



Dynamics of Crimean-Congo hemorrhagic fever virus in two wild ungulate hosts during a disease-induced population collapse

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ABSTRACT

Identifying the role that host species play in pathogen transmission and maintenance is crucial for disease control, but it is a difficult task, in particular for vector-borne and multi-host pathogens, and especially when wildlife species are involved. This is the case for a Crimean-Congo hemorrhagic fever virus (CCHFV) hotspot in north-eastern Spain, where Iberian ibex (*Capra pyrenaica*) and wild boar (*Sus scrofa*) are involved, but their roles in disease transmission are unclear. In this context, we studied the dynamics of CCHFV transmission in these two species during the collapse of an Iberian ibex population due to a sarcoptic mange outbreak. We carried out a repeated cross-sectional study measuring the trends of CCHFV seroprevalence in Iberian ibex and wild boar and their abundances. In addition, we identified the tick species present in this area on the vegetation and on wild boars, and evaluated relevant meteorological factors. Results show that while the trends in CCHFV seroprevalence in Iberian ibex and density of wild boars remained constant ($p = 1.0$ and $p = 0.8$, respectively), both the trends in Iberian ibex census and CCHFV seroprevalence in wild boars decreased significantly ($p = 0.003$ and $p = 0.0001$, respectively), and were correlated (Spearman's rank, $0.02 < p\text{-adjusted} < 0.05$). The correlation between the patterns of reduction of Iberian ibex abundance and the decrease of seroprevalence in wild boars suggests some sort of shared transmission cycle between the two species. Data from tick species in the area suggest a possible role of *Rhipicephalus bursa* in CCHFV transmission. The dynamics of CCHFV were unlikely caused by changes in meteorological variables such as temperature or water vapor pressure deficit. Further studies will be needed to confirm these hypotheses.

1. Introduction

Most emerging pathogens, including zoonotic agents, can infect multiple species [1]. Identifying the role of host species in pathogen transmission in a particular system is crucial for disease control [2]. This is particularly challenging for vector-borne and multi-host pathogens since interactions among the pathogen, vectors, and multiple hosts add layers of complexity to these systems [3]. Understanding the transmission dynamics is even more difficult when wildlife species are

involved because information on morbidity, mortality, or infection susceptibility of those species is difficult to obtain [4].

Crimean-Congo hemorrhagic fever virus (CCHFV) is a vector-borne pathogen mainly transmitted by *Hyalomma* spp. ticks, which are in expansion in southern Europe [5]. In humans, CCHFV infection can cause a fatal hemorrhagic systemic disease [6]. The natural cycle of CCHFV involves ticks of different stages that feed on several domestic and wild animal species, and occasionally on humans. *Hyalomma* spp. larvae and nymph stages usually feed on small mammals such as

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lagomorphs, hedgehogs, or rodents, or on ground-feeding birds, while adults mostly feed on wild and domestic ungulates [7]. Immature stages of some *Hyalomma* spp. can occasionally feed on ungulates when small mammals are not abundant [8]. Ticks acquire the virus through co-feeding or feeding on viremic hosts, and through transovarian and transstadial transmission. As *Hyalomma* spp. ticks become infected for life, they are considered both vectors and reservoirs of CCHFV [9]. Therefore, while other tick species have been attributed a role in CCHFV transmission, it is assumed that only *Hyalomma* spp. ticks are able to maintain an active focus of CCHFV [9]. Since CCHFV infected animals are asymptomatic with short viremias, serological surveys are essential to investigate CCHFV eco-epidemiology in wildlife, define endemic areas, and assess the exposure of potential hosts [10].

In Spain, by 2022, twelve Crimean-Congo Hemorrhagic Fever (CCHF) cases in humans have been diagnosed since the first one in 2013, and CCHF is considered an emerging infectious disease [11]. In central and southern Spain, red deer (*Cervus elaphus*) is considered an indicator of CCHFV infection risk, and it is the species that presents the highest CCHFV seroprevalences [12]. In contrast, in the northern and eastern Mediterranean regions of Spain (Catalonia and Valencian Community, respectively), where red deer is absent or scarce, hotspots of CCHFV exposure in wild ungulates have also been detected [13,14]. A serological study on CCHFV carried out between 2014 and 2020 in Catalonia detected a focus of high antibody seropositivity in Iberian ibex (*Capra pyrenaica*) in *Ports de Tortosa i Beseit Natural Park* (PTiBNP), with a lower seroprevalence in Eurasian wild boar (*Sus scrofa*) [13]. These two ungulates are the most abundant wild mammalian species that host adult ticks in the PTiBNP, while other species such as roe deer (*Capreolus capreolus*) or red deer are scarce and absent, respectively [15]. Antibodies against CCHFV were not detected in wildlife, including Iberian ibex and wild boars, from areas of Catalonia far from this hotspot [13]. Another study carried out in the Valencian region (to the south of Catalonia) detected two other hotspots of CCHFV [14]. A high seropositivity (> 90%) was detected in wild ruminants, in Iberian ibex in both foci and in mouflon (*Ovis aries musimon*) which was present only in one of the foci; while a much lower seroprevalence (<20%) was detected in wild boars. In these two CCHFV foci of the Valencian region, the seroprevalences and the populations of Iberian ibexes and wild boars have not fluctuated significantly in the last 10 years [14,16]. Those findings suggest that Iberian ibexes, wild boars and occasionally mouflons, are involved in the CCHFV circulation in the eastern Mediterranean region. However, little is known about the distribution and abundance of CCHFV-competent tick species in this region.

In 2014, an outbreak of sarcoptic mange was detected in Iberian ibex in the PTiBNP surrounding area, presumably introduced by local livestock [17]. Sarcoptic mange is a skin disease caused by *Sarcoptes scabiei* mite, which excavates the superficial epidermal layers causing a catabolic systemic inflammatory disease that leads to high mortalities, especially in wild Caprinae populations [18]. The sarcoptic mange epidemic in the PTiBNP progressed slowly but ended up in an 86.1% reduction of the Iberian ibex population by 2021 (*Departament d'Acció Climàtica, Alimentació i Agenda Rural* (DACC), i.e., Department of Climate Action, Food and Rural Agenda, personal communication).

