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Prevalence of *Coxiella burnetti* infection in wild and farmed ungulates

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19 **Abstract**

20 The aim of this study was to evaluate by serology and PCR analyses the prevalence of *C.*
21 *burnetti* infection in ungulates in Spain. Sera were collected from red deer (*Cervus elaphus*;
22 n=116), roe deer (*Capreolus capreolus*; n=39), fallow deer (*Dama dama*; n=13) and cattle
23 (n=79). Sera were tested for anti-*C. burnetii* antibody detection by means of an
24 immunofluorescence antibody assay (IFA) and *C. burnetii* DNA was amplified by PCR in
25 samples from ungulates that had antibodies to phase II antigens. Twenty-nine, fifteen and
26 thirty-nine percent of the red deer, roe deer and cattle had antibodies against *C. burnetii*,
27 respectively. None of the fallow deer sera tested positive. Seroprevalence was statistically
28 higher in farmed than in wild red deer and higher in northern than in southern populations,
29 whereas an inverse pattern was observed for the roe deer. Most of the seropositive animals
30 had only anti-*C. burnetii* phase II antibodies, thus showing the acute nature of infections in
31 the sampled ungulates. These results show that *C. burnetii* circulates in wild ungulates in
32 Spain and suggest that they can act as pathogen reservoirs for both domestic animals and
33 humans.

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35 Keywords: *Coxiella*; Q fever; tick; vector-borne diseases; wildlife

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43 Introduction

44 Q fever is a worldwide zoonosis produced by the gram-negative bacteria, *Coxiella*
45 *burnetii*. Multiple hosts can serve as reservoir of infection, including many wild and
46 domestic mammals, birds and ticks (Willeberg et al., 1980; Marrie et al., 1986; Webster et
47 al., 1995; Dunbar et al., 1998; Maurin and Raoult, 1999; Komiya et al., 2003). However,
48 domestic ruminants represent the most frequent source of *C. burnetii* infection in humans
49 (Maurin and Raoult, 1999).

50 Over 40 tick species are naturally infected with *C. burnetii*, including
51 *Rhipicephalus*, *Haemaphysalis*, *Amblyomma*, *Dermacentor*, *Ixodes* and *Otobius* species.
52 However, ticks are not considered essential in the natural cycle of *C. burnetii* in livestock
53 because other sources of infection are more important in animals that live in close contact
54 (Maurin and Raoult, 1999). In contrast, ticks may play a significant role in the transmission
55 of coxiellosis among wild vertebrates (Marrie et al., 1986; Maurin and Raoult, 1999).

56 Despite efforts to eradicate coxiellosis from cattle, goat and sheep herds, the disease
57 remains a serious risk for human and animal health in Spain (Tellez et al., 1988; Maurin
58 and Raoult, 1999; Maltezou and Raoult, 2002; Bolaños et al., 2003; Marrie, 2004;
59 Cardenosa et al., 2006; Oporto et al., 2006; Sanz et al., 2006). The disease seems to be
60 more prevalent in the Basque and Navarra provinces in northern Spain than in the central
61 and southern regions of the country (Tellez et al., 1988; Maurin and Raoult, 1999). Recent
62 research efforts have been focused on the characterization of *C. burnetii* infection in
63 domestic ruminants and humans (reviewed by Woldehiwet, 2004; Kazar, 2005). However,
64 little is known about the prevalence of *C. burnetii* in wild ungulates (Enright et al., 1971;
65 Ejercito et al., 1993; Marrie et al., 1993) and to our knowledge, *C. burnetii* infection has not
66 been characterized in Spanish wild and farmed cervids. Wild and farmed cervid populations

67 are growing in Spain and in other countries due to their value as game animals, thus
68 increasing the risk for disease transmission to humans and domestic animals (Gortázar et
69 al., 2006; Vicente et al., 2005).

70 The objective of the study reported herein was to evaluate by serology and PCR
71 analyses the prevalence of *C. burnetii* infection in wild and farmed cervids from two
72 regions in northern and southern Spain.

73 **Materials and Methods**

74 Study sites

75 In southern Spain, we collected sera of Iberian red deer (wild and farmed),
76 European roe deer and cattle in a 3,000 hectares fenced hunting estate (LO) located in the
77 Natural Park of “Los Alcornocales” in the province of Cádiz, Andalucía. We also collected
78 European roe deer and cattle sera from a neighbouring hunting estate (OJ). In northern
79 Spain, Iberian red deer, European roe deer and fallow deer serum samples were collected in
80 Asturias region. The number of samples through ruminant species and habitat are
81 summarized in table 1.

