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SHORT COMMUNICATION

Serological survey of selected infectious diseases in mouflon (*Ovis aries musimon*) from south-central Spain

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Abstract Serum samples from 101 mouflons (*Ovis aries musimon*) collected from July 2002 to January 2006 were tested for antibodies against *Anaplasma* spp., *Brucella* spp., bovine viral diarrhea virus, *Chlamydophila abortus*, *Coxiella burnetii*, *Mycobacterium avium* ssp. *paratuberculosis*, and Maedi-Visna virus. Mouflon came either from extensive farms or high ungulate density fenced hunting estates. Antibodies were detected against *Anaplasma* spp. (22.2%), *C. burnetii* (4.0%), *M. avium* ssp. *paratuberculosis* (1.0%) and *C. abortus* (1.0%). According to our results, mouflons could participate as a wild reservoir in the epidemiology of *Anaplasma* spp. infection and maybe Q fever, but they do not seem to contribute in the epidemiology of the rest of the studied infectious diseases in south-central Spain.

Keywords Epidemiology · Ungulate · Wildlife · *Anaplasma* spp. · *Coxiella burnetii*

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Introduction

Mouflon (*Ovis aries musimon*) is a wild ungulate introduced in the Iberian Peninsula during the second half of the twentieth century (Rodríguez-Luengo et al. 2002). The estimated population size in Spain numbers 15,000 mouflons, most of them in private hunting estates (Santiago-Moreno et al. 2003). Fencing and feeding wild ungulates are common management practices in south-central Spain (Höfle et al. 2004; Vicente et al. 2006). As an introduced wild species, mouflons may play a significant role in the epidemiology of several infectious diseases potentially shared with other native wild ungulates, domestic ungulates, and even human beings (Gortázar et al. 2007). The principal wild ungulates also present in the area are the Iberian red deer (*Cervus elaphus hispanicus*) and the European wild boar (*Sus scrofa*). Domestic livestock, mainly cattle but also goats and free-roaming domestic pigs, occasionally share grazing and watering places with wild ungulates (Vicente et al. 2002). In a large serological survey including mouflon, no antibodies were found against leptospiral antigens in this species (Slavica et al. 2008), so this pathogen was not included in the present study. Also, a recent serosurvey of wild ruminants from Spain for antibodies against bluetongue confirmed antibodies in 13% of the mouflons tested (Ruiz-Fons et al. 2008a). However, little information on the prevalence and distribution of many other important infectious diseases among mouflons in Spain is available. The objective of this serosurvey is to determine the seroprevalence of *Anaplasma* spp., *Brucella* spp., bovine viral diarrhea virus (BVDV), *Chlamydophila abortus, Coxiella burnetii*, *M. avium* ssp. *paratuberculosis* (MAP), and Maedi-Visna virus (MVV) in mouflons from south-central Spain.

Materials and methods

From July 2002 to January 2006, blood samples were obtained from 101 mouflons from three different study areas (Sierra Morena, Montes de Toledo, and Guadiana basin) in south-central Spain (38°46′07″–40°96′30″ N, 3°23′15″–4°55′47″ W). Most samples were collected during hunting season (late fall–early winter). The mouflons were either shot in fenced private hunting areas with high ungulate density and blood immediately collected from the heart during field necropsies (89 individuals), or physically restrained in farms and blood obtained from the jugular vein (12 individuals). Serum was obtained after centrifugation and stored at −20°C until analyzed.

Serologic tests and techniques employed are reported in Table 1. All of them are available commercial kits for domestic ruminants. All sera were tested in duplicate.

Results

Serologic results are presented in Table 2. No antibodies were detected against *Brucella* spp., BVDV, and MVV. Antibodies were detected against *C. abortus* (1.0%; 95% confidence interval, 0.0–2.9%), MAP (1.0%; 95% confidence interval, 0.0–3.0%), *C. burnetii* (4.0%; 95% confidence interval, 0.1–7.8%), and *Anaplasma* spp. (22.2%; 95% confidence interval, 13.6–30.9%).