Since the abundance of wild ungulates seems to have a significant effect on the risk of exposure to CCHFV [19], this sarcoptic mange outbreak represented an interesting scenario to evaluate how changes in the community of hosts modulated the transmission of CCHFV. Therefore, we designed a retrospective multi-year cross-sectional study to evaluate the trends of CCHFV seroprevalence in both Iberian ibexes and wild boars, as well as their population trends between 2013 and 2022. Understanding the dynamics of CCHFV in an area also requires knowledge about the structure of the local community of ticks, known or potential vectors of CCHFV. Thus, we carried out a field study to identify the tick species present in the PTiBNP, particularly focusing on the detection of CCHFV vectors. Moreover, some meteorological factors are known to influence the transmission of CCHFV, either through the effect

on viral replication and spread within the tick (mainly determined by temperature), or through the effect of meteorological factors on tick abundance. Therefore, we also evaluated whether changes in those factors throughout the study period may have influenced the dynamics of CCHFV transmission in the PTiBNP.

2. Methods

PTiBNP (40°48'28"N, 0°19'7"E) is a calcareous mountain range with highly complex orography characterized by valleys, ravines and steep gradients. The main habitats are pine and oak forests with altitudes ranging from 300 to 1442 m above sea level. The predominant ungulate species are Iberian ibex and wild boar whereas roe deer is found in much lower densities [15]. We selected all available serum samples of wild boars ($n = 288$) and Iberian ibexes ($n = 88$) collected in the PTiBNP between 2013 and 2022 as part of the routine wildlife disease surveillance in Catalonia. Some of these samples had been tested, and the results were published in Espunyes et al. [13]. No samples from Iberian ibexes were available between 2014 and 2017 due to a hunting ban because of the detection of sarcoptic mange. Samples were analyzed using the CCHF Double Antigen Multi-species ELISA kit (iDvet, Montpellier, France), which has a sensitivity of 98.9% (CI_{95%}: 96.8%–99.8%) and a specificity of 100% (CI_{95%}: 99.8%–100%) [20]. Data on the population trends of Iberian ibex and of wild boar in the PTiBNP were provided by the DACC of the Government of Catalonia. Wild boar densities (animals/km²) were estimated based on the method developed by Sáez-Royuela and Tellería using data from collective hunts [21]. Iberian ibex censuses were obtained through total counts, except for 2016 and 2017 when the counts could not be carried out in the whole area, and estimates were obtained by linear regression.

Annual CCHFV seroprevalences were grouped according to hunting seasons (September–April) with their 95% exact confidence intervals. In order to evaluate whether the seroprevalences in Iberian ibex and wild boar followed any (increasing, decreasing, or unchanged) trend throughout the study period, a chi-squared test for trend [22], was carried out. Mann-Kendall tests were performed to evaluate the trends in the densities of Iberian ibex and wild boar [23]. Finally, considering the non-parametric nature of the data, a Spearman's rank correlation test was carried out to evaluate a possible correlation between the seroprevalences of CCHFV in wild boar and the densities of Iberian ibex. To account for the small number of observations (six, i.e., 2016–2017 to 2021–2022), the p -value was adjusted based on the exact distribution [24]. All statistical analyses were carried out using R software version 4.2.1 [25], and packages tidyverse [26], binom [27] and trend [28].

To assess whether meteorological factors may have impacted the dynamics of CCHFV in the PTiBNP, we used a knowledge-driven modelling approach (i.e., based on the existing knowledge about the causal relationships with the pathogen of interest) [29]. We selected ambient temperature and water vapor pressure deficit in the atmosphere (VPD) as the factors to be evaluated for their potential effect on the dynamics of CCHFV transmission in the PTiBNP [19,30]. Ambient temperature influences the replication and spread of the virus within ticks and also regulates tick development. VPD influences tick mortality and has been found to be more relevant in defining the niche of ixodid ticks such as *H. marginatum* than other water-related variables such as evapotranspiration or soil humidity [30]. Hourly temperature data for the area (PTiBNP) and period of study (2009–2023) was obtained from Copernicus Climate Change Service [31] and averaged to obtain mean temperatures by month, while monthly VPD values were obtained from TerraClimate (<http://www.climatologylab.org/terraclimate.html>, accessed June 2023). We also calculated accumulated temperature and VPD in the complete year and accumulated VPD in winter [30] for the hunting seasons 2016–2017 to 2021–2022. To evaluate the trends of those three measures, Mann-Kendall tests were carried out, while possible correlations between those measures and the seroprevalences of CCHFV in wild boar, were evaluated by Spearman's rank correlation

tests, with adjusted p -values [24].

Sampling of questing ticks was performed by dragging a 1×1 m white cloth through the vegetation in three different 1 km transects [32], all in areas where both wild boar and Iberian ibex were known to be present. Furthermore, six (two per transect) carbon dioxide-based traps (CO₂ traps) baited with dry ice were placed on 50×50 cm white cloths, to target species with host-seeking behavior (e.g., adult *Hyalomma* spp.) [33]. CO₂ traps were placed at least 200 m far from each dragging transect and left for 2 h. Cloth-dragging and CO₂ trapping were performed three times in the same transects during May and July 2023. Moreover, we collected ticks from wild boars ($n = 7$) hunted by rangers from the PTiBNP as a part of a wild boar population control program, during May and June 2023. Adults and nymphs were identified alive after collection using $35\times$ and $64\times$ magnifying lenses and taxonomic keys [34]. Data from ticks collected on Iberian ibex from PTiBNP in Varela-Castro et al. [35] is also displayed in a table for a more comprehensive comparison.

