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Infectious diseases surveillance of the Iberian ibex (*Capra pyrenaica victoriae*) in Western Spain: health and conservation implications

Vigilancia de enfermedades infecciosas de la cabra montés (*Capra pyrenaica victoriae)* en el oeste de España: implicaciones para la salud y la conservación

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Abstract

A survey was conducted between 2004 and 2011 to identify potential pathogens present in the main subpopulation of Iberian ibex subspecies *Capra pyrenaica victoriae*, located in Gredos Mountain range in Western Spain. Samples were collected from 144 animals and subjected to specific procedures to detect the presence of, or evidence of exposure to, viral, bacterial and parasitic agents. A wide diversity of parasites was found, including 17 species of gastrointestinal nematodes and lungworms, nine species of Coccidia and three of Cestoda. Overall, 54% (75/138) of animals were parasitized by ticks (*Rhipicephalus bursa*, *Ixodes ricinus*, *Haemaphysalis sulcata*, and *Hyalomma marginatum marginatum*). Null detection of *Sarcoptes scabiei* mites, causal agent of sarcoptic mange, must be highlighted. Serological assays revealed exposure to *Babesia ovis* (43%, 56/129), *Neospora caninum* (13%, 14/108), *Oestrus* spp. (10%, 4/42), *Coxiella burnetii* (10%, 5/50), *Mycobacterium bovis* (6%, 3/50), and *Toxoplasma gondii* (3%, 4/137). We did not detect the presence of antibodies against *Chlamydia psittaci*, *Brucella* spp., *Mycobacterium avium* subspecies *paratuberculosis*, Visna-Maedi virus and Bluetongue virus. Despite the fact that major pathogens for animal health were not evidenced, the implementation of periodic passive surveillance programs directed to both wildlife and livestock is highly desirable to predict outbreaks that may compromise survival of such wild goat subspecies.
Keywords*: Capra pyrenaica victoriae*, surveillance, infectious agents, conservation.

Resumen

Se realizó un estudio entre 2004 y 2011 para identificar posibles patógenos presentes en la principal subpoblación de cabra montés, subespecie *Capra pyrenaica victoriae*, ubicada en la Sierra de Gredos en el oeste de España. Se recolectaron muestras de 144 animales y se sometieron a procedimientos específicos para detectar la presencia, o evidencia de exposición a agentes virales, bacterianos y parasitarios. Se encontró una amplia diversidad de parásitos, incluidas 17 especies de nematodos gastrointestinales y vermes pulmonares, nueve especies de coccidios y tres de cestodos. En total, el 54% (75/138) de los animales estaban parasitados por garrapatas *(Rhipicephalus bursa, Ixodes ricinus, Haemaphysalis sulcata* y *Hyalomma marginatum marginatum*). Debe destacarse la ausencia de ácaros *Sarcoptes scabiei*, agente causal de la sarna sarcóptica. Las técnicas serológicas revelaron exposición a *Babesia ovis* (43%, 56/129), *Neospora caninum* (13%, 14/108), *Oestrus* spp. (10%, 4/42), *Coxiella burnetii* (10%, 5/50), *Mycobacterium*

bovis (6%, 3/50) y *Toxoplasma gondii* (3%, 4/137). No detectamos la presencia de anticuerpos contra *Chlamydia psittaci, Brucella* spp., *Mycobacterium avium* subespecie *paratuberculosis*, virus Visna-Maedi y virus de la lengua azul. A pesar del hecho de que no se evidenciaron los principales patógenos para la sanidad animal, la implementación de programas periódicos de vigilancia pasiva dirigidos tanto a la fauna silvestre como al ganado es necesaria para predecir brotes que puedan comprometer la supervivencia de esta subespecie de cabra montés.
Palabras clave*: Capra pyrenaica victoriae*, vigilancia, agentes infecciosos, conservación.

