Received: 5 December 2023 | Revised: 25 April 2024 | Accepted: 10 May 2024

DOI: 10.1111/zph.13155

ORIGINAL ARTICLE

WILEY

Occurrence of *Coxiella burnetii* in wild lagomorphs and their ticks in Spanish Mediterranean ecosystems

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Funding information
Ministerio de Ciencia e Innovación; Universidad de Córdoba; European Union "NextGenerationEU"; European Regional Development Fund; Instituto de Salud

Abstract

Background: *Coxiella burnetii*, the causative agent of Q fever, is a zoonotic multi-host vector-borne pathogen of major public health importance. Although the European Food Safety Authority has recently made the monitoring of this bacterium in wildlife a priority, the role of wild lagomorphs in the transmission and maintenance of *C. burnetii* is poorly understood.

Aims: The aims of this study were to determine the prevalence and risk factors associated with *C. burnetii* circulation in European wild rabbits (*Oryctolagus cuniculus*) and Iberian hares (*Lepus granatensis*) and to assess the presence of this pathogen in ticks that feed on them in Mediterranean ecosystems in Spain, the country with the highest number of reported cases of Q fever in Europe.

Methods: A total of 574 spleen samples were collected from 453 wild rabbits and 121 Iberian hares, and 513 ticks (processed in 120 pools) between the 2017/2018 and 2021/2022 hunting seasons.

Results: *C. burnetii* DNA was detected in 103 (17.9%; 95% CI: 14.8–21.1) of the 574 wild lagomorphs tested. By species, prevalence was 16.3% (74/453; 95% CI: 12.9–19.7) in the European wild rabbit and 24.0% (29/121; 95% CI: 16.4–31.6) in the Iberian hare. At least one positive lagomorph was found on 47.9% of the 96 hunting estates sampled and in every hunting season since 2018/2019. Two risk factors associated with *C. burnetii* infection were as follows: outbreak of myxomatosis on the hunting estate in the month prior to sampling and high tick abundance observed by gamekeepers on the hunting estate. *C. burnetii* DNA was also found in 33 of the 120 (27.5%; 95% CI: 19.5–35.5) tick pools tested. The pathogen was detected in 66.7% (4/6), 29.2% (26/89) and 21.4% (3/14) of *Haemaphysalis hispanica*, *Rhipicephalus pusillus* and *Hyalomma lusitanicum* pools respectively.

Conclusions: This study provides new epidemiological data on *C. burnetii* in European wild rabbits and is the first survey on this zoonotic pathogen performed in Iberian

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hares. Our results indicate widespread endemic circulation of *C. burnetii* and highlight the importance of both wild lagomorph species as natural reservoirs of this zoonotic bacterium in Mediterranean ecosystems in southern Spain, which may be of public and animal health concern. The high prevalence and wide diversity of positive tick species suggest the possible role of ticks in the epidemiological cycle of *C. burnetii*, with the potential risk of transmission to sympatric species, including humans.

KEYWORDS
European wild rabbit, Iberian hare, Q fever, risk factors, tick

1 | INTRODUCTION

Coxiella burnetii (family *Coxiellaceae*) is a globally distributed obligate intracellular zoonotic bacterium that causes Q fever, a disease with a major impact on public and animal health (European Food Safety Authority and European Centre for Disease Prevention and Control [EFSA & ECDC], 2022). The European Food Safety Authority (EFSA) recently included this disease as a priority for the establishment of a coordinated surveillance system (European Food Safety Authority [EFSA] et al., 2023).

Q fever in humans is mainly acquired zoonotically not only through environmental contamination, the result of infected animals excreting bacteria in their faeces, but also through foodborne and tickborne transmission (EFSA, 2014). More rarely, *C. burnetii* can be transmitted through blood transfusions and congenital or sexual transmission (Centers for Disease Control and Prevention [CDC], 2019). In Europe, more than 700 human cases per year and a fatality rate of around 1.7% have been reported in recent years; Spain has the highest number of cases (EFSA & ECDC, 2022).

Domestic ruminants are considered to be the main reservoirs of *C. burnetii* (Maurin & Raoult, 1999). In these species, the disease is frequently associated with reproductive disorders, which have a considerable economic impact due to production losses and the cost of implementing control programmes (van Asseldonk et al., 2013). However, this bacterium has a complex eco-epidemiology and its host range has expanded in recent decades so that it is currently considered a multi-host pathogen (Celina & Cerný, 2022). Different studies suggest that wildlife plays a major epidemiological role in the maintenance and transmission of *C. burnetii*, not only to other wildlife species but also to domestic animals and humans (González-Barrio et al., 2015a, 2021). It should also be noted that, in recent years, an increased number of human cases of Q fever have been associated with exposure to wildlife (González-Barrio & Ruiz-Fons, 2019). Ticks are known to be important vectors of transmission of *C. burnetii* among wild animals (Moraga-Fernández et al., 2023), either via tick saliva at the site of the bite or by direct contact or inhalation of tick faeces (Philip, 1948). Different studies point out that these parasites may act as reservoirs of the bacterium in nature since ticks can spread the pathogen to their progeny by transovarial transmission (Walker & Fishbein, 1991). To date, the bacterium has been detected in more than 30 tick species collected from wildlife, some of which have already been shown to be competent vectors (Duron et al., 2015; Eldin et al., 2017).