Discussion

Most of the tests used in this work have been validated for domestic sheep. However, mouflon is closely related to domestic sheep, so these tests should therefore be able to detect positive animals as well in mouflon. BVDV enzyme-linked immunosorbent assay (ELISA) tests have been standardized (Graham et al. 1997) and used for detection of antibodies against border disease virus (BDV) in sheep (Ataseven et al. 2006; Berriatúa et al. 2006), although given pestivirus diversity, some serotypes could have passed undetected. Both BVDV and BDV of sheep belong to the genus *Pestivirus* (Thiel et al. 2005), and serological cross reaction have been demonstrated (Krametter-Frötscher et al. 2007). To our knowledge, no previous data on *Pestivirus* in mouflon is available, although they cause border disease in domestic sheep (Nettleton et al. 1998) and a newly described pestivirus has become an emerging disease in Pyrenean chamois (Hurtado et al. 2004; Marco et al. 2007, 2008; Pioz et al. 2007). Nevertheless, no antibodies against BVDV or MVV, a lentivirus which affects sheep and goats with a high prevalence in Spain (Luján et al. 1993), were found in the sera of the mouflon considered in this study. Indirect ELISA has proven to be more reliable than standard Rose Bengal test to diagnose *Brucella melitensis* infection in sheep (Ferreira et al. 2003). The absence of antibodies against *Brucella* spp. agrees with previous reports of low prevalence of anti-*Brucella* antibodies in mouflon (Gourreau et al. 1993; Hubalek et al. 1993; León-Vizcaíno et al. 1985), suggesting that mouflon is not likely to play a significant role in the epidemiology of brucellosis (Cerri et al. 2002).

In ruminant species *C. abortus* is an important pathogen, which mainly induces abortion and genital infections, and can also cause enteritis, conjunctivitis, mastitis, pneumonia polyarthritis and meningoencephalitis (Longbottom and Coulter 2003). The prevalence of *C. abortus* antibodies found in this study (1.0%) is lower than the 37% previously reported in a high density free-ranging population of mouflon in southern Spain (Cubero-Pablo et al. 2000). Mouflon density is not homogeneous throughout its distribution area in south-central Spain, but it reaches its higher values (up to 3.2 mouflon/km^2) in Cazorla, Segura y Las Villas Natural Park (Blanco 1998) and is scattered in lower density spots in private hunting areas in most of its distribution range in south-central Spain (Blanco 1998, Santiago-Moreno et al. 2003). The high prevalence of anti-*Chlamydophila* spp. antibodies found in mouflons from Cazorla, Segura y las Villas Natural Park (Cubero-Pablo et al. 2000) could therefore be related to the higher density of the continuous population of mouflon in this area, where it also shares habitat with high densities of other wild

Table 1 Serologic tests employed for serological assay of mouflons sera sampled in south-central Spain from 2002 to 2006

Agent	Reference
Anaplasma spp.	*Anaplasma* Antibody Test Kit, cELISA 282-2®, VMRD, Coetzee et al. (2007)
Brucella spp.	Ingezim *Brucella* Small Ruminants, 13.BM.K1®, Ingenasa, Ferreira et al. (2003)
Bovine viral diarrhea virus	HerdChek BVDV Antibody Test Kit® Idexx, Ataseven et al. (2006)
Chlamydia psittaci	CHEKIT *Chlamydophila abortus* Antibody ELISA Test Kit®, Idexx, Vretou et al. (2007)
Coxiella burnetti	Chekit-Q-Fever enzyme immuno-assay kit®, Bommeli, Arricau-Bouvery et al. (2003)
Mycobacterium avium ssp. *Paratuberculosis*	ELISA Paratuberculosis serum and milk P07110® Pourquier®, Gumber et al. (2006)
Maedi-Visna virus	Elitest®, Hyphen Biomed, Varea et al. (2001)

Table 2 Serologic prevalence of selected infectious diseases (positive sera/tested sera) in mouflons sera sampled in south-central Spain from 2002 to 2006, according to the geographic area and the management system

Region		Sera analyzed	*Anaplasma* spp.	*Brucella* spp.	BVDV	*Chlamydophila abortus*	*Coxiella burnetii*	*M. avium* ssp. *paratuberculosis*	MVV
Montes de Toledo (Estates)		55	12/51	0/55	0/55	1/55	4/55	1/55	0/55
Sierra Morena	Estates	18	5/16	0/18	0/17	0/18	0/18	0/17	0/16
	Farms	12	2/12	0/12	0/12	0/12	0/12	0/12	0/12
	Total	30	7/28	0/30	0/29	0/30	0/30	0/29	0/28
Guadiana Basin (Estates)		16	1/11	0/14	0/16	0/16	0/16	0/16	0/5
Total		101	20/90	0/99	0/100	1/101	4/101	1/100	0/88

BVDV Bovine viral diarrhea virus, *MVV* Maedi-Visna virus

ungulates, namely red deer (*C. elaphus*), Spanish ibex (*Capra pyrenaica*), and wild boar (*Sus scrofa*), when compared to the lower density and smaller discontinuous populations in the private hunting areas of this study.