Introduction

At present, two subspecies of Iberian ibex, to note *Capra pyrenaica victoriae* and *C. p. hispanica* are present in the north-west and south and east in the Iberian Peninsula, respectively (Acevedo & Real 2011). *Capra p. victoriae* population was formerly located mainly in central areas of Spain, such as the Gredos mountain range (Acevedo & Real 2011). In the 90s, following proposal of the International Union for Conservation of Nature (IUCN), some individuals from Gredos Nacional Game Reserve (Ávila province, 40°15'N, 5°16'W) were translocated to Galicia (Northwestern Spain) aiming strengthen its conservation status after establishment of additional populations of *C. p. victoriae* (Herrero & Pérez 2008, Acevedo *et al.* 2009). Despite evidence of an stable population, continuous attention should be paid because a wrong population management may favor the overcoming of the biological load capacity of game reserves; since most of *C. p. victoriae* population is confined to only one nucleus, an epizootic event, as outbreaks of sarcoptic mange (López-Olvera *et al.* 2015) could wipe out or severely deplete the present cluster of this subspecies. In addition, it was shown that surveillance data on the presence of infectious agents might contribute to guarantee the sustainability of reserves and species preservation (Pérez 2002). The aim of this study was to assess the presence of, or exposure to, pathogens within *C. p. victoriae* population that may compromise its health status and survival.

Materials and methods

In the study area of Sierra de Gredos, *C. p. victoriae* has an estimated population density of 15-18 animals/km^2 (Pérez *et al.* 2002). The study area is located within the Regional Game Reserve "La Sierra" (630 to 2,395 meters above sea level and 139.1 km^2) at the North of Cáceres province in Western Spain (40º17′ to 40º06′ N, 5º43′ to 5º32′ W) (Fig. 1). There were about 2,800 Iberian ibexes (*C. p. victoriae*) in the reserve in 2011 (Nieto Remedios

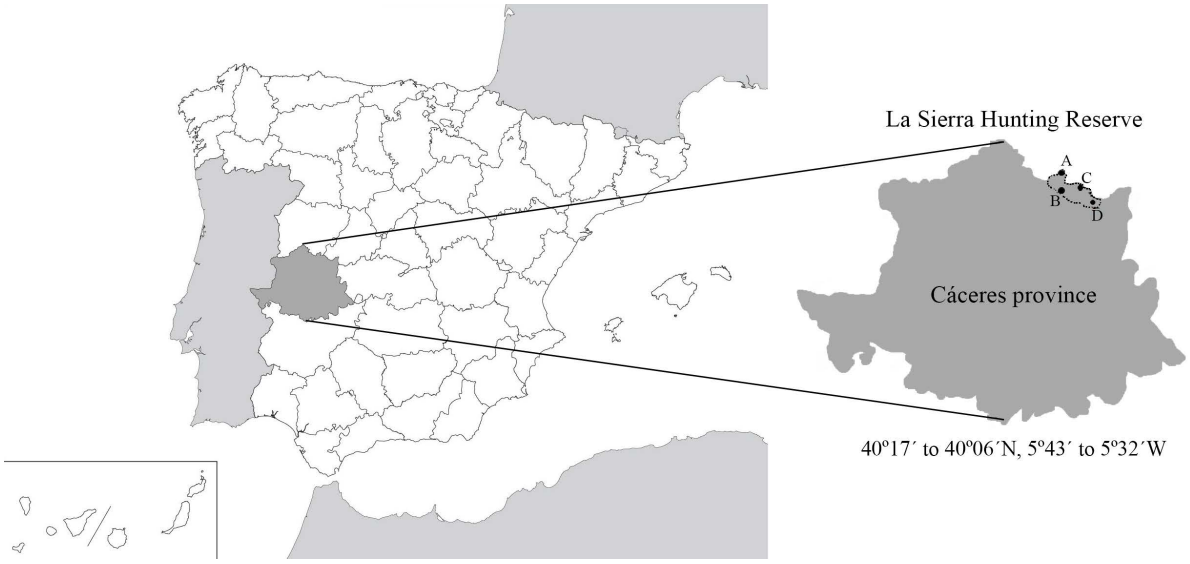

Figure 1. Sampling sites in La Sierra Hunting Reserve in Western Spain. Municipalities: A. Tornavacas municipality (n= 60 animals harvested), B. Guijo de Santa Bárbara (n= 46), C. Viandar de la Vera (n= 2), D. Losar de la Vera (n= 36).

