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SHORT REPORT
Wide exposure to *Coxiella burnetii* in ruminant and feline species living in a natural environment: zoonoses in a human–livestock–wildlife interface

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SUMMARY

Assessment of the role of wild and domestic hosts as potential reservoirs of misdiagnosed zoonoses, such as Q fever by *Coxiella burnetii*, is an important public health issue today both for wildlife conservation and management of disease in human–livestock–wildlife interface. This study used ELISA, an indirect antibody, to research (2003–2013) *C. burnetii* infection in seven free-living wild and domestic ruminant species and in European wildcats (*Felis silvestris*). The animals studied were 0 European wildcats, 21 Spanish ibex (*Capra pyrenaica*), 314 red deer (*Cervus elaphus*), 556 fallow deer (*Dama dama*), 211 European mouflon (*Ovis aries musimon*), eight roe deer (*Capreolus capreolus*), 407 bovines (*Bos taurus*) and 3739 sheep (*Ovis aries*). All the animals shared the same habitat in the Serranía de Cuenca Natural Park (Castile-La Mancha, Spain). The study area is an example of human–domestic–wildlife interface where people and domestic animals live in close proximity to wildlife. Observed *C. burnetii* seropositive frequencies were: 33·3% European wildcats, 23·8% Spanish ibex, 22·5% domestic sheep 1·5% red deer, 1·4% European mouflon, 0·24% cattle, 0·18% fallow deer and 0% roe deer. The study found a wide *C. burnetii* prevalence of previous and present exposure in wild and domestic ruminant hosts in the Serranía de Cuenca Natural Park and reports the first evidence of *C. burnetii* exposure in free-living European wildcats.

Key words: Epidemiology, European wildcat, one health, public health, Q fever, wild ruminant.

Q fever (coxiellosis in animals), caused by *Coxiella burnetii*, is an important worldwide zoonosis, with a very broad host range [1]. Forty percent of primary infections in humans are symptomatic and clinically polymorphic. The illness can be acute or chronic and may have serious sequelae, such as endocarditis, debilitating post-Q fever fatigue and granulomatous lesions in bone and soft tissues [1]. *C. burnetii*, recently included in the order Legionellales, has been detected in many host species, including vertebrate and invertebrate taxa. It can be found in ticks and other arthropods, but unlike other members belonging to its former family, Rickettsiaceae, *C. burnetii* maintenance is not dependent on arthropod transmission. The main mode of infection is via inhalation of contaminated aerosols from parturient secretions of infected animals. Considering that cattle, sheep and goats are the most commonly reported sources of infection for humans, it is striking that *C. burnetii* infection has scarcely been observed in wildlife species from areas with consolidated human–livestock–wildlife contact. Given the recent upsurge of outbreaks in humans

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reported in many parts of the world [2–4], the aim of this study was to analyse *C. burnetii* infection in eight free-living wild and domestic species, including locally vulnerable species.

The species studied shared the same habitat in the Serranía de Cuenca Natural Park (SCNP) (Central Spain, 1° 51′–2° 03′ West, 40° 12′–40° 28′ North), a partially protected area (25 724 ha) with a traditional Mediterranean mountainous landscape. The survey was conducted between 2003 and 2013. The sample size used in this study was proportional to the population density of each species in the study territory. The sample comprised 21 Spanish ibex (*Capra pyrenaica*), 314 red deer (*Cervus elaphus*), 556 fallow deer (*Dama dama*), 211 European mouflon (*Ovis aries musimon*) and eight roe deer (*Capreolus capreolus*). The European wildcat (*Felis silvestris*) population in central Spain has, for its part, remained stable in recent years. Considering a minimum home range of 1·95 km^2/animal [5], we estimated a maximum wildcat population of 128 for the SCNP. We also took into account the minimum seroprevalence reported for wild or stray cats in similar studies [6] to calculate the minimum sample size required for wildcats from the SNCP. Assuming an expected seroprevalence of 25% (±3%) and a 95% confidence level, a minimum sample of 11 wildcats in the SNCP would be necessary. In consequence, samples were taken from nine wildcats. Samples from domestic animals totalled 407 cattle (*Bos taurus*) and 3739 sheep (*Ovis aries*), which were located in the SNCP at the time of sampling. Samples from domestic animals were obtained following the World Organisation for Animal Health (OIE) notifiable diseases official control and eradication programmes. Blood samples from wild ruminants were extracted from the jugular vein in live animals trapped with fixed capture boxes or directly from the heart or thoracic cavity in sport-hunted animals. Wildcats were trapped with fixed box traps, were anesthetized with a mixture of ketamine hydrochloride (Imalgene; Merial, France) and xylazine (Rompún; Bayer, Spain) and were released after reanimation at the same place of capture. Samples from wildcats were taken from the radial vein.