3. Results

Throughout the study period, 100% of the Iberian ibexes tested had antibodies against CCHFV. Regarding wild boars, the seroprevalence gradually declined throughout hunting seasons (66.7% in 2016–2017 to 4.2% in 2021–2022; Table 1, Fig. 1). This trend in seroprevalence reduction was statistically significant (X^2 (df = 1) = 59.9, $p < 0.0001$). The census of Iberian ibexes also had a statistically significant decreasing trend ($\tau = -0.64$, $p = 0.0028$), but no statistically significant trend was observed for the density of wild boars ($p = 0.80$) or the seroprevalence in Iberian ibexes ($p = 1.0$) (Fig. 1). However, there was a statistically significant (Spearman's) correlation (r_s) between the seroprevalences of CCHFV in wild boar and the abundance of Iberian ibexes during the study period ($r_s = 0.942$, $0.02 < p$ -adjusted < 0.05).

A total of 499 ticks were harvested from the vegetation, 250 by dragging and 249 by CO₂ traps. Among them, 367 (89.2%) were *Rhipicephalus bursa*, while *Haemaphysalis punctata*, *Rhipicephalus sanguineus* sensu lato (s.l.) and *Hyalomma marginatum* were less frequent (Table 2). One single specimen of *Dermacentor marginatus* and another of *Rhipicephalus pusillus* were also found.

A total of 118 ticks were collected from wild boars, with *Rhipicephalus bursa* being the most abundant (44.9%) and present in all the individuals, followed by *Rhipicephalus sanguineus* (28.8%) and *Hyalomma marginatum* (20.4%). Six *Hyalomma lusitanicum* (5.1%) and one *Dermacentor marginatus* (0.8%) were also identified (Table 2).

The results of the monthly temperatures and the monthly VPD values in the PTiBNP for the period 2009 to 2022 showed a rather regular pattern (Fig. 2). None of the cumulative meteorological variables showed any statistically significant trend: annual accumulated temperature ($\tau = -0.33$, $p = 0.45$), annual accumulated VPD ($\tau = -0.07$, p

= 1.00), and accumulated VPD in winter ($\tau = 0.33$, $p = 0.45$). Moreover, none of them was correlated with the seroprevalence of CCHFV in wild boars: annual accumulated temperature ($r_s = 0.26$, $0.2 < p$ -adjusted < 0.5), annual accumulated VPD ($r_s = 0.09$, $0.5 < p$ -adjusted), and accumulated VPD in winter ($r_s = 0.26$, $0.2 < p$ -adjusted < 0.5).

4. Discussion

The epidemiology of CCHF is complex and spatiotemporally heterogeneous since biotic (host-vector-pathogen) and abiotic (e.g., climate, habitat) interactions vary among ecosystems [36]. Identifying the main drivers for the transmission of CCHFV in different systems is critical to implement measures to reduce the risk to people.

Red deer is considered an indicator of CCHFV infection risk in extensive areas of central and southern Spain [12]. However, in the eastern Mediterranean ecoregion the density of red deer is low and, in the PTiBNP, CCHFV circulation involved Iberian ibex and wild boar, although with different levels of seroprevalence [13]. Our repeated cross-sectional study in the PTiBNP showed that the seroprevalence of CCHFV in Iberian ibex remained at 100% even though sarcoptic mange dramatically reduced its population. In contrast, the CCHFV seroprevalence in wild boars decreased significantly yet its density did not show any increasing or decreasing trend. In fact, the reduction in the seroprevalence of CCHFV in wild boars was found to be significantly correlated to the decrease in the abundance of Iberian ibexes. This correlation suggests a possible connection between the CCHFV transmission cycles in Iberian ibex and wild boar, potentially through the sharing of common tick vectors.

The influence of the community of ungulates on CCHFV transmission was evidenced by the results of Cuadrado-Matías et al. [19], who found that the abundance of hosts was the most influential determinant of the inter-annual variation in CCHFV exposure risk. However, the precise mechanism that explains the epidemiological scenario in the PTiBNP is difficult to ascertain. Whether Iberian ibexes can develop a CCHFV viremia after the bite of an infected tick and then transmit the virus to uninfected ticks — potentially amplifying the viral infection in parasitizing ticks — is not known. In that case, the reduction of the population of Iberian ibexes would have decreased the number of infected ticks available to transmit CCHFV to wild boars. The few CCHFV experimental infections used to evaluate viremias in ungulates were carried out several decades ago in livestock species such as cattle and sheep, and they reported short-term viremias (2–9 days) followed by the development of anti-CCHFV antibodies [10]. As indicated by several authors [9,10,37], further experimental infections are needed, including in wild ruminants but also in wild boars, to determine their potential role as amplifiers of CCHFV. Even in the absence of viremic hosts, there are other mechanisms that may explain the maintenance of CCHFV foci. Tick co-feeding allows the transmission of the virus present in the saliva of an infected tick to uninfected ticks feeding nearby [38]. This mechanism has been thoroughly explored for tick-borne encephalitis virus [39,40], but its importance for CCHFV transmission remains to be determined [8]. Some tick species, rather than hosts, are considered the true natural reservoirs of CCHFV. Competent tick species allow the replication of CCHFV in their tissues, which later enables the transstadial transmission of the virus from larva to nymph or from nymph to adult [9]. Transmission from adult female ticks to their eggs (i.e., vertical transmission) is also possible, although this mechanism seems to be less efficient in the case of CCHFV [37]. If vertebrate hosts can only develop short-term viremias at first exposure [37], a more likely explanation for the correlation between the Iberian ibex population decline and the decrease in the seroprevalence of CCHFV in wild boars would be that Iberian ibex is important for the maintenance of tick vectors of CCHFV. Therefore, the knowledge about the local community of ticks and which species could act as vectors of CCHFV, is critical.