Impacts

- Widespread circulation of *Coxiella burnetii* in wild rabbits and Iberian hares and wide variety of tick species positive for *C. burnetii* DNA.
- First report of *C. burnetii* infection in the Iberian hare.
- The prevalence detected in both wild lagomorphs and their ticks may be of public and animal health concern and underlines the need to establish a coordinated surveillance system for Q fever in wild lagomorph species.

It has been suggested that the European wild rabbit (*Oryctolagus cuniculus*) is a natural reservoir of *C. burnetii* (González-Barrio et al., 2015b) and human infections have already been linked to this species (Marrie et al., 1986). Transmission can be direct, while handling hunted animals being prepared for consumption, for example, or indirect, either by tick bite or by coming into contact with contaminated environment, given the high resistance of the bacterium. In Spanish Mediterranean ecosystems, both the European wild rabbit and the Iberian hare (*Lepus granatensis*) are the most important small game species in terms of hunting interest, distribution and abundance, and constitute an important source of human food, generally destined for home consumption and without veterinary controls. Although the EFSA has highlighted the need for the surveillance of *C. burnetii* in wild lagomorphs (ENETWILD-consortium et al., 2023), to date, there is very little information on these species and their associated ticks. The aims of this study, therefore, were to molecularly investigate the presence and associated risk factors of *C. burnetii* in spleen samples from European wild rabbits and Iberian hares and ticks feeding on these lagomorph species in Mediterranean ecosystems of southern Spain.

2 | MATERIALS AND METHODS

2.1 | Study design and sampling

Between the 2017/2018 and 2021/2022 hunting seasons, a cross-sectional epidemiological study was carried out in the region of Andalusia (southern Spain; 36°N-38°60′N, 1°75′W–7°25′W), the second largest in Spain with an area of 87,300km^2, occupying more than 17% of the national territory. For the European wild rabbits, sample size was calculated as a minimum of 385 animals, assuming a prevalence of 50%, with a 95% confidence interval (95% CI) and a desired precision of ±5%. Whenever possible, 60 wild rabbits were sampled in each of the eight provinces comprising the study area, in order to ensure a 95% probability of detecting at least one positive animal, assuming a minimum within-province prevalence of 5% (Thrusfield & Christley, 2018). Sampling sites (hunting estates) were randomly selected in each province. On each of these hunting estates (Figure 1), hunters kindly provided between 3 and 25 (mean: 11.9) wild rabbits for sampling. A total of 453 wild rabbits from 38 hunting estates distributed across all eight provinces were ultimately sampled during the study period. Between hunting seasons 2017/2018 and 2021/2022, samples were also collected from 121 Iberian hares from 66 hunting estates in the same study area using convenience sampling. The spleens were removed from these animals aseptically and stored in individually labelled plastic tubes. After visual inspection, a total of 513 ticks feeding on 127 and 30 of the sampled rabbits and hares, respectively, were also collected using tweezers. Spleen and tick samples were kept refrigerated until arrival at the laboratory and immediately frozen at −20°C.

Information on each individual was recorded, including species, location, year of sampling, age (according to body weight and body length; Morris, 1972), kidney fat index and sex. During each sampling, epidemiological data related to the hunting estates were also gathered through personal interviews with gamekeepers using a standardized questionnaire (Table S1). The information obtained included the characteristics of the hunting estate, the presence of disease and control measures, management practices and presence of other sympatric species.

2.2 | Tick identification and pooling

Ticks collected in the present study were already identified in a previous survey conducted by Remesar et al. (2021). Identification to species level was performed using morphological keys (Pérez-Eid, 2007), and a subset of each tick species was further molecularly analysed (Norris et al., 1996) to confirm microscopic identification. Tick specimens collected from the same hunting estate were pooled according to species, development stage and host species.

