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ORIGINAL PAPER

Prevalence of antibodies against selected agents shared between Cantabrian chamois (*Rupicapra pyrenaica parva*) and domestic goats

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Abstract Southern chamois (*Rupicapra pyrenaica*) share the habitat with domestic ungulates, and may, therefore, play a role in the epidemiology of shared agents. The objective of this study was to determine the seroprevalence for *Brucella* spp., *Mycobacterium avium* ssp. *paratuberculosis* (MAP), pestivirus, and *Sarcoptes scabiei* in Cantabrian chamois (*Rupicapra pyrenaica parva*) and compare these data with those of sympatric domestic goats (*Capra hircus*). From 2005 to 2008, blood samples were obtained from 236 adult Cantabrian chamois in two different populations, the western one and the eastern one. Seroprevalence for *Brucella* spp. and pestivirus was assessed using commercial ELISA kits, whereas specifically designed ELISA tests were used for MAP and *S. scabiei*. No antibodies against *Brucella* spp. were detected. Conversely, antibodies against MAP, pestivirus (chamois 3.8%; goat 2.3%), and *S. scabiei* (chamois 11.9%; goat 12.8%) were detected in both species. Seroprevalence for MAP was significantly higher for domestic goats (26%) than for chamois (9.7%). In chamois, seroprevalence for pestivirus was higher in the west (6.5%) than in the east (range 0–1.8%), whereas seroprevalence for *S. scabiei* followed the opposite trend (west 4.6%; east 16.7–21.4%). We suggest that certain diseases could circulate between Cantabrian chamois and domestic goat populations, and domestic livestock may suppose a threat for the health status of sympatric Cantabrian chamois.

Keywords Serosurvey · Cantabrian chamois · *Mycobacterium avium* ssp. *paratuberculosis* · Sarcoptic mange · Pestivirus · Domestic goat

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Introduction

Southern chamois (*Rupicapra pyrenaica*) is a wild mountain ungulate belonging to the Bovidae family and Caprinae subfamily. Two subspecies can be found in Spain: the Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) in the Pyrenees and the Cantabrian chamois (*Rupicapra pyrenaica parva*), endemic from the Cantabrian Mountains and occupying the western limit of the *Rupicapra* genus distribution area (Fig. 1). A western and an eastern population of Cantabrian chamois, physically separated by anthropogenic barriers, have been defined (Catusse et al. 1996; Shackleton and the IUCN/SSC Caprinae Specialist Group 1997; Pérez-Barbería and García-González 2004). For the purpose of this study,

the eastern population is shared by two different political regions: Asturias and León.

In 1993, the eastern chamois populations were affected by sarcoptic mange, the disease becoming endemic since then (Fernández-Morán et al. 1997). Sarcoptic mange is considered absent in the western population, without clinical cases of mange detected up to date in this area, where Cantabrian chamois population has continuously increased throughout the last years, reaching a density of 14.35 Cantabrian chamois per square kilometer in 2007. This compares to the 6.50 chamois per square kilometer registered for the mange-affected eastern population in 2007 (Anonymous 2008a, 2008b). Domestic livestock in Asturias and León includes approximately 80,000 domestic goats (*Capra hircus*; Anonymous 2008c), some of which share habitat in summer with more than 13,500 Cantabrian chamois (ca. 3,400 in the western nucleus, ca. 5,300 in the eastern Asturias one, and ca. 4,800 in the eastern León nucleus) (Pérez-Barbería and García-González 2004; Anonymous 2008a, 2008b).

