited in GenBank.

episode of mortality, the chamois population in Andorra was 939 and in 2010 it dropped to 555, a decrease of 41%. In the Enclar HR, the decline of the population was higher (from 415 to 173 chamois, a decrease of 58%) than for the whole of Andorra. Game keepers observed the final, presumably BDV-infected clinical case at the beginning of 2011 when a chamois with alopecia was observed. However, it was not possible to capture this animal and, subsequently, no more clinical cases or dead chamois were found or reported.

TABLE 2. Virus neutralization titers against four pestivirus strains found in four antibody-positive Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) from Andorra. Neutralizing antibody titers are expressed as the reciprocal of the highest dilution that neutralized 100 tissue culture infective doses ($TCID_{50}$) in all cultures.[a]

Chamois	BVDV-1 NADL	BDV- 1 137/4	BDV-4 ESP-97	BDV-4 Aran 1
a	40	320	320	160
b	320	1,280	320	160
c	10	80	80	320
d	80	80	320	640

[a] BVDV = bovine viral diarrhea virus; BDV = border disease virus.

DISCUSSION

The overall apparent prevalence of antibodies against pestivirus found in Pyrenean chamois in Andorra (5%) between 2001 and 2009 was low when compared with other areas of the Pyrenees, where antibody prevalence ranged from 49% to 70% (Pioz et al., 2007; Marco et al., 2011). These results suggest that, despite being surrounded by severely infected populations, pestiviruses were not circulating before the outbreak of disease in Andorra. A single case of a chamois affected by the disease was described in 2002 (Arnal et al., 2004) near the border with Spain but was most probably related to the BDV outbreak that occurred between 2001 and 2002 in the Alt Pallars-Aran NHR (Marco et al., 2007). In 2005, a second affected chamois was detected, most probably linked to the 2005 outbreak in the NHR of Cerdanya-Alt Urgell (Marco et al., 2009). Sheep grazing, which has been associated with BDV transmission to Pyrenean chamois and which may be responsible for a high antibody prevalence in some areas (Martin et al., 2011), is not common in Andorra. Thus, the low prevalence of antibodies against pestivirus detected in Andorra suggests that Andorran chamois were highly susceptible to infection in the case of contact with BDV-infected chamois.

The absence of antibodies in roe deer and mouflon from Andorra agrees with the low circulation of pestiviruses in chamois. BDV infection in roe deer is rare (Riekerink et al., 2005; Marco et al., 2008), and previous studies performed in the neighboring Alt Pallars-Aran NHR also indicate a very low antibody prevalence in mouflon (Marco et al., 2008). However, in this area BDV was circulating extensively in Pyrenean chamois. Martin et al. (2011) described a pestivirus antibody prevalence of 61% in mouflons from the southern French Alps. This high prevalence could be associated with greater susceptibility in mouflon to BDV-6 (the BDV type circulating in this part of the French Alps) infection rather than to

TABLE 3. Summary of information available about the three border disease virus RNA-positive Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) from Andorra.[a]

Chamois no.	Cause of death	Collection date	Age and sex	Necropsy findings	Available samples	Antibodies	Virus name and accession number
1	Hunted	December 2009	6-yr-old male	Apparently healthy	Spleen serum	No	ANDORRA-1 HE615083
2	Unknown (scavenged)	October 2009	NA	Cachexia	Brain	ND	ANDORRA-2 HE615084
3	Unknown (scavenged)	January 2010	1-yr-old male	Cachexia, pneumonia	Spleen	ND	ANDORRA-3 HE615085

[a] NA = not available; ND = not done.

BDV-4 or with high infection pressure from domestic animals.

Specific antibodies against BDV in wild boars were not detected, possibly because our sample size was small. Antibodies against BDV in wild boars have been detected in other parts of the Pyrenees, probably associated with a BDV virus of chamois origin (Rosell, unpubl.). Thus, in light of the outbreak in Andorran chamois, studies with a greater number of samples are needed if the transmission of BDV infection to this species is to be fully investigated.