2.3 | Molecular analysis

Total DNA from spleen samples was extracted with the commercial NucleoSpin Tissue® kit (Macherey-Nagel, Germany), while

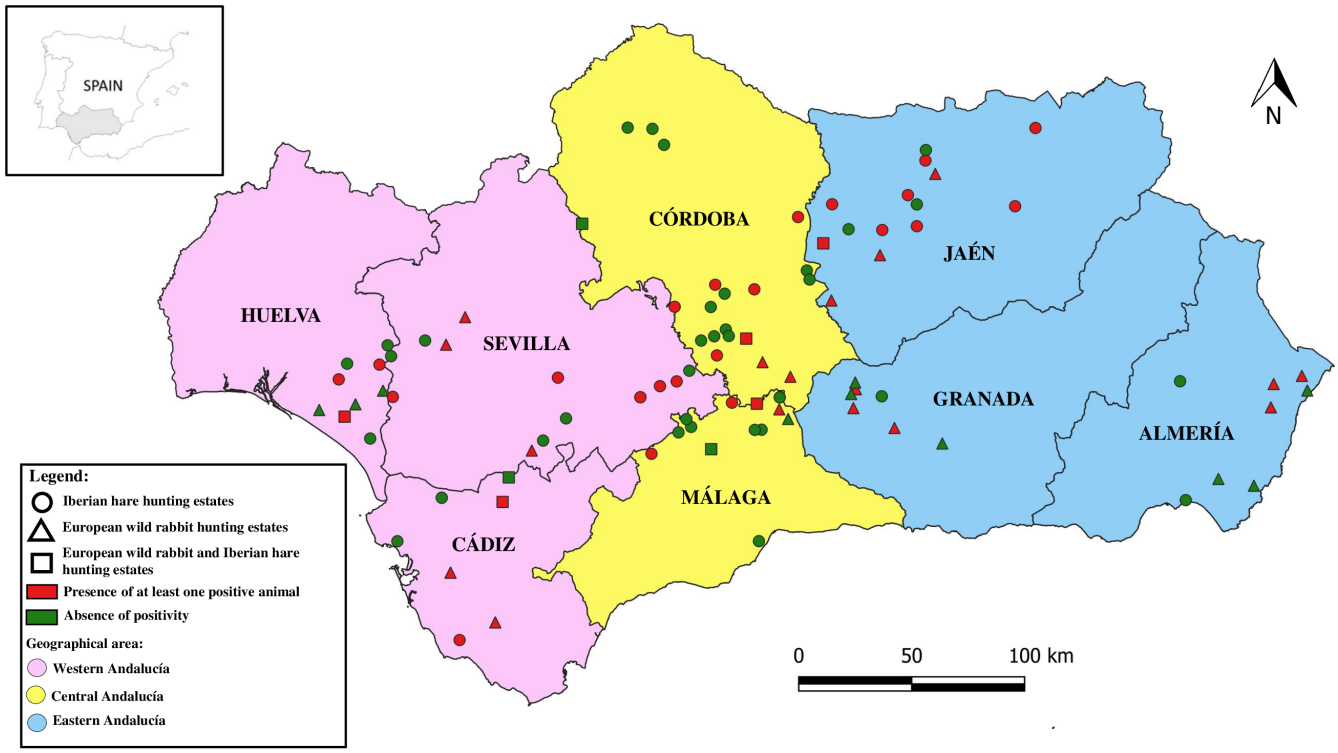

FIGURE 1 Spatial distribution of the prevalence of *Coxiella burnetii* infection among wild lagomorphs in the study region (Andalusia, southern Spain).

DNA from tick pools was obtained using the commercial High Pure PCR Template Preparation Kit® (Roche Diagnostics, Mannheim, Germany), both following the manufacturer's instructions. Extracted DNA was stored at −80°C until molecular analyses. To test for the presence of *C. burnetii* DNA, real-time PCR (RT-PCR; Bio-Rad CFX Connect Real-Time PCR System, Feldkirchen, Germany) was performed, targeting the IS1111a insertion element of this pathogen, as previously described (Tilburg et al., 2010), using GoTaq®qPCR Master Mix technology (Promega). One positive control in duplicate, obtained after DNA extraction of *C. burnetii* phase I antigens derived from the Serion® ELISA classic *C. burnetii* (Serion GmbH, Würzburg), and two non-template controls were used in each run of PCR.

2.4 | Statistical analyses

The prevalence of *C. burnetii* in the wild lagomorphs was determined from the proportion of positive animals to the total number of individuals analysed by PCR, whereas the percentage of *C. burnetii*-positive ticks was calculated by taking into account the number of pools analysed, using the two-sided exact binomial test, with 95% CI in both. Cut-off points for continuous variables were determined at the 33rd and 66th percentiles. Pearson's Chi-square or Fisher's exact test was first used, as appropriate, to screen for associations between the prevalence of *C. burnetii* in wild lagomorphs and the percentage of positive tick pools with explanatory variables. All variables with a $p < 0.05$ in the bivariate analysis were selected for further analysis. Collinearity between pairs of variables was then tested by Cramer's V coefficient, and finally, a generalized estimating equations (GEE) analysis was carried out to study the effect of the variables selected from the bivariate analysis. The number of positive animals and tick pools was assumed to follow a binomial distribution, and 'hunting estate' was included as the subject variable. Forward selection was used for introduction of variables, starting with the variable with the lowest *p*-value in the bivariate analysis, until all remaining variables showed statistically significant values ($p < 0.05$). SPSS 25.0 software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA) was used for all statistical analyses.