It is accepted that *Hyalomma* spp. are the main vectors and reservoirs of CCHFV, and in Spain, the two established species are *H. lusitanicum*

Table 1

Seropositivity against CCHFV in Iberian ibex and wild boar in the Ports de Tortosa i Besit Natural Park by hunting season. Data is displayed by n° of positives / total; seroprevalence percentage (95% exact confidence interval), — no samples available. Hunting seasons 2014–2015 and 2015–2016 are not included in the table because there were no samples available. * Part of these results were already published in Espunyes et al. (2021) [13].

Season	Iberian ibex	Wild boar
2012–2013	13 / 13; 100% (75.3% - 100.0%)	—
2013–2014	9 / 9; 100% (66.4% - 100.0%)	—
2016–2017	—	4 / 6; 66.7% (22.3% - 95.7%)
2017–2018*	16 / 16; 100% (79.4% - 100.0%)	23 / 47; 48.9% (34.1% - 63.9%)
2018–2019*	41 / 41; 100% (91.4% - 100.0%)	18 / 31; 58.1% (39.1% - 75.4%)
2019–2020*	9 / 9; 100% (66.4% - 100.0%)	15 / 54; 27.8% (16.5% - 41.6%)
2020–2021	—	7 / 78; 8.8% (3.7% - 17.6%)
2021–2022	—	3 / 72; 4.2% (0.9% - 11.7%)
Total	88 / 88; 100% (95.9% - 100.0%)	66 / 288; 22.9% (18.2% - 28.2%)

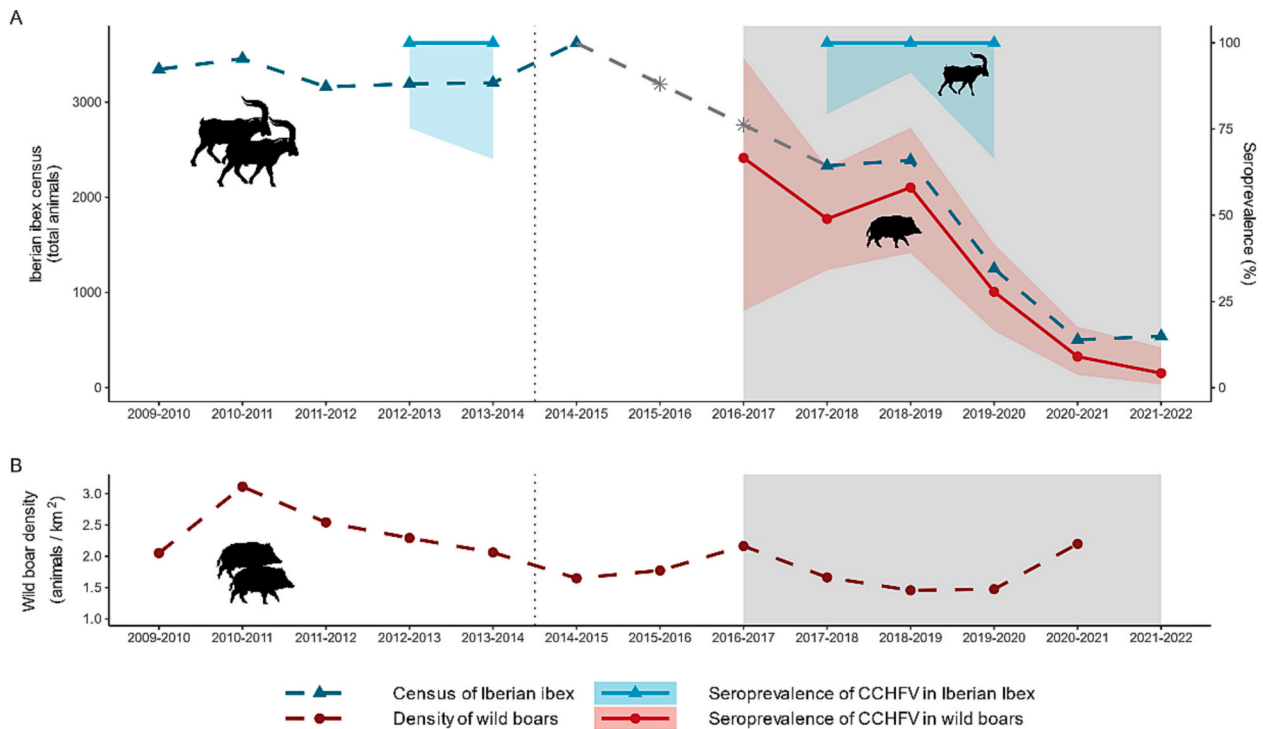


Fig. 1. A: Trends in the seroprevalences of CCHFV antibodies in Iberian ibexes and wild boars (with their 95% confidence intervals), in relation to Iberian ibex censuses for the period between 2009 and 2022 in Ports de Tortosa i Beselit Natural Park (PTiBNP). B: Trend in the census of wild boars for the same area and period. The dotted vertical line indicates sarcoptic mange introduction in the surrounding of PTiBNP (2014). Grey dashed line with asterisks in Iberian ibex census trend indicates the years when total animal counts were obtained through linear regression (with the previous and the next animal counts). Grey area indicates the study period.

Table 2

Ticks collected from vegetation and wild boars during May and July 2023 (present study), and from Iberian ibex during 2011–2015 (Valera-Castro et al. [35]) in Ports de Tortosa i Beselit Natural Park (PTiBNP).

