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FIRST SEROPOSITIVE CASES OF *COXIELLA BURNETII* IN RED DEER POPULATIONS IN THE SOUTHWEST IBERIAN PENINSULA

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Abstract: The aim of this study was to evaluate the seroprevalence of *Coxiella burnetii* in different red deer populations and to investigate role of red deer densities, livestock, and habitat on seroprevalence. The serosurvey revealed 5 positive cases out of 137 sera (3.64%) that occurred in two of the three study areas. This study documents the first cases of *Coxiella burnetii* in red deer in the southwest Iberian peninsula. A relationship between deer density and *Coxiella* seroprevalence was not found. Results revealed that indirect transmission through ticks between livestock and red deer might be associated with higher prevalence. The timing of shelter area usage may influence the contact between ticks and red deer by favoring transmission. *Coxiella burnetii* in red deer may be associated with infertility or early abortions with reabsorption. Further research is needed to evaluate its epidemiology and effect on the disease dynamics of red deer in the southwest Iberian peninsula.

Key words: *Coxiella burnetii*, red deer, disease, management, Mediterranean, ungulates.

INTRODUCTION

Coxiella burnetii is an intracellular gram-negative bacterium within the Coxiellaceae family; and the agent of Q fever, a disease with a worldwide distribution first described in Australia.[11] This disease has a very wide host range, all of them vertebrates, including humans and ruminants, either wild or domestic.[2] Transmission of the disease can be direct or indirect. Direct transmission takes place through aerosols, aborted fetal residues, or infected secretions.[23] It can also be indirectly transmitted through ticks of the genera *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Ixodes*, *Otobius*, and *Rhipicephalus*).[23] The incidence of *Coxiella*-infected ticks in livestock in Spain was reported to be around 7.7%.[33]

Traditionally, the disease has been assumed to have a double cycle, including domestic and wild animals. In the domestic animal cycle, the transmission appears to be mostly direct; whereas in the wildlife cycle, ticks may play an important role with some small rodents and lagomorphs as reservoirs. The wildlife cycle is connected with the domestic animal cycle because ticks spread the agent to domestic animals, such as cattle or sheep.[1] Ticks may not be essential for the transmission of *C. burnetii* within the livestock cycle.[3]

Many authors have described the effects of seasonal changes on disease presentation.[23,31] These variations have been mainly associated with the elimination of contaminants at abortion sites[14] during the calving season and with the biologic cycle of ticks. *Coxiella burnetii* has an unusual resistance to heat, being able to survive for several weeks under desiccation.[27] It can also disseminate to surrounding areas by wind.[23,31,32] It can survive for 20 days in water,[38] up to 2 wk in aerosols, and up to 5 wk in contaminated soil.[20,37]

Coxiella burnetii infection is characterized by occasional mild respiratory signs and reproductive failures,[12,19] and can be confused with brucellosis or chlamydiophilosis.[2]

Indirect immunofluorescence assay testing[5] has shown the prevalence of *Coxiella* in domestic animals in Mediterranean countries to be around 14% in cattle; ELISA testing shows prevalence of 9% in sheep and 13% in goats.[22] There are few studies of *Coxiella* in wild ungulates, despite its zoonotic importance. Ruiz-Fons et al.[28] evaluated the prevalence of *Coxiella* in red deer in Spain under different regions and management conditions and reported a higher prevalence in northern areas (5.6%) and in southern farmed deer (40%) kept at higher densities. However, this study reported no positive cases in southern free-living red deer populations.

The aim of this study was to investigate the prevalence of *Coxiella burnetii* in different populations of southwestern Spain and to determine the effect of red deer densities and management practices on prevalence.

MATERIAL AND METHODS

Study area

The study was conducted in three areas in the southwestern Iberian Peninsula, Sierra de San

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Figure 1. Location of studied areas. The circle represents Sierra de San Pedro, the triangle represents Monfragüe National Park, both in Extremadura, and the star represents Sierra de Hornachuelos in Andalucía.

Pedro and Monfragüe National Park in Extremadura, and Sierra de Hornachuelos in Andalucía (Fig. 1). Vegetation in these areas is characterized by holm oak (*Quercus ilex*) and cork oak (*Quercus suber*) scrublands with some scattered pasturelands and small areas of crops. The climate is Mediterranean with mild winters and hot and dry summers. During the study period (2005–2006), annual precipitation was below average for the area, with only 300 mm of accumulation in the autumn (133.41) and spring (237.84; Agencia Estatal de Meteorología).