82 Animals and sample preparation

83 Wild cervids from Asturias region were necropsied after being hunter harvested.
84 Blood from shot wild cervids was collected directly from the heart or thoracic cavity into
85 sterile tubes without anticoagulant. Wild cervids from southern Spain were life-captured by
86 gamekeepers with fixed capture boxes and bled by cervical puncture. Cattle were bled
87 during the tuberculosis control campaign. All samples were collected in 2004 and 2005.
88 Blood from captured/farmed wild cervids and cattle from southern Spain was collected into
89 sterile tubes with and without anticoagulant (EDTA) and maintained at 4°C until arrival at
90 the laboratory. Plasma and serum were then separated after centrifugation and stored at -

91 20°C. DNA was extracted from sediments of serum samples using TriReagent (Sigma, St.
92 Louis, MO, USA). Ticks were collected from cervids and cattle and classified as previously
93 described (Ruiz-Fons et al., 2006).

94 *C. burnetii* serologic tests

95 Serum antibodies were determined for *C. burnetii* using an indirect
96 immunofluorescence antibody (IFA) test (Fuller Laboratories, Fullerton, CA, USA) and
97 following manufacturer's recommendations. The test for *C. burnetii* uses phase I and II
98 antigens with a cut-off dilution of the test serum of 1:16 (Torina et al., 2007). The IFA test
99 has been previously used to test deer sera for anti-*Coxiella burnetii* antibodies (Yasumoto
100 et al., 1997).

101 *C. burnetii* *htpB* polymerase chain reaction (PCR) and sequence analysis

102 The *C. burnetii* *htpB* gene, coding for a 62 kDa antigen (Vodkin and Williams,
103 1988) was amplified by nested PCR as previously reported (To et al., 1996). To check the
104 specificity of the PCR, amplified fragments were resin purified (Wizard, Promega) and
105 cloned into the pGEM-T vector (Promega) for sequencing both strands by double-stranded
106 dye-termination cycle sequencing (Secugen SL, Madrid, Spain). At least two independent
107 clones were sequenced for each PCR analyzed.

108 Statistical analysis

109 A chi-square test was used to test for the distribution patterns of *C. burnetii*
110 seroprevalence between sampling area (northern vs. southern), ungulate species and habitat
111 (wild vs. farmed). The same statistical test was used to evaluate the differences in the anti-
112 phase I and II antibodies seroprevalence in cattle from LO farm. Statistical analyses were
113 performed using the SPSS v14.0 (SPSS Inc., Chicago, USA) package. The *p* value was set
114 at 0.05.

115 **Results**

116 Evidence of *C. burnetii* infection was found in red deer in northern and southern
117 Spain and in roe deer and cattle in southern Spain. Seroprevalence of *C. burnetii* in the
118 sampled ruminants was 15.4% in roe deer, 5.6% in wild red deer, 40% in farmed red deer
119 and 39% in cattle. None of the fallow deer analyzed showed evidence of contact with *C.*
120 *burnetii*. Seroprevalence level through species, habitat and location is shown in table 1.

121 *Hyalomma marginatum marginatum* and *Hy. lusitanicum* were found parasitizing
122 on red deer and cattle. Roe deer had infestations with *Hy. m. marginatum*. *Rhipicephalus*
123 *bursa* and *R. pusillus* were collected from roe deer and cattle and red deer and roe deer,
124 respectively. *R. (Boophilus) annulatus* was found on cattle only. *Ixodes ricinus* and
125 *Haemaphysalis concinna* were found infesting red deer and roe deer in Asturias.

126 The observed seroprevalence to *C. burnetii* antigens was higher in the southern red
127 deer population ($\chi^2=4.85$, $df=1$, $p<0.05$; Table 1) and it was higher in farmed than in wild
128 animals ($\chi^2=9.05$, $df=1$, $p<0.01$; Table 1). Furthermore, the observed seroprevalence of *C.*
129 *burnetii* infection in wild red deer was higher in northern than in southern populations
130 (Table 1). However, the opposite result was obtained for wild roe deer with higher observed
131 seroprevalence in the southern population ($\chi^2=8.27$, $df=1$, $p<0.01$; Table 1).

132 In the study reported herein, anti-phase I antibodies were found in three cattle only
133 in the farm LO in southern Spain (Table 1), which statistically differed from the number of
134 anti-phase II antibodies positive cattle from the same farm ($\chi^2=22.6$, $df=1$, $p<0.001$).
135 Interestingly, the observed seroprevalence of *C. burnetii* in cattle was higher in farm LO
136 than in farm OJ in Andalucía, southern Spain ($\chi^2=21.5$, $df=1$, $p<0.001$; Table 1).