2012), estimated by direct counting performed by rangers. The sampling site constitutes a mountain area with restrictions to domestic livestock access that may limit the spread of potential pathogens; in addition, Iberian ibexes are well adapted to heights over 1,200 m and do not essentially compete with other wild ungulates making them very dependent on their capacity to gain resources as seen elsewhere with the North American mountain goat (*Oreamnos americanus*) (Festa-Bianchet 2008). Samples were collected from 144 animals (99 males, 43 females, and 2 unidentified) that were hunted for population control (n= 143) under regional license (Decree 65/2001) and one (7-month-old male) was dead-found. The average age of specimens, calculated based on the number of horn growth rings (Fandos & Vigal 1988), was 11.4 years old (range: 7 months to 21 years); 10.4 years in males and 12.2 years in females. Limited number of samples was collected for several animals due to the destruction of some organs by the cavitation effect of the shot.

An array of direct and indirect diagnostic methods specifically employed for the detection of exposure to parasitic, bacterial and viral agents was used; corresponding methodology is shown in Tables 1 and 2. During in-field necropsies, cavities were carefully examined and viscera collected for further comprehensive endoparasites harvesting at the laboratories of the University of Extremadura. Blood samples were directly taken from heart cavity by using BD Vacutainer® collection tubes (Whole-blood PLUS K_2EDTA and PLUS Serum), and sera samples were obtained after centrifugation (10 min, 1200 xg) and kept frozen until analyses. In addition, cotton-swabs (Deltalab, Amies medium) were taken from skin, eye conjunctiva, middle ear, preputial and vaginal mucosa, lung and mediastinal lymph nodes, liver, ileocecal valve and cecum for culture purposes (Table 2). Collected samples were preserved at 4ºC until direct examination and procedure. Identification of parasites was based on dichotomous keys (Yamaguti 1961, Pellerdy 1974, Estrada-Peña 2000). The collected specimens are available in a proper repository under collection reference CM-055/09-UEx at the Veterinary Faculty of the University of Extremadura, Cáceres.

Results and discussion

After coprological analysis, a high prevalence and a wide diversity of parasites was found (Table 1); the whole subpopulation investigated was parasitized by at least one genus, including 15 species of gastrointestinal nematodes (93.1%, 121/130) (*Ostertagia ostertagi, O. trifurcata, Teladorsagia circumcincta, T. colubriformis, T. vitrinus, T. capricola, Trichostrongylus axei, Haemonchus contortus, Oesophagostomum venulosum, O. columbianum, Bunostomum trigonocephalum*) and *Nematodirus* spp (50%, 65/130) (*Nematodirus battus* and *N. filicollis*), *Trichuris* spp. (0.8%, 1/130) and *Toxocara vitulorum* (0.8%, 1/130); two species of lungworms: *Dictyocaulus filaria* larvae (1.6%, 2/130) and *Muellerius capilaris* (97.3%, 110/113) (also detected in lungs in all cases); eight species of intestinal Coccidia (97.7, 127/130) (*E. arloingi, E. intrincata, E. ninakohlyakimovae, E. parva, E. faurei, E. gonzalezi, E. ovina, E. crandallis*), two species of Cestoda, *Moniezia expansa* (23.1%, 3/13), and *M. benedeni* (61.5%, 8/13), and one species of Trematoda: *Dicrocoelium dentriticum* (20.8%, 30/144).