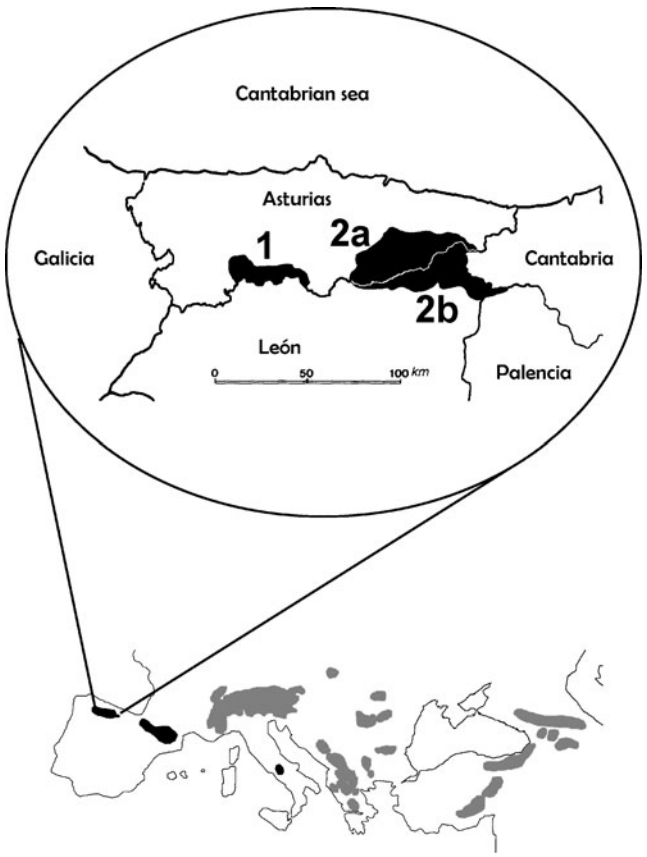

Fig. 1 Chamois distribution in Europe and study areas in north–western Spain. Adapted and modified from Catusse et al. 1996 and Fernández-Morán et al. 1997. *Gray* northern chamois (*Rupicapra rupicapra*) populations, *Black* southern chamois (*Rupicapra pyrenaica*) populations, *1* western Cantabrian chamois (*Rupicapra pyrenaica parva*) population, *2a* eastern Cantabrian chamois (*Rupicapra pyrenaica parva*) population in Asturias, *2b* eastern Cantabrian chamois (*Rupicapra pyrenaica parva*) population in León

This commingling may play a significant role in the epidemiology of several infectious agents since cross-transmission may occur as livestock graze in mountain pastures (Gauthier et al. 1991; Cubero-Pablo et al. 2000; Gortázar et al. 2007). Transmission of agents has been suggested in both directions, with wildlife acting as reservoirs of infectious agents which affect domestic animals and livestock being a source of infection for wild mountain ruminants (Bengis et al. 2002; Simpson 2002; Gaffuri et al. 2006).

Antibodies against several infectious agents of ruminants have been detected in chamois (*Rupicapra* spp.). Antibodies against viral pathogens include bovine herpesvirus 1, encephalomyocarditis virus (Gentile et al. 2000), bovine parainfluenza type 3 virus, bovine respiratory syncytial virus (Gaffuri et al. 2006), and pestivirus (Gentile et al. 2000; Arnal et al. 2004; Hurtado et al. 2004; Gaffuri et al. 2006; Marco et al. 2007; Pioz et al. 2007; Marco et al. 2008). Regarding bacterial agents, antibodies have been detected against *Brucella melitensis* (Garin-Bastuji et al. 1990), *Mycoplasma conjunctivae* (Giacometti et al. 2002; Gaffuri et al. 2006), *Salmonella enterica*, *Coxiella burnetii* (Pioz et al. 2008), *Leptospira interrogans* (Gentile et al. 2000), and *Chlamydophila abortus* (Pioz et al. 2008; Salinas et al. 2009). Finally, antibodies against two protozoa, *Neospora caninum* and *Toxoplama gondii*, have also been reported (Gentile et al. 2000; Gaffuri et al. 2006).

In spite of such information regarding other species or subspecies of chamois, only limited information on the seroprevalence of infectious agents in Cantabrian chamois is available (Fernández-Morán et al. 1997; Gauss et al. 2006; Almería et al. 2007). The objective of this study was determining the seroprevalence for *Brucella* spp., *Mycobacterium avium* ssp. *paratuberculosis* (MAP), pestivirus, and *Sarcoptes scabiei* in the Cantabrian chamois. This was compared with data on sera from sympatric domestic goats sharing summer pastures with the chamois, in order to detect potentially shared diseases between both species.

Materials and methods

From 2005 to 2008, blood samples were obtained from 236 adult Cantabrian chamois, either hunted (N=213) or life captured with drive-nets (N=23), an already reported method to capture chamois (López-Olvera et al. 2009a). Two different chamois populations were defined according to geographical criteria, the eastern one and the western one, the latter covering an area shared by two different public administrations, León and Asturias (Fig. 1). The

sampled area represents more than 75% of the current distribution area of Cantabrian chamois. Since hunting programs are aimed at maintaining population structure balance, animals from both sexes and all ages were included in our sample, thus being representative of the studied population.