The results of VNT from three positive chamois suggest that these animals came into contact with a BDV, although in one chamois no differences between titers against BDV and BVDV were detected. The absence of greater differences between the different BDV strains could be due to serologic cross-reactivity, which has been reported as occurring between all members of the genus *Pestivirus* (Schirrmeier et al., 2004). Two of the positive chamois were hunted in 2005 and 2006. Given that they were hunted in western Andorra near the Spanish border, these animals could have been infected with a BDV from chamois from the Alt Pallars-Aran NHR. However, infection with a BDV originating from sheep cannot be ruled out. The antibody-positive chamois detected in autumn 2009 were hunted at the same time as the first chamois carcasses were observed and, thus, could be related to the onset of the disease outbreak. During the same period, we detected BDV in a hunted healthy chamois collected during the surveillance program (chamois 1).

The detection of BDV in the hunted chamois and in two of the three analyzed carcasses, together with the field observations and decrease in the population, suggest that the high chamois mortality detected in Andorra between 2009 and 2010 was related to an outbreak of BDV infection. The resulting sequences of viruses isolated in the three virus-positive chamois showed that these animals were infected with the BDV-4 genotype, the same BDV type identified as the etiologic agent for the BDV-associated disease in chamois (Cabezon et al., 2011). The lack of viral RNA detection in the third carcass could have been caused by viral RNA degradation, as the carcass was highly decomposed. Despite being surrounded by severely affected areas since 2001, no clinical cases consistent with BDV infection or any significant mortality were observed in Andorra in its Pyrenean chamois population until the end of 2009. Several factors including behavior probably played a part in this delay, For example, Pyrenean chamois tend to form spatial clusters, and mixing between different groups rarely occurs. This could reduce pathogenic contamination between animals of different groups, as described in the case of an infectious keratoconjunctivitis (Crampe at al., 2007). In addition, chamois in Andorra are concentrated in reserves, and this type of distribution could have delayed virus transmission.

The total number of carcasses found between 2009 and 2010 in the Enclar HR was exceptional when compared with the number of dead chamois that are usually found each year (3–5 animals). The finding of the first RT-PCR–positive chamois in this area, and the clustering of the three isolated viruses with those isolated in diseased chamois from the Alt Pallars-Aran NHR (Chamois-Spain-02, Chamois 1, ARAN-1, ARAN-2, ARAN-3, and ARAN-4; Fig. 2), suggest that the virus entered from this reserve, which borders on the west of Andorra, in autumn of 2009. The chronology of the carcasses found, and the presumptive clinical cases observed from that time onward, would seem to indicate that the virus spread through the chamois population in a northeasterly direction until it reached the east of the country in spring 2010. The overall estimated mortality in Andorra was 41% while the estimated mortality in the Enclar HR was 58%, higher than in the

rest of Andorra. This difference is probably related to the higher chamois population density in Enclar, which would have facilitated virus transmission.

This study has shown that the question of pestivirus infections in chamois is not limited to the Spanish Pyrenees and that it could potentially expand to neighboring populations. We conclude that the severe decline in the chamois population in Andorra observed in 2010 was associated with an outbreak of disease due to BDV infection and that the source of infection was the neighboring chamois population of Alt Pallars-Aran NHR in Spain.

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LITERATURE CITED

Arnal, M. C., D. Fernandez-de-Luco, L. Riba, M. Maley, J. Gilray, K. Willoughbi, S. Vilcek, and P. F. Nettleton. 2004. A novel pestivirus associated with deaths in Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*). Journal of General Virology 85: 3653–3657.

Cabezon, O., R. Velarde, G. Mentaberre, L. Fernández-Sirera, E. Casas-Díaz, J. López-Olvera, E. Serrano, R. Rosell, C. Riquelme, S. Lavín, J. Segales, and I. Marco. 2011. Experimental infection with chamois border disease virus causes long-lasting viraemia and disease in Pyrenean chamois (*Rupicapra pyrenaica*). Journal of General Virology 92: 2494–2501.

Collett, M. S., R. Larson, S. K. Belzer, and E. Retzel. 1988. Proteins encoded by bovine viral diarrhea virus: The genomic organization of a pestivirus. Virology 165: 200–208.

Crampe, J. P., R. Bon, J. F. Gerard, E. Serrano, P. Caens, E. Florence, and G. González. 2007. Site fidelity, migratory behaviour, and spatial organization of female isards (*Rupicapra pyrenaica*) in the Pyrenees National Park, France. Canadian Journal of Zoology 85: 16–25.