In this study, six species of *Eimeria (E. intrincata, E. parva, E. faurei, E. gonzalezi, E. ovina,* and *E. crandallis*) and three nematode species (*O. trifurcata, Trichostrongylus longispicularis* and *O. columbianum*) were identified for the first time infecting Iberian ibexes. Most of the results on prevalence and speciation (parasitofauna) can be comparable with previous data obtained in different Iberian ibex populations, especially those found in Southern territories (Pérez *et al*. 2003). The prevalence of *Eimeria* spp. (97.7%) observed here was higher than that reported in ibexes from Southern Spain, being the sympatric ruminants active reservoirs of numerous coccidian and helminthic species of interest in wildlife (Pérez *et al*. 2003).

The occurrence of peritoneal *Cysticercus tenuicollis* was considerably lower (7.0%, 10/144) than that (27%) documented in Cazorla Natural Park, Southern Spain (Pérez 2002). Those differences might be due to lower hunting pressure using dogs in the study area, less rural population and tourism. In addition, *Sarcocystis* sarcocysts were particularly prevalent among Iberian ibexes (84%), with infection rates higher than the 27% previously observed in Andalusian animals (Pérez 2002), and similar to the 86% found in Alpine ibex (*Capra ibex*) by Cornaglia *et al*. (1998). Sarcocysts observed in H&E-stained sections were thin-walled and appeared smooth like *Sarcocystis hircicanis*; but diagnosis was only possible at the genus level. The prevalence of *D. dendriticum* found in this study (21%, 30/144) was remarkably higher than it (0.5-

Table 1. Summary of direct investigations on parasites on Iberian ibexes (*Capra pyrenaica victoriae*) from Western Spain.

Parasite	Detection method	Prevalence (%)	Parasite burden ± SD (Range)
Eimeria spp.[1]	Coprology[2]	127/130 (97.7)	7583±33860 OPG (100-183500)
Sarcocystis spp.	Trypsin digestion[3] and histological[4] examination of diaphragm muscle tissue	42/50 (84.0)	-
Dicrocoelium dentriticum	Coprology	30/144 (20.8)	-
Moniezia spp.	Coprology	13/130 (10.0)	1153±2088 EPG (100-8200)
Cysticercus tenuicollis (*Taenia hydatigena*)	Examination of cavities (abomasum serosa, liver capsule and omentum of the small intestine)	10/144 (6.9)	(1-3)[5]
Gastrointestinal nematodes[1] (Strongylida order)	Coprology and collection after examination of viscera	121/130 (93.1)	704±1080 EPG (100-6100)
Nematodirus spp.	Coprology	65/130 (50.0)	128±92 EPG (100-600)
Trichuris spp.	Coprology	1/130 (0.8)	100 EPG
Toxocara vitulorum	Coprology	1/130 (0.8)	100 EPG
Dictyocaulus filaria	Coprology and lung examination	2/130 (1.6)	100 EPG
Muellerius capillaris	Coprology and lung examination	110/113 (97.3)	-
Ticks	Examination (harvesting)	75/138 (54.3)	6 (1-53)
Sarcoptes scabiei	Skin scrapings[6]	0/144 (0.0)	-

SD: Standard deviation. OPG: oocysts per gram of feces. EPG: eggs per gram of feces. [1] Copro-culture for species identification (20-25ºC, 75-80% RH, 2% potassium dichromate, 5-7 days). [2] Coprology: fecal float in ClNa solution, sedimentation in tap water, and McMaster method for quantification. [3] Trypsin digestion (1% trypsin solution, pH 7.4, 8-10 min; light microscopy examination). [4] Hematoxylin and eosin staining. [5] No. of *Cysticercus* spp. vesicles in peritoneal cavity. [6] Skin samples were cleared up using 20% KOH.

Table 2. Summary of investigations by using serology and culture growth assays on parasitic, bacterial and viral processes on Iberian ibexes (*Capra pyrenaica victoriae*) from Western Spain.