Blood samples were collected by jugular venipuncture in the live chamois and by heart puncture in the hunted ones. Blood was allowed to clot at room temperature and centrifuged less than 24 h after collection. Sera were also obtained from 219 domestic goats through the Official Health Testing Programme of the Spanish government for *Brucella abortus*. All sera were stored at −20°C until analyzed.

A commercial competitive enzyme-linked assay (ELISA; INGEZIM BRUCELLA BOVINE 1.2.BB.K.1, INGENASA, Madrid, Spain) was used for the detection of antibodies against *Brucella* spp. according to the manufacturer's recommendations. This ELISA detects antibodies against the protein–polysaccharide complex LPS-S of *Brucella*, and it has been previously used in mouflon (*Ovis aries musimon;* López-Olvera et al. 2009b).

An ELISA BVD/Mucosal Disease p80 kit (Institut Pourquier, Montpellier, France) was used to search for pestivirus antibodies, according to the procedure described by the manufacturer, as previously described in Pyrenean chamois (Pioz et al. 2007). The test detects antibodies directed against protein p80/125, common to all pestivirus strains. Samples with a percentage of inhibition equivalent or less than 40% were considered positive.

Antibodies to MAP were detected using a specifically designed adsorbed ELISA test following protocols reported previously for cattle and sheep (Garrido 2001; Sevilla 2007), as previously used and reported for red deer (*Cervus elaphus*) (Reyes-García et al. 2008). This test detects antibodies against MAP protoplasmatic antigen 3. The cutoff was set as the mean optical density value of the negative controls added to three times the standard deviation of all negative control.

Antibodies against *S. scabiei* were determined using an "in house" ELISA test based in the use of a recombinant antigen. This test detects the Ssλ20 antigen of the parasite, located in the mite's organs, the integument of the epidermis, and the spaces surrounding its vital organs (Casais et al. 2007). This test was characterized by 100% sensitivity and 97% specificity in both red deer and southern chamois (Casais et al. 2007), although field trials of the test in red deer revealed a slight decrease of specificity (97%) but a stronger decrease of sensitivity, which was 75% (Oleaga et al. 2008).

A chi-square analysis was performed for all agents, using the PROC FREQ procedure of SAS® System for Windows V8 (SAS Institute Inc., Cary, NC, USA), to detect seroprevalence differences between species, among areas for both species, and among years for southern chamois.

Results

Table 1 shows the serologic results for both Cantabrian chamois and domestic goat in the different study areas. No antibodies were detected against *Brucella* spp. either in chamois or goat. Conversely, antibodies against MAP, pestivirus, and *S. scabiei* were detected in both species.

Seroprevalence for MAP was significantly ($P<0.05$) higher in domestic goats than in Cantabrian chamois for the total and for the west and León areas. Differences in MAP antibodies seroprevalence in goats were also significant among areas.

Seroprevalence for pestivirus in Cantabrian chamois was statistically higher in the western area than in the eastern area. Moreover, pestivirus seroprevalence significantly ($P<0.05$) increased throughout the years in this species (Table 2).

Seroprevalence for *S. scabiei* in Cantabrian chamois was statistically ($P<0.05$) higher in the eastern area than in the western area.

Discussion

The absence of antibodies against *Brucella* spp. in all the animals examined has been previously reported in the Cantabrian chamois (Fernández-Morán et al. 1997). Our results seem to further confirm that *Brucella* spp. is apparently not circulating within the wild chamois population and the domestic goat flocks in the study area.

Wild ruminants may become MAP-infected by contact with infected domestic livestock (Riemann et al. 1979; Marco et al. 2002; Deutz et al. 2005; Kopecna et al. 2006), but transmission from wildlife to domestic livestock has also been suspected (Chiodini and Van Kruiningen 1983; Greig et al. 2003). Absence of antibodies against MAP had been previously reported in Cantabrian (González-Quirós et al. 1996; Fernández-Morán et al. 1997) and Abruzzo (*Rupicapra pyrenaica ornata*) chamois (Gentile et al. 2000). However, paratuberculosis (or specific antibodies) has been recently reported in the fallow (*Dama dama*) (Marco et al. 2002; Balseiro et al. 2008) and red deer (Reyes-García et al. 2008) in the Cantabrian Mountains. Our results indicate that paratuberculosis is present in the study area both in Cantabrian chamois and domestic goats, livestock probably being a source of MAP-infection for Cantabrian chamois due to their higher seroprevalence of antibodies against MAP. Nevertheless, these results should be considered with caution, since ELISA has demonstrated

