



**HAL**  
open science

## Prevalence of infection in wild and farmed ungulates

Francisco Ruiz-Fons, Óscar Rodríguez, Alessandra Torina, Victoria Naranjo,  
Christian Gortázar, José de La Fuente

► **To cite this version:**

Francisco Ruiz-Fons, Óscar Rodríguez, Alessandra Torina, Victoria Naranjo, Christian Gortázar, et al.. Prevalence of infection in wild and farmed ungulates. *Veterinary Microbiology*, 2007, 126 (1-3), pp.282. 10.1016/j.vetmic.2007.06.020 . hal-00532284

**HAL Id: hal-00532284**

**<https://hal.science/hal-00532284v1>**

Submitted on 4 Nov 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Accepted Manuscript

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

Authors: Francisco Ruiz-Fons, Óscar Rodríguez, Alessandra Torina, Victoria Naranjo, Christian Gortázar, José de la Fuente



PII: S0378-1135(07)00319-7  
DOI: doi:10.1016/j.vetmic.2007.06.020  
Reference: VETMIC 3742

To appear in: *VETMIC*

Received date: 8-3-2007  
Revised date: 21-6-2007  
Accepted date: 25-6-2007

Please cite this article as: Ruiz-Fons, F., Rodríguez, Ó., Torina, A., Naranjo, V., Gortázar, C., de la Fuente, J., Prevalence of *Coxiella burnetti* infection in wild and farmed ungulates, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.06.020

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18

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

Francisco Ruiz-Fons<sup>1</sup>, Óscar Rodríguez<sup>1</sup>, Alessandra Torina<sup>2</sup>, Victoria Naranjo<sup>1</sup>, Christian Gortázar<sup>1</sup>, José de la Fuente<sup>1,3</sup>

<sup>1</sup>Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13071 Ciudad Real, Spain.

<sup>2</sup>Istituto Zooprofilattico Sperimentale della Sicilia, Via G. Marinuzzi n°3, 90129 Palermo, Italy.

<sup>3</sup>Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA.

\*Corresponding author: Francisco Ruiz-Fons. Phone: + 34 926 29 54 50; Fax: + 34 926 29 54 51. e-mail: josefrancisco.ruiz@uclm.es

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.

34

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

36

37

38

39

40

41

42

## 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.

#### 176 **Acknowledgements**

177 This research was supported by grants "Epidemiología de zoonosis transmitidas por  
178 garrapatas en Castilla – La Mancha" (06036-00 ICS-JCCM), GC05-006 (Consejería de  
179 Sanidad, JCCM), "Epidemiología de las enfermedades compartidas entre ungulados  
180 silvestres y ganado en España: aproximación multidisciplinar" (MEC AGL2005-07401)  
181 and FEDER. This is a contribution to the agreement between IREC and Principado de  
182 Asturias. V. Naranjo is funded by Junta de Comunidades de Castilla – La Mancha (JCCM),  
183 Spain. We are grateful to two anonymous reviewers for improving this manuscript.

#### 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., Martin-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.

Accepted Manuscript

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 (%) <sup>a</sup>
				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) <sup>b</sup>
	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>

<sup>a</sup> Only samples from seropositive animals were tested by *C. burnetii htpB* gene nested PCR.

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