137 *C. burnetii* DNA was amplified by PCR in samples from wild and farmed red deer
138 and cattle that had antibodies to phase II antigens (Table 1). As expected, the sequence of

139 the amplicons corresponded to the *C. burnetii* *htpB* gene. Sequence analysis evidenced that
140 all sequences were identical to the sequence reported for the Nine Mile phase I isolate
141 (Genbank accession number M20482; Vodkin and Williams, 1988).

142 **Discussion**

143 This is to our knowledge the first report on *C. burnetii* infection in Spanish wild
144 ungulates. As abovementioned, there is a need of research on pathogens circulating in wild
145 ungulate populations due to its increasing demographic trend and especially due to their
146 possible role as pathogen reservoirs for domestic animals and humans (see for example
147 Ruiz-Fons et al., 2007).

148 The seroprevalence of *C. burnetii* found in wild roe (15.4%) and red deer (5.6%)
149 populations in Spain is within the range reported for other wild cervids. Recent
150 seroepidemiological studies in wild cervids have shown anti-*C. burnetii* antibody
151 prevalence of 16.5% and 1.5% in moose (*Alces alces*) and white-tailed deer (*Odocoileus*
152 *virginianus*) in Nova Scotia, USA, respectively (Marrie et al., 1993) and 69% and 56% in
153 Hokkaido deer (*Cervus nippon yesoensis*) and Japanese deer (*Cervus nippon centralis*) in
154 Hokkaido, Japan, respectively (Ejercito et al., 1993). We found no fallow deer to show
155 evidence of contact with *C. burnetii*, but the number of samples tested was smaller than in
156 other host species included in the study. Alternatively, this species may have innate
157 resistance to infection.

158 Our results suggest that in farmed red deer, animal-to-animal contact may increase
159 the risk of *C. burnetii* transmission. Additionally, differences in the observed prevalence of
160 *C. burnetii* infection in wild cervid populations may depend upon living environments
161 (Komiya et al., 2003), which are different between northern and southern Spain.
162 Management widely differs between the areas sampled in northern and southern Spain.

163 While sampled wild cervids in southern Spain live in fenced estates, northern wild cervids
164 live in natural conditions. Feeders for both red and roe deer are present in the sampled
165 southern estates and cervids use to concentrate on them, thus increasing the risk of *C.*
166 *burnetii* and other pathogens transmission between individuals (Acevedo et al., 2007).

167 Anti-*C. burnetii* phase II antibodies are generally associated with acute infections
168 whereas in chronic infections, the antibody level to phase I is usually higher than that to
169 phase II (Maurin and Raoult, 1999). Our results suggest that most of the animals analyzed
170 had acute *C. burnetii* infections, which may correlate with heavy tick infestations in these
171 animals. Tick species found are common on wild ungulates in Spain (de la Fuente et al.,
172 2004; Ruiz-Fons et al., 2006) and have been suspected to play an important role as vectors
173 of *C. burnetii* among wild vertebrates (Marrie et al., 1986; Maurin and Raoult, 1999).
174 Nevertheless, in order to definitively determine the real role of wild ungulates as *C. burnetii*
175 reservoirs for domestic animals and humans, an experimental approach is needed.