Blood samples collected were put into separator tubes (Venoject II, Terumo Europe, Leuven, Belgium), and centrifuged at 1800 ***g*** for 10 min to obtain sera, which were stored at −20 °C until analyses were performed. Both wild and domestic ruminant sera were tested for antibodies against *C. burnetii* using the commercially available CHEKIT* Q-Fever Antibody ELISA test kit (IDEXX, Switzerland), which uses ELISA plates coated with an inactivated mixture of *C. burnetii* phase I and II antigens (Nine Mile strain). The monoclonal secondary antibody used in this ELISA blocks an epitope that is shared by all the species belonging to the suborder Ruminantia (J. I. Salido, Technical Service Europe South IDEXX, personal communication). Samples were tested in duplicate and optical densities (ODs) were normalized using the formula:

$$\mathrm{S/P\%} = \frac{(\mathrm{OD_{sample}} - \mathrm{OD_{negative\ control}})}{(\mathrm{OD_{positive}} - \mathrm{OD_{negative\ control}})} \times 100.$$

Following the manufacturers' instructions, sera with S/P $\geqslant$40% was considered positive, S/P <30% was deemed negative, and results in the interval 30%$\leqslant$ S/P<40% were taken as inconclusive. Moreover, according to the manufacturers, the assay has a 99% diagnostic sensitivity (Se) and a 88·57% specificity (Sp). Wildcat sera were similarly tested at 1:400 dilution in duplicates following IDEXX Q-fever ELISA instructions. To adapt the ELISA to wildcat sera, we had to make some modifications along the lines of a previous validation assay performed with stray cat sera (M. G. Candela, unpublished results). Briefly, modifications made in the validated stray-cat assay included: (i) using a peroxidase-conjugated affinity purified goat anti-cat IgM (Bethyl Laboratories Inc., USA), which was titrated and used at a 1:5000 dilution in PBS; (ii) including stray-cat sera as positive and negative controls to normalize tested sera as described above. Corrected OD values of the stray-cat sera were plotted through a binomial distribution that separates positive and negative curves enabling us to select the best control sera. The stray cats selected as controls were seronegative to *Toxoplasma gondii, Chlamydophila* spp., *Bartonella felis,* feline immunodeficiency virus, feline leukaemia virus, feline coronavirus and feline panleukcopenia virus; (iii) using a 50% S/P% cut-off point, which has been shown to provide the highest specificity when using a receiver-operating curve analysis in the stray-cat samples [7].

χ^2 test, or when appropriate, Fisher's exact test, was used to test differences between species in the proportion of animals displaying serological evidence of exposure to *C. burnetti* and significance was considered at $P < 0{\cdot}05$ for a double-tailed test. Odds ratios [with 95% Cornefield confidence intervals (CI)] were used to estimate the risk of *C. burnetii* exposure associated to different host species.

Antibodies against *C. burnetii* were detected in all species tested, except in roe deer. Seroprevalence was

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Table 1. *Prevalence of present/previous exposure to* Coxiella burnetii

Species	Positive	D	Negative	P	No.	OR	95% CI	Yates χ^2	*P*
Wildcat	3	—	6	33·3	9	NP	NP	2·62	0·1
Spanish ibex	5	15	1	23·8	21	2	0·75–5·32	1·1	0·29
Domestic sheep	708	194	2837	18·9	3739	24·87	14·9–41·4	309·63	⩽0·05
Red deer	5	2	307	1·59	314	0·09	0·03–0·21	42·66	⩽0·05
European mouflon	3	1	207	1·42	211	0·08	0–0·24	28·54	⩽0·05
Fallow deer	1	0	555	0·17	556	0·009	0–0·05	100·36	⩽0·05
Cattle	1	3	403	0·24	407	0·01	0–0·07	69·52	⩽0·05
Roe deer	0	8	0	0	8	NP	NP	NP	NP

Odds ratio (OR) of the exposure to *C. burnetii*. χ^2 difference test
D, Doubtful; P, prevalence of exposure (frequency expressed in %); No., total number of samples; CI, confidence interval; NP, not performed (it is not possible when the sample is small).