3 | RESULTS

C. burnetii DNA was detected in 103 (17.9%; 95% CI: 14.8–21.1) of the 574 wild lagomorphs analysed. Prevalence by species was 16.3% (74/453; 95% CI: 12.9–19.7) in the European wild rabbit and 24.0% (29/121; 95% CI: 16.4–31.6) in the Iberian hare. The pathogen was detected in animals sampled during each hunting season since 2018/2019, with prevalence values ranging from 12.7% (48/379; 95% CI: 9.3–16.0) in 2020/2021 to 33.3% (12/36; 95% CI: 17.9–48.8) in 2019/2020 (Table 1). Positive animals were detected on 46 of 96 (47.9%; 95% CI: 37.9–57.9) hunting estates and in every province in the study region (Figure 1).

The final GEE model showed that the risk of being infected by *C. burnetii* was significantly higher in lagomorphs from hunting estates that had reported outbreaks of myxomatosis during the month prior to sampling (19.9%) than in those with no outbreaks (4.3%) ($p = 0.028$). In addition, significantly higher positivity was found in animals from hunting estates where gamekeepers observed a high tick abundance (22.0%) than in those where a high tick abundance was not detected (7.9%) ($p = 0.022$) (Table 2).

Five tick species were identified: *Rhipicephalus pusillus*, *Rhipicephalus sanguineus* sensu lato, *Haemaphysalis hispanica*, *Hyalomma lusitanicum* and *Ixodes ventalloi* (Table 3). *Coxiella burnetii* DNA was detected in 33 (27.5%; 95% CI: 19.5–35.5) of the 120 tick pools tested (Table 3). According to tick species, *C. burnetii* DNA was found in 29.2% (26/89; 95% CI: 19.8–38.7) of *R. pusillus* pools, 21.4% (3/14; 95% CI: 0.0–42.9) of *H. lusitanicum* pools and 66.7% (4/6; 95% CI: 29.0–100) of *H. hispanica* pools. None of the *R. sanguineus s.l.* (0/9; 95% CI: 0.0–33.6) or *I. ventalloi* (0/2; 95% CI: 0.0–84.2) pools tested positive (Table 3). Positive tick pools were observed during all hunting seasons sampled, and at least one positive pool was found on 20 of 42 (47.6%; 95% CI: 32.5–62.7) hunting estates (Figure 2). *C. burnetii*-positive lagomorphs were detected on 10 (50%) of these 20 hunting estates, and on 14 (63.6%) of the 22 hunting estates where *C. burnetii* DNA was not present in ticks.

The final GEE model for tick pools identified the variable 'lagomorph species' as potentially associated with the positivity to *C. burnetii* found in these parasites. In connection with this, the frequency of positive tick pools collected from the European wild rabbits (33.0%; 30/91; 95% CI: 23.3–42.6; $p = 0.031$; OR = 4.3 (95% CI: 1.1–16.3)) was significantly higher than the 10.3% (3/29; 95% CI: 0.0–21.4) detected in those collected from Iberian hares.

4 | DISCUSSION

In view of the current epidemiological situation of Q fever in Europe, *C. burnetii* has been recognized as an important zoonotic pathogen of public health concern, with Q fever being listed by the EFSA as a priority disease for coordinated surveillance under a One Health approach. During the study period, a total of 1402 human cases were reported in Spain, with Andalusia being the region with the second highest number of reported cases after the Canary Islands (EFSA et al., 2023).

The present study provides new epidemiological data on *C. burnetii* in wild lagomorphs and adds information relevant to the role of ticks in the epidemiology of this zoonotic bacterium in wild animals. To date, only four studies have assessed the presence of *C. burnetii* DNA in European wild rabbits, all of them carried out in Spain. The prevalence found in wild rabbit populations (16.3%) in Andalusia in the present study was lower than in those detected in central regions of this country, where 43.4%–47.2% of European wild rabbits were positive (González et al., 2019; Sánchez et al., 2022). By contrast, lower prevalence values ranging between 1.5% and 4.4% were

TABLE 1 Distribution of the prevalence of *Coxiella burnetii* in wild lagomorphs in Andalusia (southern Spain) according to animal and hunting estate variables and results of bivariate analysis