Pathogen	Method	Seroprevalence (%)	Culture growth (%)
Babesia ovis	Serology: IFAT (Habela *et al.* 1990)	56/129 (43.4)	-
Toxoplasma gondii	Serology: ELISA (ID Screen® Toxoplasmosis Indirect Multi-species, IDVET, Montpellier, France)	4/137 (2.9)	-
Neospora caninum	Serology: ELISA (*N. caninum* Antibody Test Kit, cELISA, VMRD, Inc. Pullman, WA, USA)	14/108 (12.9)	-
Oestrus spp.	Serology: ELISA (Alcaide *et al.*, 2005)	4/42 (9.5)	-
Coxiella burnetii	Serology: ELISA (LSIVET Ruminant Milk/Serum Q Fever, Lissieu, France)	5/50 (10.0)	-
Brucella spp.	Serology: Rose Bengal test (Alton *et al.*, 1988), CFT (Alton *et al.*, 1988), AGIDT (Ingezim IDR, Ingenasa, Madrid, Spain) Culture: samples from preputial and vaginal mucosa; Farrell medium (Ewalt, 1989)	0/50 (0)	0/50 (0)
Mycobaterium avium ssp. *paratuberculosis*	Serology: ELISA (Parachek 2, Prionics Ag, Schlieren-Zurich, Switzerland) Culture: sample from ileocecal valve; Herrold Egg Yolk enriched with mycobactin J and sodium pyruvate	0/50 (0)	0/50 (0)
Mycobacterium bovis	Serology: ELISA (IDEXX *M. bovis* ELISA, IDEXX Laboratories, Westbrook, ME) Culture: sample from lung and mediastinal lymph nodes; Lowenstein-Jensen	3/50 (6.0)	0/50 (0)
Mycoplasma spp.	Culture: sample from eye conjunctiva and middle ear; Blood Agar and McConkey Agar, and biochemical identification by API® strips (BioMérieux).	-	0/50 (0)
Salmonella spp.	Culture: sample from liver and ileum; Blood Agar and McConkey Agar, and biochemical identification by API® strips (BioMérieux).	-	0/50 (0)
Staphylococcus spp.	Culture: sample from skin; Blood Agar and McConkey Agar, and biochemical identification by API® strips (BioMérieux).	-	50/50 (100)
E. coli	Culture: sample from ileum; Blood Agar and McConkey Agar, and biochemical identification by API® strips (BioMérieux).	-	50/50 (100)
Pasteurella spp.	Culture: sample from lung; Blood Agar and McConkey Agar, and biochemical identification by API® strips (BioMérieux).	-	16/50 (32.0)
Clamydia spp.	Serology: CFT (Ornithosis-Complement Fixation Test, Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany)	2/50 (4.0)	-
Blue Tongue virus	Serology: ELISA (Bluetongue Antibody Test Kit, cELISA, VMRD, Inc. Pullman, Washington, USA)	0/50 (0)	-
Visna-Maedi virus	Serology: AGIDT (MAEDITECT 1000 Test Kit, Veterinary Laboratories Agency, New Haw, UK)	0/50 (0)	-

IFAT: immunofluorescence antibody test; ELISA: Enzyme-Linked ImmunoSorbent Assay; AGID: agar gel immunodiffusion test; CFT: Complement Fixation Test.

1.8%) previously documented in Southern Spain (Pérez 2002, Alasaad *et al*. 2008).

Ticks presence was observed in 54% (75/138) of animals, among those, 96% (72/75) were infested by *Rhipicephalus bursa*, 20% (15/75) by *Ixodes ricinus*, 20% (15/75) by *Haemaphysalis sulcata*, and 9.3% (7/75) by *Hyalomma marginatum marginatum*, interestingly these tick species were identified for the first time in the Iberian ibex. Infestation levels seemed to be similar to those (58%) previously found in Southern Spain (Pérez 2002). Again, pasture sharing with wildlife species such as Red deer (*Cervus elaphus*) and Eurasian Wild boar (*Sus scrofa*) may favor infestation by ticks (Ruiz-Fons *et al*. 2006). Importantly, ticks collected from sympatric Red deer near the study area have been recently demonstrated to harbor pathogens such as *Coxiella burnetii, Babesia ovis*, *Borrelia burgdorferi* and the Crimean-Congo Hemorrhagic Fever virus (Estrada-Peña *et al*. 2012). Despite no cases of infestation by *Sarcoptes scabiei* mites were detected, a serological analysis to discard possible contact with the parasite would have been desirable; currently no standardized assay for wild ruminants is available (Valldeperes *et al*. 2019).