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184 **References**

- 185 Acevedo, P., Vicente, J., Höfle, U., Cassinello, J., Ruiz-Fons, F., Gortázar, C.,
186 2007. Estimation of European wild boar relative abundance and aggregation: a novel
187 method in epidemiological risk assessment. *Epidemiol. Infect.* 135, 519-527.
- 188 Bolaños, M., Santana, O.E., Angel-Moreno, A., Perez-Arellano, J.L., Limiñana,
189 J.M., Serra-Majem, L., Martín-Sánchez, A.M., 2003. Seroprevalence of infection by
190 *Coxiella burnetii* in Canary Islands (Spain). *Eur. J. Epidemiol.* 18, 259-262.
- 191 Cardenosa, N., Sanfeliu, I., Font, B., Munoz, T., Nogueras, M.M., Segura, F., 2006.
192 Short report: seroprevalence of human infection by *Coxiella burnetii* in Barcelona
193 (northeast of Spain). *Am. J. Trop. Med. Hyg.* 75, 33-35.
- 194 de la Fuente, J., Naranjo, V., Ruiz-Fons, F., Vicente, J., Estrada-Peña, A., Almazán,
195 C., Kocan, K.M., Martín, M.P., Gortázar, C., 2004. Prevalence of tick-borne pathogens in
196 ixodid ticks (Acari: Ixodidae) collected from European wild boar (*Sus scrofa*) and Iberian
197 red deer (*Cervus elaphus hispanicus*) in central Spain. *Eur. J. Wildl. Res.* 50, 187-196.
- 198 Dunbar, M.R., Cunningham, M.W., Roof, J.C., 1998. Seroprevalence of selected
199 disease agents from free-ranging black bears in Florida. *J. Wildl. Dis.* 34, 612-619.
- 200 Ejercito, C.L., Cai, L., Htwe, K.K., Taki, M., Inoshima, Y., Kondo, T., Kano, C.,
201 Abe, S., Shirota, K., Sugimoto, T., Yamaguchi, T., Fukushi, H., Minamoto, N., Kinjo, T.,
202 Isogai, E., Hirai, K., 1993. Serological evidence of *Coxiella burnetii* infection in wild
203 animals in Japan. *J. Wildl. Dis.* 29, 481-484.
- 204 Enright, J.B., Franti, C.E., Behymer, D.E., Longhurst, W.M., Dutson, V.J., Wright,
205 M.E., 1971. *Coxiella burnetii* in a wildlife-livestock environment. Distribution of Q fever in
206 wild mammals. *Am. J. Epidemiol.* 94, 79-90.
- 207 Gortázar, C., Acevedo, P., Ruiz-Fons, F., Vicente, J., 2006. Disease risks and
208 overabundance of game species. *Eur. J. Wildl. Res.* 52, 81-87.

- 209 Kazar, J., 2005. *Coxiella burnetii* infection. Ann. N. Y. Acad. Sci. 1063, 105-114.
- 210 Komiya, T., Sadamasu, K., Kang, M.I., Tsuboshima, S., Fukushi, H., Hirai, K.,
211 2003. Seroprevalence of *Coxiella burnetii* infections among cats in different living
212 environments. J. Vet. Med. Sci. 65, 1047-1048.
- 213 Maltezou, H.C., Raoult, D., 2002. Q fever in children. Lancet Infect. Dis. 2, 686-
214 691.
- 215 Marrie, T.J., Schlech, W.F., Williams, J.C., Yates, L., 1986. Q fever pneumonia
216 associated with exposure to wild rabbits. Lancet 1, 427-429.
- 217 Marrie, T.J., Embil, J., Yates, L., 1993. Seroepidemiology of *Coxiella burnetii*
218 among wildlife in Nova Scotia. Am. J. Trop. Med. Hyg. 49, 613-615.
- 219 Marrie, T.J., 2004. Q fever pneumonia. Curr. Opin. Infect. Dis. 17, 137-142.
- 220 Maurin, M., Raoult, D., 1999. Q fever. Clin. Microbiol. Rev. 12, 518-553.
- 221 Oporto, B., Barandika, J.F., Hurtado, A., Moreno, B., Aduriz, G., García-Pérez,
222 A.L., 2006. Incidence of ovine abortion by *Coxiella burnetii* in Northern Spain. Ann. N. Y.
223 Acad. Sci. 1078, 498-501.
- 224 Ruiz-Fons, F., Fernández de Mera, I.G., Acevedo, P., Höfle, U., Vicente, J., de la
225 Fuente, J., Gortázar, C., 2006. Ticks (Acari: Ixodidae) parasitizing Iberian red deer (*Cervus*
226 *elaphus hispanicus*) and European wild boar (*Sus scrofa*) from Spain: geographical and
227 temporal distribution. Vet. Parasitol. 140, 133-142.
- 228 Ruiz-Fons, F., Segalés, J., Gortázar, C., 2007. A review of viral diseases of the
229 European wild boar: Effects of population dynamics and reservoir role. Vet. J., in press.
- 230 Sanz, J.C., de los Rios, R., Martin, F., Tebar, M.A., Jado, I., Anda, P., 2006.
231 Aplicación de cuatro métodos de ELISA (dos para IgM y dos para IgG) para el diagnóstico
232 serológico de un brote de fiebre Q. Enferm. Infecc. Microbiol. Clin. 24, 178-181.