33% (95% CI 2·53–64·13) in European wildcats, 24% (95% CI 5·59–42·03) in Spanish ibex, 19% (95% CI 17·68–20·19) in domestic sheep, 1·6% (95% CI 0·21–2·98) in red deer, 1·4% (95% CI 0·00–3·02) in European mouflon, 0·2% (95% CI 0–0·727) in cattle, and 0·1% (95% CI 0–0·53) in fallow deer (Table 1). The odds ratio (OR) of seropositivity was significantly higher in domestic sheep compared to the other species ($P < 0·05$) (Table 1).

Our results indicate that *C. burnetii* infection is widespread in wild and domestic species that share the same habitat in the SCNP, and suggest that some (mainly sheep and Spanish ibex) may play a significant role in the Q fever–coxiellosis epidemiological cycle in the protected area studied. The presence of animals displaying serological evidence of exposure to *C. burnetii* does not necessarily imply that animals remain infected and shed organisms into the environment. However, the reasonably high prevalence of previous exposure in these populations suggests that infection is probably widespread within the animals in the SCNP, and that these animals may potentially be a source of infection for other animals and humans. It is important to note that a variety of human activities (rearing domestic herds, hunting, forestry works, ecotourism) are carried out in this human–domestic–wildlife interface, where people and their domestic animals live in close proximity to wildlife.

Q fever has been diagnosed in animals and people in Spain with variable seroprevalence [4, 8]. It has also been recognized as an important public health problem in other European rural areas in The Netherlands [2] and in France [3]. Furthermore, it has been associated with living and working near ruminant farms or manure-covered fields [3, 4], where the wind factor increases the risk of infection [9]. The risk of infection can also increase in the spring months, when most of the births in wild and domestic ruminants occur. Most human Q fever cases in Spain [8] and Southern France [3] also appear in spring. The high *C. burnetii* infection found in sheep from the SCNP and their potential role in human infection requires researching.

The ELISA technique used in the survey successfully detected the immunoglobulin response against *C. burnetii* in the ruminant species analysed, except in roe deer. This technique, which is recommended by the OIE [10], shows high sensitivity and good specificity. The IDEXX Q-fever ELISA can be used in different ruminant species, since it uses an unspecific conjugate that binds to a common IgG epitope present in the suborder Ruminantia (IDEXX technical services, personal communication). This kit was previously used and assessed by our research group in a *C. burnetii* study on roe deer [11].

Our study shows for the first time that free-living European wildcats are exposed to infection with *C. burnetii,* which suggests they should be considered a part of the epidemiological cycle of *C. burnetii*, in the same way as stray and pet cats [6, 12, 13]. Seroepidemiological studies conducted in stray cats in Japan and the UK have reported high prevalence values of antibodies to *C. burnetii,* ranging from 33·3% to 72·67% [6, 12]. Although it is difficult to compare reported seroprevalence due to the different methodologies used, these studies provide an overview of the level of infection in free-roaming felines and highlight their potential zoonotic risk to humans. In addition, domestic felines have been recently considered as both a reservoir [12, 13] and an important route of infection to humans [13].

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As with many other infectious diseases in humans, IgM antibodies appear first in response to *C. burnetii* infection and persist for at least 1 year. IgG appears later and can persist for several years [14]. Since the antigen used by our ELISA is a mix between phases I and II of *C. burnetii*, we have not been able to distinguish between phases. However, if the immunological pattern developed by wildcats mimics the features observed in humans, the use of an anti-IgM conjugate in the modified ELISA could maximize the detection of acute infection in the sampled animals.

The wide home range of the wildcats and the difficulties in capturing them, as well as the comparatively low density of this feline species, may explain why few wildcats have been tested and the wide confidence range observed in *C. burnetii* seroprevalence. Wildcats were captured reasonably far from each other and seroprevalence detected in our study is in agreement with those reported by other authors [6, 12]. Nevertheless, it should be borne in mind that a possible explanation for the prevalence detected could be infections acquired in agonistic encounters between males, or the fact that some of the animal tested belonged to a litter born from an infected female. Most of the epidemiological features of *C. burnetii* transmission in wildcats remain to be elucidated.

In summary, this study confirms the exposure to infection in wild and domestic animals in the SCNP, including wildcats, whose exposure to infection has not been previously described. There is a clear need for further interdisciplinary studies involving veterinary, biological and medical services to improve our understanding of the complexity of the Q fever–coxiellosis epidemiology in its natural environment in Spain.

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DECLARATION OF INTEREST

None.

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