Variable	Categories	No. positives/Overall[a]	Seroprevalence (%)	*p*
Data recorded from the sampled animals				
Species	Wild rabbit	74/453	16.3	**0.038**
	Iberian hare	29/121	24.0	
Age	Adult	73/405	18.0	0.846
	Subadult	25/129	19.4	
	Young	5/33	15.2	
Sex	Male	44/279	15.8	0.099
	Female	59/291	20.3	
Kidney fat index	0	33/135	24.4	0.059
	1	24/139	17.3	
	2	17/108	15.7	
	3	10/92	10.9	
Body weight (kg)	0.4–0.9	32/165	19.4	0.347
	1.0–1.2	22/143	15.4	
	1.3–3.1	26/116	22.4	
Body length (cm)	19–37	31/159	19.5	0.917
	38–40	27/142	19.0	
	41–59	20/114	17.5	
Hunting season	2017/2018	0/1	0.0	**<0.001**
	2018/2019	9/44	20.5	
	2019/2020	12/36	33.3	
	2020/2021	48/379	12.7	
	2021/2022	34/114	29.8	
Hunting estate's characteristics				
Geographical area	Western	39/186	21.0	**0.007**
	Central	19/181	10.5	
	Eastern	45/207	21.7	
Burrow density	High	69/405	17.0	**0.002**
	Medium	11/30	36.7	
	Low	5/65	7.7	
High abundance of ticks in the hunting estate	Yes	63/286	22.0	**<0.001**
	No	11/140	7.9	
High abundance of fleas in the hunting estate	Yes	52/264	19.7	0.067
	No	22/162	13.6	
Presence of rabbit feeders	Yes	42/297	14.1	**0.027**
	No	43/203	21.2	
Feed supplementation in rabbits	Yes	34/216	15.7	0.298
	No	51/284	18.0	
Presence of swamps	Yes	20/82	24.4	**0.040**
	No	65/418	15.6	
Presence of troughs	Yes	69/419	16.5	0.283
	No	16/81	19.8	
Presence of streams	Yes	52/262	19.8	**0.048**
	No	33/238	13.9	

(Continues)

TABLE 1 (Continued)

Variable	Categories	No. positives/Overall[a]	Seroprevalence (%)	*p*
The hunting estate is weeded	Yes	12/109	11.0	**0.037**
	No	73/391	18.7	
Presence of artificial burrows	Yes	3/35	8.6	0.122
	No	82/465	17.6	
Fenced hunting estate	Yes	5/37	13.5	0.375
	No	80/463	17.3	
Lagomorph density (animal/km^2)	High (51–100)	3/27	11.1	0.057
	Medium (26–50)	5/48	10.4	
	Low (11–25)	6/38	15.8	
	Very low (0–10)	31/118	26.3	
Detection of outbreaks of other infectious diseases				
Outbreaks of myxomatosis in the last year	Yes	84/477	17.6	0.074
	No	1/23	4.3	
Outbreaks of RHD in the last year	Yes	68/405	16.8	0.450
	No	17/95	17.0	
Outbreaks of myxomatosis in the last month	Yes	81/408	19.9	**<0.001**
	No	4/92	4.3	
Outbreaks of RHD in the last month	Yes	24/115	10.9	0.133
	No	61/385	15.8	
Presence of other sympatric species in the hunting estate				
Wild boar (*Sus scrofa*)	Yes	45/297	15.2	**0.048**
	No	54/258	20.9	
Red deer (*Cervus elaphus*)	Yes	4/57	7.0	**0.013**
	No	95/498	19.1	
Wildcat (*Felis silvestris*)	Yes	27/153	17.6	0.445
	No	58/347	16.7	
Iberian lynx (*Lynx pardinus*)	Yes	8/68	11.8	0.143
	No	77/432	17.8	
Domestic cat (*F. silvestris catus*)	Yes	74/449	16.5	0.231
	No	11/51	21.6	
Dog (*Canis familiaris*)	Yes	65/371	17.5	0.353
	No	20/129	15.5	
Cattle (*Bos taurus*)	Yes	5/25	20.0	0.223
	No	46/361	12.7	
Goat (*Capra aegagrus hircus*)	Yes	21/144	14.6	0.321
	No	30/242	12.4	
Sheep (*Ovis aries*)	Yes	26/203	12.8	0.461
	No	25/183	13.7	
Farmed rabbit (*Oryctolagus cuniculus*)	Yes	8/49	17.1	0.541
	No	77/451	16.3	
Domestic pig (*Sus scrofa domesticus*)	Yes	6/40	15.0	0.439
	No	45/346	13.0	
Climate characteristics of the hunting estate				
Mean temperature (°C)	12.3–16.8	23/209	11.0	**0.004**
	16.9–17.4	16/93	17.2	
	17.5–18.5	37/152	24.3	

TABLE 1 (Continued)

Variable	Categories	No. positives/Overall[a]	Seroprevalence (%)	*p*
Max temperature (°C)	18.9–23.0	17/171	9.9	**<0.001**
	23.1–24.2	29/194	14.9	
	24.3–27.4	20/65	30.8	
Rainfall (mm)	273.3–563.8	21/157	13.4	0.333
	563.9–597.9	34/175	19.4	
	598.0–1134.6	21/122	17.2	
Humidity (g/m^3)	33–56	10/26	38.5	0.107
	57–65	23/119	19.3	
	66–100	15/67	22.4	

Note: *p* values <0.05 in bold.