Tick species	Questing and host-seeking ticks			Wild boars (n = 7) (%)	Iberian ibex (n = 134) (%) (Results from Varela-Castro et al., (2018))
	Dragging	CO ₂ -trap	Total (%)		
<i>Rhipicephalus bursa</i>	206	239	445 (89.2%)	53 (44.9%)	601 (89.8%)
<i>Haemaphysalis punctata</i>	28	4	32 (6.4%)	–	23 (3.4%)
<i>Rhipicephalus sanguineus</i> s.l.	13	2	15 (3.0%)	34 (28.8%)	5 (0.8%)
<i>Hyalomma marginatum</i>	2	3	5 (1.0%)	24 (20.4%)	1 (0.2%)
<i>Dermacentor marginatus</i>	–	1	1 (0.2%)	1 (0.8%)	11 (1.6%)
<i>Rhipicephalus pusillus</i>	1	–	1 (0.2%)	–	–
<i>Hyalomma lusitanicum</i>	–	–	–	6 (5.1%)	–
<i>Haemaphysalis sulcata</i>	–	–	–	–	28 (4.2%)
Total	250	249	499	118	669

and *H. marginatum* [34], but little information is available about their distribution in the eastern Mediterranean region of Spain. A recent study reported a high burden of *H. lusitanicum* ticks in wild boars from the metropolitan area of Barcelona, where this tick species was detected in more than one-third (33.6%) of the animals [41]. In addition, in 2022, questing *H. lusitanicum* ticks were collected in 31 municipalities surrounding the city of Barcelona [42]. Our study is the first to report the presence of *H. lusitanicum* in the PTiBNP although in low abundance – six specimens parasitizing two wild boars. In fact, *H. lusitanicum* was not found in any of the 134 Iberian ibexes from the PTiBNP sampled between 2011 and 2015 [35], likely because Lagomorpha is absent or present in very low densities in the main areas where Iberian ibexes are present (DACC personal communication). Presence of *H. lusitanicum* has traditionally been associated with the presence of wild rabbits and hares because they are the main hosts for the immature stages [43], although, occasionally, larvae and nymphs may also feed on the same hosts as adults [8].

In contrast, *H. marginatum* was known to be present in the PTiBNP but only one specimen had been found in the 134 the Iberian ibexes evaluated by Varela-Castro et al. [35]. Absence or low levels of *H. lusitanicum* and *H. marginatum* ticks in Iberian ibexes may also be associated to a low preference of *Hyalomma* spp. ticks for this host. That would be consistent with studies in other areas of Spain in which both *H. marginatum* and *H. lusitanicum* were hardly ever detected feeding on Iberian ibex despite its presence in the area [44,45]. Nonetheless, it should be pointed out that all those studies on ticks collected from Iberian ibexes were carried out during the hunting season (October to February), while the adults of *H. lusitanicum* are found on hosts mainly between April and October (with peaks in May–June and September–October), and the adults of *H. marginatum* are found on hosts mainly between March and November (peak in April–June) [43]. Other authors report that, nowadays, adults of *H. lusitanicum* may be found feeding on ungulates throughout the entire year [8], which is consistent with the finding of Castillo-Contreras et al. [41], who detected *H. lusitanicum*

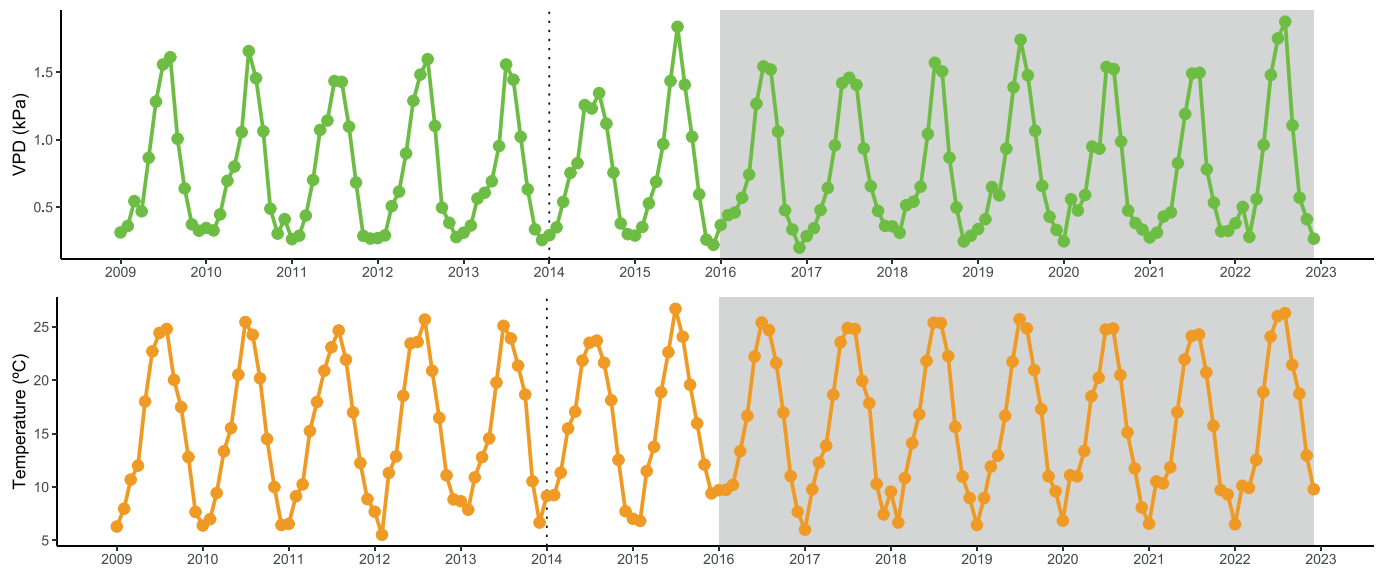


Fig. 2. Monthly vapor pressure deficit (VPD) in kilopascal (kPa) (green) and temperature in Celsius (orange) from 2009 to 2022. Grey area indicates the study period between 2016 and 2022. The dotted vertical line indicates sarcopic mange introduction in PTiBNP (2014). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ticks in wild boars from areas close to Barcelona all year round. In the case of wild boar, we found *H. marginatum* in 6 out of 7 animals (May to July 2023) suggesting a possible role in CCHFV transmission to wild boars. This finding might be related to the fact that these individuals were captured in an area where free-ranging domestic cattle are occasionally present, and *H. marginatum* is mainly associated with the presence of cattle [43]. Studies on wild boars and environmental ticks from other areas of the PTiBNP are needed to accurately evaluate the abundance and distribution of *H. marginatum* in this region. In any case, the data available points out to the absence or very low abundance of *Hyalomma* spp. ticks in Iberian ibexes of the PTiBNP [35], which does not appear to explain the 100% seroprevalence detected in this species.