Serological assays revealed exposure to *B. ovis* (43%, 56/129) falling within the range of the values previously reported in Spain (Pérez 2002). Antibodies against abortifacient coccidians like *Toxoplasma gondii* and *Neospora caninum* were found in 3% (4/137) and 13% (14/108) of animals, respectively; these observations are in contrast with the higher rates (6-27%) found in a previous survey in other populations of Iberian ibexes (García-Bocanegra *et al*. 2012a). Differences may be due to variations in definitive hosts distribution, managing of populations, and/or sampling bias. Overall, 10% (4/42) of our serum samples reacted against antigens of *Oestrus ovis*; the presence of *O. ovis* instars was confirmed in a single animal (1 out of 4) because craniotomy procedure was limited by trophy collection. Previously, in other Iberian populations, antibodies against *O. caucasicus* had been detected at higher levels (62-74%) (Pérez 2002). These discrepancies may be related to the nature of the antigenic source used (*O. ovis*) or the scarcity of *Oestrus* spp. in the sampling area.

Antibodies against *C. burnetii* were detected in this Iberian wild goat population (10%, 5/50), while their absence was reported in other studies (0%, 0/628, Marreros *et al*. 2011), this may be due to the coexistence with Red deer, natural reservoir of *C. burnetii* (González-Barrio *et al*. 2015). Absence of tuberculosis-like lesions, negative culture growth and low seroprevalence level (6%, 3/50) agreed with findings in ibexes from Northern and Southern Spain (Mentaberre *et al*. 2010, García-Bocanegra *et al*. 2012b). Tuberculosis-like lesions by *M. bovis* in Eurasian Wild boar and Red deer in the same sampling area was confirmed by Parra *et al*. (2006); absence in Iberian ibexes might be related to natural resistance to TB by *M. bovis* has suggested earlier (Mentaberre *et al*. 2010, García-Bocanegra *et al*. 2012b). The samples tested negative against *Brucella* spp. (0%, 0/50), *Salmonella* spp. (0%, 0/50), *Mycoplasma* spp. (0%, 0/50) and *M. avium* subspecies *paratuberculosis* (0%, 0/50), confirming previous data in Spain (Pérez 2002). The *Chlamydia* spp. seroprevalence level detected in this study (4%, 2/50) was markedly lower than the 24% observed in Southern Spain (Pérez 2002). Cycling of *C. psittaci* between wild and domestic hosts has been shown elsewhere. In addition, culture growth was observed for *Staphylococcus* spp. (100%, 50/50), *Escherichia coli* (100%, 50/50) and *Pasteurella* spp. (32%, 16/50). Regarding viral infections, absence of seropositive results (0%, 0/50) that indicate exposure to Bluetongue virus (BTV) was not observed in contrast with previous findings (4-11%) in Southern Spain (García-Bocanegra *et al*. 2011, Lorca-Oró *et al*., 2011). The current situation of BTV needs further attention in ungulate species sympatrically occurring in a given area (Boadella *et al*. 2010). Exposition to Visna-Maedi virus was not detected in present survey.

Despite the high variety of potential pathogens detected, or evidence of exposure, in this study (Tables 1 and 2), no evident clinical signs were observed in the infected Iberian ibexes, whose populations appeared anecdotally healthy and growing in number. Nonetheless, the major pathogens for animal health were not evidenced, and the implementation of periodic passive surveillance programs directed to both wildlife and livestock is highly desirable to predict outbreaks that may compromise survival of such wild goat subspecies.

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