Table 1 Serologic prevalence of selected infectious diseases in Cantabrian chamois (*Rupicapra pyrenaica parva*) and domestic goats (*Capra hircus*) from north–western Spain

			Mycobacterium avium ssp. *paratuberculosis*			Pestivirus			*Sarcoptes scabiei*		
		Sera analyzed	Positive	Percentage	Confidence interval (95%)	Positive	Percentage	Confidence interval (95%)	Positive	Percentage	Confidence interval (95%)
Chamois	Total	236	23	9.7%*	6.0–13.5	9	3.8%	1.4–6.3	28	11.9%	7.7–16.0
Goat	Total	219	57	26.0%*	20.2–31.9	5	2.3%	0.3–4.3	28	12.8%	8.4–17.2
Chamois	West	108	8	7.4%*	2.5–12.4	7	6.5%a*	1.8–11.2	5	4.6%a	0.7–8.6
	East—Asturias	60	8	13.3%	4.7–22.0	0	0.0%b		10	16.7%b	7.2–26.2
	East—León	56	4	7.1%*	0.4–13.8	1	1.8%b	0–5.3	12	21.4%b	10.7–32.1
	Unknown	12	3	25.0%	0–50.6	1	8.3%	0–24.7	1	8.3%	0–24.7
Goat	West	68	29	42.6%a*	30.8–54.5	0	0.0%*		8	11.8%ab	4.0–19.5
	East—Asturias	86	11	12.8%b	5.6–20.2	2	2.3%	0–5.5	7	8.1%a	2.2–14.0
	East—León	65	17	26.2%c*	15.6–36.7	3	4.6%	0–9.7	13	20.0%b	10.5–29.5

Means with different letters are statistically ($p<0.05$) different among areas for the corresponding species

*$p<0.05$ (means are statistically different between species for the corresponding area)

Table 2 Pestivirus seroprevalence in Cantabrian chamois (*Rupicapra pyrenaica parva*) from north–western Spain according to the year

Year	Positive/analyzed	Percentage
2005	0/7	0.0%a
2006	1/61	1.6%a
2007	3/153	2.0%a
2008	5/15	33.3%b

Means with different letters statistically ($P<0.05$) different from each other

to have a low sensitivity to detect MAP-infected domestic and wild ruminants, with culture and polymerase chain reaction (PCR) MAP-positive animals showing negative ELISA results (Weber et al. 1992; Nebbia et al. 2000; Juste et al. 2005). Strain characterization of MAP isolates from both Cantabrian chamois and domestic goats, as well as other domestic species such as cattle (Balseiro et al. 2003), could help to elucidate the epidemiology of this pathogen in the study area.

To our knowledge, this is the first study to provide data on antibodies against pestivirus for Cantabrian chamois. A mortality outbreak affecting Pyrenean chamois, associated to a newly described pestivirus, has been reported in the western Pyrenees, seroprevalences ranging from 62.8% to 70.3% (Hurtado et al. 2004; Frolich et al. 2005; Marco et al. 2007; Pioz et al. 2007; Marco et al. 2008). Unlike the Pyrenees outbreak, no mortality or clinical signs potentially related to pestivirus infection have been observed in our study area. Lower seroprevalences against pestivirus (from 5.6% to 25.5%) without associated virus isolation, clinical signs, or mortality have been previously reported in Pyrenean (Arnal et al. 2004), Abruzzo (Gentile et al. 2000), and Alpine chamois (*Rupicapra rupicapra*) (Baradel et al. 1988; Olde Riekerink et al. 2005; Gaffuri et al. 2006). The relatively low seroprevalence for pestivirus and the absence of mortality and clinical signs found in the present study are similar to this epidemiological situation. Domestic animals (e.g., sheep) have been considered the origin of pestivirus inducing low seroprevalence in healthy chamois populations (Olde Riekerink et al. 2005; Gaffuri et al. 2006; Marco et al. 2009), due to higher prevalence and relatively frequent detection of persistently infected farm animals (Vilcek and Nettleton 2006), although domestic ungulates can also be infected from wild ungulates through indirect contact (Uttenthal et al. 2005). New high pathogenic virus strains could cause a mortality outbreak, as it has happened in the Pyrenees (Hurtado et al. 2004; Vilcek and Nettleton 2006; Marco et al. 2007), and pestivirus strains circulating in the wildlife–domestic livestock interface should be determined to evaluate the potential risk. However, the phylogenetic grouping of a highly pathogenic pestivirus

isolated of Pyrenean chamois from France suggests that cross-specific transmission of this isolate from domestic sheep to chamois via shared pastures is unlikely (Frolich et al. 2005).