Abbreviation: RHD, rabbit haemorrhagic disease.

[a]Missing values omitted.

TABLE 2 Generalized estimating equations (GEE) analysis of risk factors associated with *Coxiella burnetii* infection in wild lagomorphs in Andalusia (southern Spain).

Variable	Categories	*p*-Value	OR 95% CI
High abundance of ticks in the hunting estate	Yes	0.022	2.3 (1.1–4.9)
	No	[a]	[a]
Outbreaks of myxomatosis in the last month	Yes	0.028	3.8 (1.2–12.7)
	No	[a]	[a]

[a]Reference Category.

observed in the Canary Islands and the south of Spain, respectively (González-Barrio et al., 2015b; Bolaños-Rivero et al., 2017).

To the best of the author's knowledge, this is the first study on *C. burnetii* in Iberian hares. Our results confirm the susceptibility of this hare species to *C. burnetii*, thereby enlarging its host range. Previous studies have evidenced circulation of this bacterium in other hare species, such as the European brown hare (*Lepus europaeus*). In Cyprus, 15 of 31 (48.4%) pools of blood samples from 247 animals of this species were positive for *C. burnetii* (Psaroulaki et al., 2014), while no infection was found in any of the 51 and 105 animals sampled in Italy (Rocchigiani et al., 2018) and Greece (Tsokana et al., 2020) respectively. A regional study conducted in northern Spain detected this pathogen in 9.1% of the 22 European brown hares sampled (Astobiza et al., 2011). The overall prevalence of *C. burnetii* detected in our study of European wild rabbits and Iberian hares (17.9%) indicates that it is actively circulating in these populations, which is of public health and animal health concern. These findings, together with the importance of wild lagomorphs as prey for a large number of species, point to the risk of interspecies transmission of *C. burnetii*, thus favouring the maintenance of this bacterium in Iberian Mediterranean ecosystems (Delibes & Hiraldo, 1981; González-Barrio et al., 2022).

The prevalence of infection was significantly higher in lagomorphs from hunting estates where there had been an outbreak of myxomatosis during the month prior to sampling (19.9%) than in those estates where there had been no cases (4.3%), suggesting a possible epidemiological link. Myxomatosis is a viral disease that causes significant immunosuppression in both rabbits and hares (Jeklova et al., 2008; García-Bocanegra et al., 2019), which may favour infection by other pathogens, including *C. burnetii*. Our result is consistent with previous studies that also detected a relationship between myxomatosis virus infection and other pathogens in European wild rabbits. García-Bocanegra et al. (2010) noted that myxomatosis virus infection may favour infection by rabbit haemorrhagic disease virus (RHDV), and Boag et al. (2013) found an increased number of *Eimeria stiedae* oocysts shed in faeces by affected rabbits. Similarly, Mason et al. (2015) found that *Toxoplasma gondii* seropositivity could be associated with exposure to myxomatosis virus, although further studies are needed to confirm this hypothesis.

C. burnetii infection in wild lagomorphs was also significantly higher in animals from hunting estates where gamekeepers had observed high tick abundance (Table 2). This, together with the detection of bacterial DNA in 27.5% of the tick pools analysed (Table 3), highlights the importance of ticks in the epidemiological cycle of *C. burnetii* in Iberian Mediterranean ecosystems. Interestingly, although the prevalence of infection in wild rabbits was lower than in Iberian hares, the frequency of positive tick pools collected from the former was significantly higher than in those collected from hares. This finding could be explained by a differential exposure of the two wild lagomorph species to vector ticks (Table 3), indicating that the European wild rabbit could play a more important role in disseminating *C. burnetii*-positive ticks. It should be noted that the European wild rabbit is widespread in Spain and is frequently present in urban and peri-urban areas, which increases the risk of

TABLE 3 Total number of ticks captured from European wild rabbits and Iberian hares in Andalusia (southern Spain) and pools processed, with percentage of pools positive for *Coxiella burnetii*, according to tick developmental stage.