Rhipicephalus bursa, on the other hand, accounted for nearly 90% of the ticks found on Iberian ibexes from the PTiBNP [35], and 44.9% and 89.2% of the ticks found, respectively, in wild boar and vegetation in the present study. Several experimental studies suggested that *Rhipicephalus* species play a certain role in the transmission of CCHFV [46,47]. In a study of cattle from across Uganda, 69.7% (292/419) of the animals had CCHFV antibodies but only one of the 1065 ticks collected on them was of the genus *Hyalomma*, and was negative to CCHFV, while 5.9% (18/304) of the *Rhipicephalus* pools were positive [48]. And, in the case of *R. bursa*, CCHFV has been found in unfed ticks, which is considered an unequivocal demonstration that the virus survived tick molting or was passed transovarially from the engorged female to the larvae via the eggs [9]. Moreover, *R. bursa* ticks appear to be the main vector of CCHFV genotype VI (Europe 2) (recently re-classified as Aigai virus) in different countries of the Balkan region [49]. Interestingly, in a study carried out in Greece in which 2000 ticks were collected from sheep and goats, most Europe 1 sequences were obtained from *Rhipicephalus sanguineus* s.l., while the majority of Europe 2 sequences were obtained from *R. bursa* [50]. It is not clear whether CCHFV strains introduced into a region could adapt to new tick species and if that association between *R. bursa* and genotype VI is driven by the competence of this tick species, or by the abundance of *R. bursa* in the region [9]. As the efficiency of *Rhipicephalus* spp. ticks as vectors of CCHFV is still not clear, further experimental studies on vector competence are needed [50]. While *R. bursa* in all its stages prefers ruminants, adult *R. bursa* ticks are also found in wild boars although in a lower burden [41,51,52], which is consistent with the findings of the present study. Therefore, the possibility of a connection between CCHFV transmission cycles in Iberian ibex and wild boars with the involvement of *R. bursa* (rather than *Hyalomma* spp.)

cannot be ruled out and deserves to be investigated. It has been generally assumed that *Hyalomma* spp. ticks are crucial for CCHFV transmission, and that in their absence/low abundance, an active focus of CCHFV cannot be maintained, but actual knowledge is lacking, and studies to address these issues are required [9].

Dermacentor marginatus was found to be the most abundant tick species in wild boars from natural areas of north-eastern Spain [53,54], coinciding with other studies carried out in Mediterranean habitats [55]. More recently, a study on ticks on wild boars from the metropolitan area of Barcelona showed that *D. marginatus* was the second most abundant species (infestation prevalence: 26.9%) after *H. lusitanicum* (33.6%), while *R. bursa* was scarce (0.2%) [41]. In the PTiBNP, *D. marginatus* was also found in the Iberian ibexes although at low levels (1.6%) [35], and we found a single *D. marginatus* in a wild boar and another in the vegetation. Although *D. marginatus* has been shown to transmit CCHFV transovarially, its role as a competent vector is still not clear [9]. Given the low burden of *D. marginatus* in Iberian ibexes, and the limited evidence about its role as a vector of CCHFV, this tick species is unlikely to have played a significant role in CCHFV transmission in the PTiBNP.

Haemaphysalis punctata was the third most abundant tick species in Iberian ibex in the PTiBNP [35], and also the second most abundant tick species in the environment. CCHFV has been found in *H. punctata* collected from ungulates on several occasions [9], and recently, a study carried out in southern Russia detected CCHFV in one ($n = 477$) *H. punctata* unfed specimen [56]. These findings indicate that *H. punctata* can also be a potential vector of CCHFV although its role in maintaining the virus is still uncertain.

If the transmission of CCHFV in Iberian ibex and wild boar in the PTiBNP are somehow interconnected, the contrast in the prevalence between the two species may be explained by a difference in the burden of CCHFV-tick vectors, as seen for example for *H. lusitanicum* which is found in lower numbers in wild boars compared to red deer [51]. The lower burden of ticks in wild boars may be influenced by behavioral features (e.g., ritual of taking mud baths to remove ectoparasites), or to a preference for habitats with a lower number of ticks [19].

A recent study in southwestern Spain detected significant interannual variations in CCHFV seroprevalence in wild boar in the absence of major demographic changes in the abundance of this species [19]. Meteorological factors may impact the transmission of CCHFV directly, by the effect on the replication and spread of the virus within the tick

(mainly determined by temperature), or indirectly, through the effect of meteorological factors on tick abundance [30,52]. We therefore evaluated whether changes in meteorological factors relevant to CCHFV transmission may have influenced the dynamics of CCHFV in the PTiBNP. The pattern of temperature and VPD remained quite regular throughout the study period, and the statistical evaluation of the cumulative values of temperature and VPD suggests that meteorological factors cannot explain the reduction of CCHFV seroprevalence in wild boars. However, as ticks are responsive only to the microclimatic conditions, conclusions based on large-area analyses should be interpreted with care [57].

An alternative explanation for the differences in the seroprevalences between Iberian ibex and wild boars is that independent transmission cycles occur in these two host species. In other words, that the ticks involved in CCHFV transmission in Iberian ibex and wild boars are not the same, and therefore may be affected differently by changes in abiotic or biotic factors.

Our findings indicate that there are some differences in the tick communities parasitizing Iberian ibex and wild boars. However, given the absence of significant variations on relevant meteorological factors, and the lack of substantial changes in the population of wild boars, the significant reduction of CCHFV seroprevalence in wild boars, appears more difficult to explain if transmission cycles are independent.

Even though serological studies can be useful to generate hypotheses about multi-host pathogen transmission dynamics, it is not acceptable to assign an epidemiological role to a host species based solely on antibody detection [58]. With serological data we cannot infer when the animals acquired the infection since antibody duration against CCHFV in wildlife species is unknown. The limited evidence available is from domestic ungulates, and indicates that antibody duration is long-lasting [59] and that reinfections can occur [10].