Statistically, higher pestivirus seroprevalence of Cantabrian chamois as compared to goats in the western area suggests that the virus could circulate in the chamois population alone, or that other domestic ungulates (like sheep or cattle) are participating in the epidemiology of pestivirus. The increase along time of pestivirus seroprevalence in the chamois population, together with the higher seroprevalence against pestivirus found in the western population, agree with the increase in Cantabrian chamois population density in this area, which could ease transmission and circulation of the virus. However, since the ELISA used is unspecific and cross-reactions between different pestivirus exist, virus neutralization tests should be used to determine pestivirus strains circulating in domestic ruminants and Cantabrian chamois in the study area, in order to evaluate the potential risk of disease and mortality for both wildlife and livestock.

Sarcoptic mange, caused by the mite *S. scabiei*, affects both livestock and wildlife (Menzano et al. 2007; Morner 1992). A sarcoptic mange epizootic affecting the eastern population of Cantabrian chamois was first detected in 1993 and has since then become endemic in this area, without detected clinical cases of sarcoptic mange in the western chamois population up to date (González-Quirós et al. 1996; Fernández-Morán et al. 1997). The statistically significant higher seroprevalence of antibodies against *S. scabiei* in the eastern population (both in Asturias and León) when compared with the western population agrees with this epidemiological situation. Chamois can be infected with *S. scabiei* mites from domestic goats and develop disease (Lavín et al. 2000), and domestic ruminants are the most probable origin of the sarcoptic mange epidemic which affected Cantabrian chamois in 1993 (Fernández-Morán et al. 1997), as exposed in other outbreaks of sarcoptic mange in wild Bovidae species (Vyrypaev 1985; León-Vizcaino et al. 1999; González-Candela et al. 2004; Rossi et al. 2007). Conversely to Spanish ibex, antimange antibodies have a certain protective effect in southern chamois (Lastras et al. 2000), and therefore, the six seropositive Cantabrian chamois found in the western population are probably related to contact with mites from mange-infected domestic goats. The western population can still be considered mange-free, since *S. scabiei* causes high morbidity in chamois, and clinical cases seem to be required to confirm that a chamois population is affected by mange (Rossi et al. 1995; Fernández-Morán et al. 1997; Rossi et al. 2007).

Statistically, significant higher mange seroprevalence of the goats from León compared to Asturias within the eastern population is probably related to differences in the sanitary management of the two administrations involved (Pérez-Barbería and García-González 2004). However, no similar differences in chamois seroprevalence were found among areas. This could indicate that sarcoptic mange has become endemic in the eastern chamois population (both in Asturias and León) and is self-maintained independently from domestic livestock. The presence of mange antibodies in the Western population of Cantabrian chamois and the almost statistically significant (P=0.07) higher seroprevalence of goats in this area suggest that domestic goats are a threat of a new mange epidemic for the currently unaffected western chamois population. Measures to control mange, including treating domestic livestock and monitoring animal movements between affected and unaffected zones, together with effective measures to reduce Cantabrian chamois population, especially in the high-density Western area, should be undertaken in order to prevent the spread of this disease and the emergence of new mange epizootics.

To summarize, this is the first study to provide systematic data on seroprevalence of several infectious diseases in the Cantabrian chamois population. MAP, pestivirus, and sarcoptic mange are present in the population of Cantabrian chamois and domestic goats in the Cantabrian Mountains. Although higher MAP seroprevalence of goats seems to suggest that they could be maintaining this disease, a long-time MAP surveillance including strain characterization in wildlife and domestic livestock is needed in order to better understand the role of Cantabrian chamois and the sympatric domestic population in the epidemiology of paratuberculosis. Presence of antibodies against pestivirus has been reported for the first time in Cantabrian chamois. Surveillance of pestivirus and strain determination in domestic ungulates and Cantabrian chamois is crucial for the early detection of new or known pestivirus which could cause a mortality outbreak. Domestic goats could be a risk regarding sarcoptic mange in the currently chamois mange-free western range. Control measures should be taken to prevent new epizootics in the chamois population, such as the previously reported ones in the Cantabrian Mountains and the Pyrenees, caused by mange and pestivirus, respectively. By contrast, the absence of relevant disease such as *Brucella* in chamois and the lower seroprevalence of MAP suggest that chamois do not carry sanitary risks for domestic goats in the study area.

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