Host species	No of ticks	Nymph positives/ no of pools	% Positives	No of ticks	Adult female positives/no of pools	% Positives	No of ticks	Adult male positives/no of pools	% Positives	N.o of ticks	Total positives/ no of pools	% Positives
European wild rabbit												
Rhipicephalus pusillus	161	12/28	42.9	127	6/24	25.0	111	5/20	25.0	399	23/72	31.9
Rhipicephalus sanguineus s.l.	0	–	–	0	–	–	0	–	–	0	–	–
Haemaphysalis hispanica	13	4/6	66.7	0	–	–	0	–	–	13	4/6	66.7
Hyalomma lusitanicum	35	3/11	27.3	0	–	–	0	–	–	35	3/11	27.3
Ixodes ventalloi	0	–	–	3	0/2	0.0	0	–	–	3	0/2	0.0
Total	209	19/45	42.2	130	6/26	23.1	111	5/20	25.0	450	30/91	33.0
Iberian hare												
R. pusillus	1	0/1	0.0	14	2/8	25.0	20	1/8	12.5	35	3/17	17.6
R. sanguineus s.l.	0	–	–	0	–	–	23	0/9	0.0	23	0/9	0.0
H. hispanica	0	–	–	0	–	–	0	–	–	0	–	–
H. lusitanicum	1	0/1	0.0	4	0/2	0.0	0	–	–	5	0/3	0.0
I. ventalloi	0	–	–	0	–	–	0	–	–	0	–	–
Total	2	0/2	0.0	18	2/10	20.0	43	1/17	5.9	63	3/29	10.3
Total	211	19/47	40.4	148	8/36	22.2	154	6/37	16.2	513	33/120	27.5

Abbreviation: s.l., sensu lato.

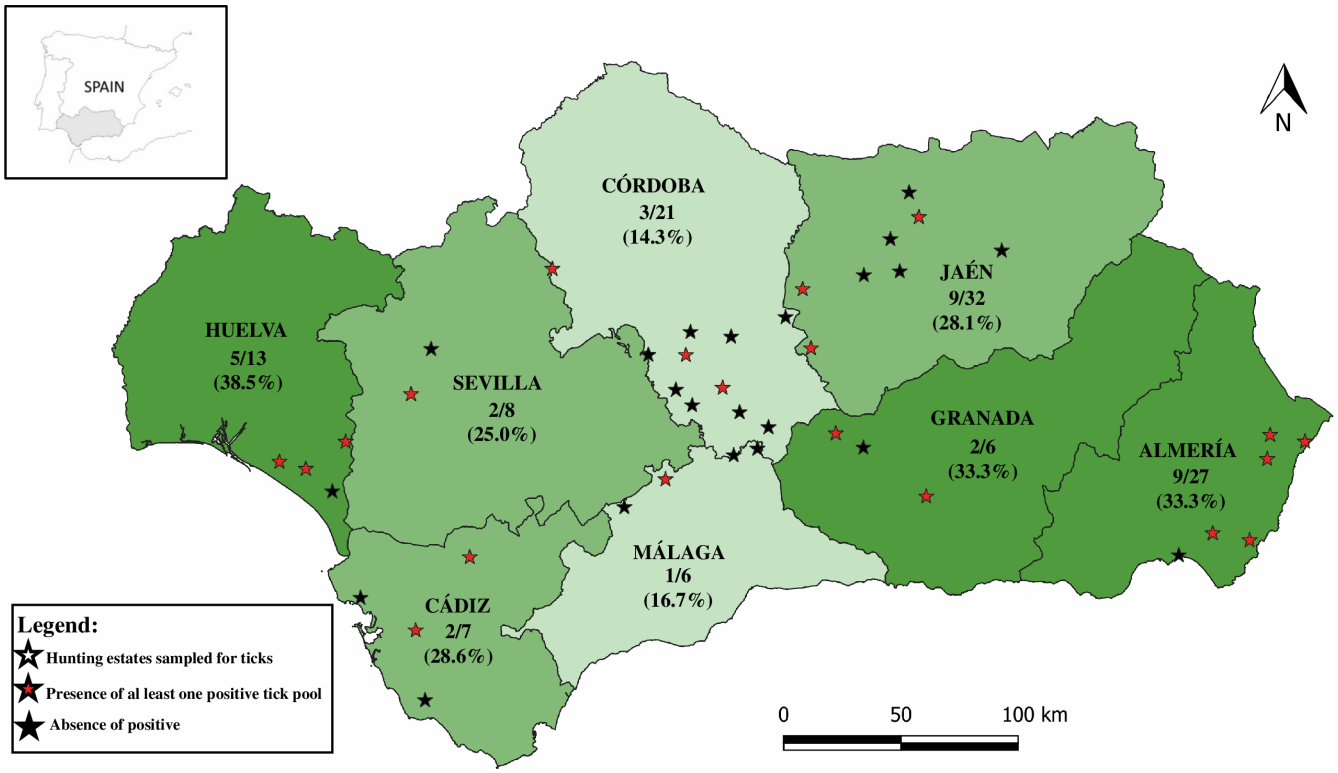

FIGURE 2 Distribution of hunting estates where tick samples were collected from wild lagomorphs and frequency of positive pools by province in the study region.

zoonotic transmission of *C. burnetii*, as has been shown for other vector-borne pathogens (Jiménez et al., 2014).