5. Conclusions

The correlation between the patterns of reduction of Iberian ibex abundance and the decrease of seroprevalence in wild boars suggests an epidemiological link between the two species. Published data indicates very low abundance of *Hyalomma* spp. ticks on Iberian ibexes of the PTiBNP, which could hardly explain the 100% seroprevalence detected in this species. In contrast, *Rhipicephalus bursa* is very abundant on Iberian ibexes, on wild boars and in the environment, pointing out for a possible role of *R. bursa* in CCHFV transmission in the area. Meteorological factors such as temperature or water vapor pressure deficit do not seem to have played a significant role on the dynamics of CCHFV in the area. Further studies will be needed to confirm these hypotheses.

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CRedit authorship contribution statement

Laura Carrera-Faja: Writing – original draft, Investigation, Methodology. **Johan Espunyes:** Writing – review & editing. **Jesús Cardells:** Supervision. **Xavier Fernández Aguilar:** Writing – review & editing. **Lola Pailler-García:** Formal analysis, Visualization. **Sebastian Napp:** Conceptualization, Formal analysis, Methodology, Writing – review & editing. **Oscar Cabezón:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

- [1] M.E.J. Woolhouse, L.H. Taylor, D.T. Haydon, Population biology of multihost pathogens, *Science* 292 (2001) 1109–1112, <https://doi.org/10.1126/science.1059026>.
- [2] J.P. Webster, A. Borlase, J.W. Rudge, Who acquires infection from whom and how? Disentangling multi-host and multi-mode transmission dynamics in the 'elimination' era, *Philos. Trans. R. Soc. B* 372 (2017) 20160091, <https://doi.org/10.1098/rstb.2016.0091>.
- [3] F. Dantas-Torres, Climate change, biodiversity, ticks and tick-borne diseases: the butterfly effect, *Int. J. Parasitol. Parasites. Wildl.* 4 (2015) 452–461, <https://doi.org/10.1016/j.ijppaw.2015.07.001>.
- [4] M. Viana, R. Mancy, R. Biek, S. Cleaveland, P.C. Cross, J.O. Lloyd-Smith, D. T. Haydon, Assembling evidence for identifying reservoirs of infection, *Trends Ecol. Evol.* 29 (2014) 270–279, <https://doi.org/10.1016/j.tree.2014.03.002>.
- [5] N. Fernández-Ruiz, A. Estrada-Peña, Towards new horizons: climate trends in Europe increase the environmental suitability for permanent populations of *Hyalomma marginatum* (Ixodidae), *Pathogens* 10 (2021) 95, <https://doi.org/10.3390/pathogens10020095>.
- [6] Ö. Ergönül, Crimean-Congo haemorrhagic fever, *Lancet Infect. Dis.* 6 (2006) 203–214, [https://doi.org/10.1016/S1473-3099\(06\)70435-2](https://doi.org/10.1016/S1473-3099(06)70435-2).
- [7] D.A. Bente, N.L. Forrester, D.M. Watts, A.J. McAuley, C.A. Whitehouse, M. Bray, Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity, *Antivir. Res.* 100 (2013) 159–189, <https://doi.org/10.1016/j.antiviral.2013.07.006>.
- [8] F. Valcárcel, L. Elhachimi, M. Vilá, L. Tomassone, M. Sánchez, S.M. Selles, M. Kouidri, M.G. González, R. Martín-Hernández, A. Valcárcel, N. Fernández, Emerging *Hyalomma lusitanicum*: from identification to vectorial role and integrated control, *Med. Vet. Entomol.* (2023) 1–35, <https://doi.org/10.1111/mve.12660>.
- [9] A. Gargili, A. Estrada-Peña, J.R. Spengler, A. Lukashew, P.A. Nuttall, D.A. Bente, The role of ticks in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus: a review of published field and laboratory studies, *Antivir. Res.* 144 (2017) 93–119, <https://doi.org/10.1016/j.antiviral.2017.05.010>.
- [10] J.R. Spengler, É. Bergeron, P.E. Rollin, Seroepidemiological studies of Crimean-Congo hemorrhagic fever virus in domestic and wild animals, *PLoS Negl. Trop. Dis.* 10 (2016), e0004210, <https://doi.org/10.1371/journal.pntd.0004210>.
- [11] European Centre for Disease Prevention and Control, Cases of Crimean–Congo haemorrhagic fever in the EU/EEA, 2013–present. <https://www.ecdc.europa.eu/en/crimean-congo-haemorrhagic-fever/surveillance/cases-eu-since-2013>, 2022 (accessed 22 September 2022).
- [12] R. Cuadrado-Matías, B. Cardoso, M.A. Sas, I. García-Bocanegra, I. Schuster, D. González-Barrio, S. Reiche, M. Mertens, D. Cano-Terriza, L. Casades-Martí, S. Jiménez-Ruiz, J. Martínez-Guijosa, Y. Fierro, F. Gómez-Guillamón, C. Cortázar, P. Acevedo, M.H. Groschup, F. Ruiz-Fons, Red deer reveal spatial risks of Crimean-Congo haemorrhagic fever virus infection, *Transbound. Emerg. Dis.* 69 (2022) e630–e645, <https://doi.org/10.1111/tbed.14385>.
- [13] J. Espunyes, O. Cabezón, L. Pailler-García, A. Dias-Alves, L. Lobato-Bailón, I. Marco, M.P. Ribas, P.E. Encinosa-Guzmán, M. Valldeperes, S. Napp, Hotspot of Crimean-Congo hemorrhagic fever virus Seropositivity in wildlife, northeastern Spain, *Emerg. Infect. Dis.* 27 (2021) 2480–2484, <https://doi.org/10.3201/eid2709.211105>.
- [14] L. Carrera-Faja, J. Cardells, L. Pailler-García, V. Lizana, G. Alfaro-Deval, J. Espunyes, S. Napp, O. Cabezón, Evidence of prolonged Crimean-Congo hemorrhagic fever virus Endemicity by retrospective Serosurvey, eastern Spain, *Emerg. Infect. Dis.* 28 (2022) 1031–1034, <https://doi.org/10.3201/eid2805.212335>.
- [15] X. Olivé-Boix, Guide to the Tortosa-Beseit Ports RNC, Department of Climate Action, Food and Rural Agenda. [https://agricultura.gencat.cat/ca/ambits/medi-natural/casa/guia-cacador/on-cacar/reserves-nacionals-casa/ports-tortosa-beseit/index.html#googtrans\(caen\)](https://agricultura.gencat.cat/ca/ambits/medi-natural/casa/guia-cacador/on-cacar/reserves-nacionals-casa/ports-tortosa-beseit/index.html#googtrans(caen)), 2018 (accessed 05 July 2023).
- [16] Dirección General del Medio Natural y de Evaluación Ambiental, Informe técnico sobre el estado de conservación de las poblaciones cinegéticas de la Comunidad