Fernández-Sirera, L., G. Mentaberre, J. R. Lopez-Olvera, R. Cuenca, S. Lavin, and I. Marco. 2011. Haematology and serum chemistry of Pyrenean chamois (*Rupicapra pyrenaica*) naturally infected with a border disease virus. Research in Veterinary Science 90: 463–467.

Loken, T 1995. Ruminant pestivirus infections in animals other than cattle and sheep. Veterinary Clinics of North America. Food Animal Practice 11: 597–614.

Marco, I., J. R. Lopez-Olvera, R. Rosell, E. Vidal, A. Hurtado, R. Juste, M. Pumarola, and S. Lavin. 2007. Severe outbreak of disease in the southern chamois (*Rupicapra pyrenaica*) associated with border disease virus infection. Veterinary Microbiology 120: 33–41.

———, R. Rosell, O. Cabezon, G. Mentaberre, E. Casas, R. Velarde, J. R. Lopez-Olvera, A. Hurtado, and S. Lavin. 2008. Epidemiological study of border disease virus infection in Southern chamois (*Rupicapra pyrenaica*) after an outbreak of disease in the Pyrenees (NE Spain). Veterinary Microbiology 127: 29–38.

———, ———, ———, ———, ———, ———, and S. Lavin. 2009. Border disease virus among chamois, Spain. Emerging Infectious Diseases 15: 448–451.

———, O. Cabezon, R. Rosell, L. Fernandez-Sirera, A. Allepuz, and S. Lavin. 2011. Retrospective study of pestivirus infection in Pyrenean chamois (*Rupicapra pyrenaica*) and other ungulates in the Pyrenees (NE Spain). Veterinary Microbiology 149: 17–22.

Martin, C., C. Letellier, B. Caij, D. Gauthier, N. Jean, A. Shaffii, and C. Saegerman. 2011. Epidemiology of *Pestivirus* infection in wild ungulates of the French South Alps. Veterinary Microbiology 147: 320–328.

Nettleton, P. F., J. A. Gilray, P. Russo, and E. Dlissi. 1998. Border disease of sheep and goats. Veterinary Research 29: 327–340.

(OIE) World Organisation for Animal Health. 2008. Border disease. *In* Manual of diagnostic tests and vaccines for terrestrial animals. www.oie.int/manual-of-diagnostic-tests-and-vaccines-for-terrestrial-animals. Accessed December 2011.

Pioz, M., A. Loison, P. Gibert, D. Dubray, P. Menaut, B. Le Tallec, M. Artois, and E. Gilot-Fromont. 2007. Transmission of a pestivirus infection in a population of Pyrenean chamois. Veterinary Microbiology 119: 19–30.

Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty percent endpoints. American Journal of Hygiene 27: 493–497.

Riekerink, R. G. M. O., A. Dominici, H. W. Barkema, and A. J. de Smit. 2005. Seroprevalence of pestivirus in four species of alpine wild ungulates in the High Valley of Susa, Italy. Veterinary Microbiology 108: 297–303.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

SCHIRRMEIER, H., G. STREBELOW, K. DEPNER, B. HOFFMANN, AND M. BEER. 2004. Genetic and antigenic characterization of an atypical pestivirus isolate, a putative member of a novel pestivirus species. Journal of General Virology 85: 3647–3652.

STEVENSON, M 2011. Package EpiR. http://cran.r-project.org/web/packages/epiR/epiR.pdf. Accessed July 2012.

THIEL, H. J., M. S. COLLETT, E. A. GOULD, F. X. HEINZ, M. HOUGHTON, G. MEYERS, L. R. PURCELL, AND C. M. RICE. 2005. Family Flaviviridae. *In* Virus taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses, C. M. Fauquet, M. A. Mayo, J. Maniloff, R. U. Deselberger, and L. A. Ball (eds.). Elsevier Academic Press, New York, New York, pp. 981–998.

VILCEK, S., AND P. F. NETTLETON. 2006. Pestiviruses in wild animals. Veterinary Microbiology 116: 1–12.

———, A. J. HERRING, J. A. HERRING, P. F. NETTLETON, J. P. LOWINGS, AND D. J. PATON. 1994. Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. Archives of Virology 136: 309–323.

———, P. F. NETTLETON, D. J. PATON, AND S. BELAK. 1997. Molecular characterization of ovine pestiviruses. Journal of General Virology 78: 725–735.

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