The antibody prevalence against paratuberculosis in mouflon here reported is low as compared to recent data published on red deer from Spain (Reyes-Garcia et al. 2008). MAP has been isolated from mesenteric lymph nodes of mouflon (Deutz et al. 2005), and interspecific transmission of specific strains has been suggested (Motiwala et al. 2004). MAP has been isolated in 3.8% of mouflons in the Czech Republic (Machackova et al. 2004), which contrasts with the 1.0% serological prevalence found in our study. The Czech mouflons came from wild nature, game parks or mouflon farms, and higher prevalence of MAP was observed in game parks. Management differences could therefore account for the differences observed in anti-MAP antibodies serological prevalence with the mouflons from fenced states considered in the present study.

C. burnetii is the causative agent of Q fever, a world-wide zoonosis endemic in Spain which can induce pneumonia, abortion, stillbirth, and delivery of weak newborns (Arricau-Bouvery and Rodolakis 2005). *Anaplasma* spp. are obligated intracellular parasites which infect erythrocytes and cause fever, weight loss, abortion, lethargy, icterus, and often death in animals older than 2 years (Ristic 1977). The only previous study determining seroprevalence of *C. burnetii* in mouflon, to our knowledge, detected antibodies against this rickettsia in the sera of the two animals analyzed (Hubalek et al. 1993). *C. burnetii* seroprevalence in wild deer from Spain has been reported to range from 0% to as high as 40% depending on the species and the management status (Ruiz-Fons et al. 2008b). Serological evidence of exposure to *Babesia ovis*, another tick-borne pathogen, was found in 12% of Spanish free-ranging mouflons (Ferrer et al. 1998), and prevalence of *Anaplasma* spp. ranging from 0% to 53% has been found in ticks from south-central Spain (de la Fuente et al. 2004). In our study, antibodies against *C. burnetii* (4.0%) and *Anaplasma* spp. (22.2%), two tick-borne diseases, were the most commonly found in the sera of the considered mouflons. All the serologically positive mouflons to *C. burnetii* came from the same very high density hunting estate, Valdejudíos, in the Montes de Toledo area (four positive out of 28 analyzed mouflons, accounting for a 14.3% prevalence in this estate). This suggests that this pathogen may be widespread and reach a relatively high prevalence when circulating within a high density population. Higher seroprevalence of *C. burnetii* in farmed deer than in wild free-ranging deer has been reported (Ruiz-Fons et al. 2008b), thus supporting the relationship between high ungulate density and high seroprevalence of *C. burnetii*. The seropositive mouflons for *C. abortus* and MAP came also from the Montes de Toledo area (the one for *C. abortus* even from the same hunting estate), and this could also be related to the higher density of ungulates in the private hunting estates from this area. Conversely, no statistically significant differences were found in the prevalence of antibodies against *Anaplasma* spp. among the three study areas, and prevalence was also similar between farmed mouflons (2/12) and mouflons from hunting estates (18/78).

Some of the pathogens considered in this study induce reproductive disorders in domestic ungulates (e.g., *C. abortus*, *C. burnetii*, *Brucella* spp.), and higher antibody titers can be found around parturition, although these high titers persist for several weeks (Office International des Epizooties 2004; Pioz et al. 2008). However, to our knowledge, no studies specifically dealing with season-related changes in the prevalence of antibodies for the three pathogens aforementioned have been published. Nevertheless, and since mouflon gives birth in spring, seropositivity could probably be higher in late spring–early summer than in the present study.

To summarize, mouflon does not seem to play an important role in the epidemiology of several infectious diseases in south-central Spain, due either to its natural resistance and/or to its low and disseminated population in private hunting areas. However, it may play a role in the interspecific transmission of vector-borne (particularly tick-borne) diseases, like anaplasmosis or Q fever, which it may share with the more abundant native red deer and wild boar.

Further research on other vector-borne diseases in mouflon, like bluetongue, ehrlichiosis, or other rickettsial diseases, would help to clarify the role of this species in the transmission of such diseases.

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