Three of the five tick species analysed had *C. burnetii* DNA. In Spain, the bacterium has previously been detected in *H. lusitanicum*, *Rhipicephalus turanicus*, *R. sanguineus*, *Rhipicephalus bursa*, *R. pusillus*, *Haemaphysalis sulcata*, *Haemaphysalis punctata* and *Dermacentor marginatus* (Körner et al., 2021). However, to the best of our knowledge, this is the first report of *C. burnetii* infection in *H. hispanica*. Positive pools of *R. pusillus* and *H. hispanica* pools were detected on 10 hunting estates where no positive wild lagomorphs were observed. The presence of positive ticks feeding on negative hosts could indicate that the pathogen was acquired at an earlier stage of tick development and from another host. This has already been evidenced in previous studies in *H. lusitanicum* and was associated with transovarial and transstadial transmission of *C. burnetii* (González et al., 2019). The same finding could also be related to recent infestation or the vector competence of these tick species. It should be noted that while *C. burnetii* has previously been detected in all positive tick species found in the present study, research on vector competence has only been conducted in *R. sanguineus* (Smith, 1941) and *H. lusitanicum* (González et al., 2020) and to date has only been demonstrated in *H. lusitanicum* (González et al., 2020). Further studies are required to assess the vector competence of these tick species.

In conclusion, our results indicate the widespread endemic circulation of *C. burnetii* in European wild rabbits, Iberian hares and ticks feeding on these two lagomorph species. The prevalence values observed in these mammals and their ticks indicate that wild lagomorphs act as reservoirs of this bacterium in Mediterranean ecosystems of southern Spain, making them potential sources of *C. burnetii* for sympatric species, including humans. Monitoring programmes for this zoonotic bacterium are justified to gain a deeper and broader understanding of the role of wildlife in the epidemiology of *C. burnetii* in different epidemiological scenarios.

ACKNOWLEDGEMENTS

We gratefully acknowledge the help of the personnel of the Epidemiological Surveillance Program in Wildlife (Regional Government of Andalusia) and all the members of the GISAZ research group in the collection of samples and epidemiological information. This work has benefited from the financial aid of research grants funded by the Spanish Ministry of Science and Innovation (project LagoHealth; REF: PID2019-111080RB-C21 and LAGOMED project REF: PRIMAS2-11-PCI2019-103698) and by the University of Córdoba (REF: UCO-FEDER-1264967). This research was also partially supported by TED2021-132599B-C22 project, funded by MCIN/AEI/10.13039/501100011033 and by the European Union 'NextGenerationEU'/PRTR Recovery, Transformation and Resilience Plan – funded by the European Union – NextGenerationEU, the research project LifeWATCH INDALO – Scientific Infrastructures for Global Change Monitoring and Adaptation in Andalusia (LIFEWATCH-2019-04-AMA-01), financed with FEDER funds (POPE 2014-2020), the Sub-modality 2.4. 'UCOLIDERA' of the 'Enrique Aguilar Benítez de Lugo' Research Plan of the University of Cordoba and CIBER-Consorcio Centro de Investigación Biomédica en Red (CB 2021), Instituto de

Salud Carlos III, Ministerio de Ciencia e Innovación and Unión Europea-Next Generation EU. S. Castro-Scholten is supported by an FPU grant from the Spanish Ministry of Universities (FPU19/06026). D. Jiménez-Martín holds a PhD contract granted by Own Research Plan of the University of Córdoba. R. Martínez was supported by a postdoctoral contract from the regional government of Andalusia. Funding for open acces charge: Universidad de Córdoba.

CONFLICT OF INTEREST STATEMENT

None of the authors of this study has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the authors upon reasonable request.

ETHICS STATEMENT

This study did not involve purposeful killing of animals. All samples were collected from legally hunted animals during the hunting seasons or by passive surveillance under Spanish and Andalusian legislation. Thus, no ethical approval was necessary.

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How to cite this article: Castro-Scholten, S., Caballero-Gómez, J., Martínez, R., Nadales-Martín, B. J., Cano-Terriza, D., Jiménez-Martín, D., Remesar, S., Jiménez-Ruiz, S., Gómez-Guillamón, F., & García-Bocanegra, I. (2024). Occurrence of *Coxiella burnetii* in wild lagomorphs and their ticks in Spanish Mediterranean ecosystems. *Zoonoses and Public Health*, *00*, 1–11. https://doi.org/10.1111/zph.13155