The three study areas were hunting estates, which combined game hunting with other uses of the land. In Sierra de San Pedro, the studied estate (2,490 ha) had cattle (300 head) and sheep (1,000 head). Livestock was extensively managed, and the use of broad-spectrum internal antihelminthics was a common practice. Cattle were maintained in the estate during winter and removed in spring. Sheep spent the whole year on the estate, although they were moved to stalls during the nights. In Monfragüe, two estates were studied (3,290 ha and 1,926 ha; 5,216 ha in total). This site had no cattle for the previous 10 yr. In Sierra de Hornachuelos, the estate (1,082 ha) had a small cattle stock that shared habitat with deer. Wild boar (*Sus scrofa*) and fallow deer (*Dama dama*) were also present in all areas.

Management for red deer was similar in all the estates, including some supplementary feeding during late summer and early autumn,[4,6,9] a practice that usually promotes aggregation at feeding sites.[7,9,29]

During the rutting period, from late August to early October, deer counts were conducted from vehicles during the late afternoon, a time of peak activity, and covering all the areas where deer gathered during the mating season or sites where supplementary food was provided. Because most animals gather at feeding and mating sites during the rut in Mediterranean areas,[8,9,29] an approximate estimation of the total number of animals in the estate could be obtained for comparing of estimated density among estates.

Animal and samples preparation

Sera ($n = 137$) were collected from red deer that were shot. Blood was collected from the thoracic cavity with sterile tubes without anticoagulant. Sera were obtained after centrifugation (Centromix II-BL, Selecta, Barcelona, 08630, Spain) and stored at -20°C until analysis. Body length (cm) and thoracic perimeter (cm)[7] was measured for each animal. In addition, for both sides of the antlers, antler length (cm), burr circumference (cm), and number of antler points[34] were also measured. For females, their reproductive status was recorded, including fetus and corpus luteum presence. As a reference value, the pregnancy rate for the previous year in the same estate was also noted. Age was estimated by counting cementum layers at the interradicular pad under the first molar[24] and also evaluated by the eruption patterns in younger animals.

The abundance of ticks from sampled individuals could not be estimated because samples were collected after 1–5 hr after death.

ELISA test

A commercial immunoenzymatic assay (ELISA Fièvre Q serum monocupule, Institut Pourquier, Montpellier, 34090, France) for the detection of phase I and phase II specific antigens for *Coxiella burnetii* was used according to the manufacturer's recommendation (Microplate reader: Opsys MR microplate reader, DYNEX Technologies Limited, Chantilly, Virginia 20151-1621, USA) and as reported in previous studies.[12,18,35]

Statistical analysis

The statistical analyses were performed with the statistic software SPSS (version 13, SPSS Inc., Chicago, Illinois, USA). Differences of the prevalence were tested by the Fisher exact test and *t*-student for independent samples (differences among means). The significance level was set at $P = 0.05$.

RESULTS

Five positive cases were found out of 137 analyzed sera (3.64%). Positive cases were found

Table 1. Characteristics of the sampled areas.

Area	Positive cases (% prevalence)	No. of samples	Density (deer/100 ha) (area in hectares)	% pregnancy (idem previous year)
Monfragüe National Park	3 (2.86)	105	40	96.3 (84.5)
Estate 1	2	55	(1,926 ha)	100 (81.88)
Estate 2	1	50	(3,290 ha)	90 (88)
Sierra de Hornachuelos	0	14	33 (1,082 ha)	100 (–)
Sierra de San Pedro	2 (11.1)	18	15 (2,490 ha)	83.3 (80.55)

among males (4 out of 108; 3.7%) or females (1 out of 20; 5%) (Fisher test, $P = 0.583$) and occurred in two of the three areas studied and in three of the four estates sampled.

Data for the areas and estates sampled are shown in Table 1. Sierra de San Pedro presented the highest prevalence while no positive case was detected in Sierra de Hornachuelos (Fisher test, $P = 0.000$). The results of population density surveys indicated that the highest deer density occurred in Monfragüe National Park and the lowest density in Sierra de San Pedro. However, there was no relationship between density and *Coxiella* prevalence (Fisher test, $P = 0.145$). Pregnancy rates of red deer appeared similar between areas.

All positive cases were adults (between 4 and 11 yr; Table 2). Morphologic measurements of these positive individuals indicated that they were bigger in body and antler size when compared to other individuals of the same age and from the same area and estate (Table 2).