- 233 Tellez, A., Sainz, C., Echevarria, C., De Carlos, S., Fernandez, M.V., Leon, P.,
234 Rezina, R., 1988. Q fever in Spain: acute and chronic cases 1981–1985. *Rev. Infect. Dis.*
235 10, 198–202.
- 236 To, H., Kako, N., Zhang, G.Q., Otsuka, H., Ogawa, M., Ochiai, O., Nguyen, S.V.,
237 Yamaguchi, T., Fukushi, H., Nagaoka, N., Akiyama, M., Amano, K., Hirai, K., 1996. Q
238 fever pneumonia in children in Japan. *J. Clin. Microbiol.* 34, 647-651.
- 239 Torina, A., Vicente, J., Alongi, A., Scimeca, S., Turlá, R., Nicosia, S., Di Marco,
240 V., Caracappa, S., de la Fuente, J., 2007. Observed prevalence of tick-borne pathogens in
241 domestic animals in Sicily, Italy during 2003-2005. *Zoonoses Public Health*, in press.
- 242 Vicente, J., Ruiz-Fons, F., Vidal, D., Höfle, U., Acevedo, P., Villanua, D.,
243 Fernández-de-Mera, I.G., Martín, M.P., Gortázar, C., 2005. Serosurvey of Aujeszky's
244 disease virus infection in European wild boar in Spain. *Vet. Rec.* 156, 408-412.
- 245 Vodkin, M.H., Williams, J.C., 1988. A heat shock operon in *Coxiella burnetii*
246 produces a major antigen homologous to a protein in both mycobacteria and *Escherichia*
247 *coli*. *J. Bacteriol.* 170, 1227–1234.
- 248 Webster, J.P., Lloyd, G., Macdonald, D.W., 1995. Q fever (*Coxiella burnetii*)
249 reservoir in wild brown rat (*Rattus norvegicus*) populations in the UK. *Parasitol.* 110, 31-
250 35.
- 251 Willeberg, P., Ruppanner, R., Behymer, D.E., Haghghi, S., Kaneko, J.J., Franti,
252 C.E., 1980. Environmental exposure to *Coxiella burnetii*: a sero-epidemiologic survey
253 among domestic animals. *Am. J. Epidemiol.* 111, 437-443.
- 254 Woldehiwet, Z., 2004. Q fever (coxiellosis): epidemiology and pathogenesis. *Res.*
255 *Vet. Sci.* 77, 93-100.

- 256 Yasumoto, M., Yanase, T., Muramatsu, Y., Morita, C., Ueno, H., 1997.
257 Seroepidemiological study of *Coxiella burnetti* in *Cervus nippon* in northern Japan. Jpn. J.
258 Zoo Wildl. Med. 2, 101-106.

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Table 1. Results of IFA test to phase I and II *C. burnetii* antigens and *htpB* gene nested PCR in wild and domesticated cervids and cattle.

Host	Habitat	Location	N° of samples	Seropositive (% ± SE at 95% CI)		Nested PCR positive/total tested (%) ^a
				Phase I	Phase II	
Red deer	Wild	Northern Spain	21	0 (0 ± 0)	2 (9.5 ± 12.9)	1/2 (50)
	Wild	Southern Spain	15	0 (0 ± 0)	0 (0 ± 0)	None tested
	Farm LO	Southern Spain	80	0 (0 ± 0)	32 (40 ± 10.8)	4/32 (12)
<i>Subtotal red deer</i>			<i>116</i>	<i>0 (0 ± 0)</i>	<i>34 (29.3 ± 8.2)</i>	<i>5/34 (14.7)</i>
Roe deer	Wild	Northern Spain	21	0 (0 ± 0)	0 (0 ± 0)	None tested
	Wild	Southern Spain	18	0 (0 ± 0)	6 (33 ± 22.4)	0/6 (0)
<i>Subtotal roe deer</i>			<i>39</i>	<i>0 (0 ± 0)</i>	<i>6 (15.4 ± 11.6)</i>	<i>0/6 (0)</i>
Fallow deer	Wild	Northern Spain	13	0 (0 ± 0)	0 (0 ± 0)	None tested
Cattle	Farm LO	Southern Spain	49	3 (6 ± 4.9)	26 (53 ± 10.6)	3/26 (12) ^b
	Farm OJ	Southern Spain	30	0 (0 ± 0)	2 (7 ± 5.1)	0/2 (0)
<i>Subtotal cattle</i>			<i>79</i>	<i>3 (3.8 ± 4.3)</i>	<i>28 (35.4 ± 10.6)</i>	<i>3/28 (10.7)</i>

^a Only samples from seropositive animals were tested by *C. burnetii htpB* gene nested PCR.

^b Samples from animals seropositive for phase I *C. burnetii* antibodies were negative for the *htpB* gene nested PCR assay.