DISCUSSION

These results document the first seropositive cases of *C. burnetii* for red deer in the Iberian peninsula. To date, positive cases have been described in this area only for farmed red deer, but with much higher prevalences (12%).[28] The results of this present study suggest that the infection may not be able to reach high prevalences in free-range red deer populations as it has been reported in farmed red deer. The high prevalence in farmed red deer[28] is probably a consequence of the close contact between animals, which facilitates the infection transmission. The studied populations maintain quite high densities of animals that could promote close contact between individuals (Sierra de San Pedro [15 deer/100 ha], Monfragüe National Park [40 deer/100 ha], and Sierra de Hornachuelos [33 deer/100 ha]). However, it seems that density is not a major factor influencing the prevalence of *Coxiella* in red deer populations, unlike for tuberculosis.[15]

The estate sampled in Sierra de San Pedro was a livestock-intensive farm, with cattle and sheep, and this site had a higher prevalence of *Coxiella* (11%) among the studied estates. Although red deer and livestock may not be in close contact, indirect transmission via ticks is likely. The main two genera of ticks occurring in the area, *Hyalomma* and *Rhipicephalus*, are susceptible to infection by *C. burnetii*.[30] This indirect transmission route may be responsible for the higher prevalence in red deer, although a more extensive survey is needed.

In Monfragüe National Park, there are no sheep or cattle to maintain the infection. The prevalences found (2.74%) may be maintained by direct contact between deer under conditions of relatively high density (close to 40 deer/ha) as described for domestic ruminants.[23,25]

In Sierra de Hornachuelos, no positive cases were found. This area is closer to that studied by Ruiz-Fons et al.,[28] who reported a 0% prevalence. This suggests that the disease may not have extended into this area, and future monitoring of the area is suggested.

At the end of summer, due to the extreme weather conditions and food shortage, animals were in poor condition and more susceptible to infections. During these times, many estates provide supplementary food, which promotes the aggregation of animals at feeding sites. These conditions may facilitate the direct transmission of *C. burnetii* as observed for sheep and cattle.[13] However, the extremely high temperatures during these months along with the low humidity in the atmosphere may reduce the viability of *Coxiella* in soil.

Another potential reason why *Coxiella* prevalence may not be elevated as expected may be differences in the biologic cycles for ticks and deer. Shelter areas in scrublands, where ticks grow and reproduce from spring to autumn, is

Table 2. Ages and morphometric data of positive cases compared to other individuals of the same age and the same area and estate (in brackets: mean ± SD).

	Positive 1	Positive 2	Positive 3	Positive 4	Positive 5
Sex:	Male	Female	Male	Male	Male
Area:	Monfragüe National Park	Monfragüe National Park	Sierra de Sierra Pedro	Sierra de Sierra Pedro	Monfragüe National Park
Age	4 (4.86 ± 3.32)	11	9 (3.52 ± 1.96)	6 (3.52 ± 1.96)	NA
P	0.014	0.000	0.000	0.000	
Body length	199.5 (183.9 ± 5.63)	176 (173.2 ± 5.32)	203 (181.97 ± 15.7)	203 (195.1 ± 3.47)	NA
P	0.000	—	0.000	0.001	—
Thoracic perimeter (cm)	123.5 (118.7 ± 4.25)	111 (109.00 ± 2.82)	126 (124.25 ± 3.30)	126 (123.71 ± 4.51)	NA
P	0.000	0.500	0.367	0.081	—
Antler length (cm)	63.6 (65.2 ± 9.33)	NA	89.1 (92.85 ± 2.82)	89.1 (82.68 ± 9.74)	NA
P	0.536	—	0.072	0.030	—
Burr circumference (cm)	17.65 (13.69 ± 2.31)	NA	24.7 (20.31 ± 2.20)	24.7 (17.29 ± 2.31)	NA
P	0.000	—	0.024	0.000	—
Number of antler points	11 (9.83 ± 2.791)	NA	13 (11.75 ± 4.19)	12 (11.29 ± 1.82)	NA
P	0.062	—	0.593	0.165	—

only used by deer during winter. From July to October, deer move to open areas where feeding is supplied. In the estate of Sierra de San Pedro, however, the same shelter areas are used longer time for deer because the scrubland areas are smaller and closer to open areas than in other estates. This could provide a closer and longer contact between ticks and deer, perhaps favoring the transmission.

Pregnancy rates were obtained in the early pregnancy season, between October and February. The lowest rate was found in Sierra de San Pedro, where the prevalence of *Coxiella* was higher. If these low pregnancy rates were associated with the presence of *Coxiella*, these results would reveal that deer would present a similar epidemiologic pattern to that described for bovine with infertility or early abortions with reabsorption.[17] Thus, in this population, direct transmission via contact with contaminated aborted tissues would not occur as in domestic small ruminants.[36]

Current epidemiologic studies indicate that Q fever should be considered a public health problem in many countries, including Italy and Spain.[23] Q fever remains an occupational hazard for persons in contact with animals, like veterinarians, slaughterhouse workers, or farmers.[10,16,26,39] In southwestern Spain, as a consequence of the game hunting activity (monteria), hunters are also in contact with potentially infected deer when the deer are processed. Further research is needed to evaluate the dynamics of this disease in red deer populations in Mediterranean environments.

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