- Valenciana. <https://agroambient.gva.es/va/web/medio-natural/estadisticas>, 2022 (accessed 05 July 2023).
- [17] G. Mentaberre, R. Velarde, M. García-Readigòs, E. Serrano, J. Jovaní, X. Olivé-Boix, Nuevo brote de sarna sarcóptica en cabra montés (*Capra pyrenaica*), in: 33èmes rencontres du Groupe d'Etudes sur l'Écopathologie de la Fauna Sauvage de Montagne (GEEFSM), Balme, Italia, 21–24 Mayo, 2015.
- [18] J.M. Pérez, J.E. Granados, J. Espinosa, A. Réez-Bravo, J.R. López-Olvera, L. Rossi, P.G. Meneguz, S. Angelone, P. Fandos, R.C., Biology and management of sarcoptic mange in wild Caprinae populations, *Mammal Rev.* 51 (2020) 82–94, <https://doi.org/10.1111/mam.12213>.
- [19] R. Cuadrado-Matías, S. Baz-Flores, A. Peralbo-Moreno, G. Herrero-García, M. A. Rivalde, P. Barroso, S. Jiménez-Ruiz, C. Ruiz-Rodríguez, F. Ruiz-Fons, Determinants of Crimean-Congo haemorrhagic fever virus exposure dynamics in Mediterranean environments, *Transbound. Emerg. Dis.* (2022), <https://doi.org/10.1111/tbed.14720> tbed.14720.
- [20] M.A. Sas, L. Comtet, F. Donnet, M. Mertens, Z. Vatanever, N. Tordo, P. Pourquier, M.H. Groschup, A novel double-antigen sandwich ELISA for the species-independent detection of Crimean-Congo hemorrhagic fever virus-specific antibodies, *Antivir. Res.* 151 (2018) 24–26, <https://doi.org/10.1016/j.antiviral.2018.01.006>.
- [21] C. Sáez-Royuela, J.L. Tellería, Las batidas como método de censo en especies de caza mayor: aplicación al caso del jabalí (*Sus scrofa* L.) en la provincia de Burgos (Norte de España), *Doñana, Acta Vertebrata* 15 (1988) 215–223.
- [22] P. Dalgaard, Introductory Statistics with R. R package version 2.0–8. <https://CRAN.R-project.org/package=ISwR>, 2020.
- [23] C. Libiseller, A. Grimvall, Performance of partial Mann-Kendall tests for trend detection in the presence of covariates, *Environmetrics* 13 (2002) 71–84, <https://doi.org/10.1002/env.507>.
- [24] P.H. Ramsey, Critical values for Spearman's rank order correlation, *J. Educ. Stat.* 14 (1989) 245–253.
- [25] R Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2022. <https://www.R-project.org/>.
- [26] H. Wickham, M. Averick, J. Bryan, W. Chang, L. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. Pedersen, E. Miller, S. Bache, K. Müller, J. Ooms, D. Robinson, D. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, H. Yutani, Welcome to the Tidyverse, *J. Open Source Softw.* 4 (2019) 1686. <https://doi.org/10.21105/joss.01686>.
- [27] S. Dorai-Raj, Binomial Confidence Intervals for Several Parameterizations. R package version 1.1–1, 2022.
- [28] T. Pohlert, Non-Parametric Trend Tests and Change-Point Detection. R package version 1.1.4, 2020.
- [29] D.U. Pfeiffer, T.P. Robinson, M. Stevenson, K.B. Stevens, D.J. Rogers, A. C. Clements, *Spatial Analysis in Epidemiology*, OUP Oxford, 2008.
- [30] A. Estrada-Peña, The climate niche of the invasive tick species *Hyalomma marginatum* and *Hyalomma rufipes* (Ixodidae) with recommendations for modeling exercises, *Exp. Appl. Acarol.* 89 (2023) 231–250, <https://doi.org/10.1007/s10493-023-00778-3>.
- [31] H. Hersbach, B. Bell, P. Berrisford, G. Biavati, A. Horányi, J. Muñoz Sabater, J. Nicolas, C. Peube, R. Radu, I. Rozum, D. Schepers, A. Simmons, C. Soti, D. Dee, J.N. Thépaut, ERA5 hourly data on single levels from 1940 to present, in: Copernicus Climate Change Service (C3S) Climate Data Store (CDS), 2023, <https://doi.org/10.24381/cds.adbb2d47>.
- [32] J. Salomon, S.A. Hamer, A. Swei, A beginner's guide to collecting questing hard ticks (Acari: Ixodidae): a standardized tick dragging protocol, *J. Insect Sci.* 20 (2020) 11, <https://doi.org/10.1093/jisesa/ieaa07329>.
- [33] R. Cuadrado-Matías, L. Casades-Martí, A. Peralbo-Moreno, S. Baz-Flores, E. García-Manzanilla, F. Ruiz-Fons, Testing the efficiency of capture methods for questing *Hyalomma lusitanicum* ticks (vector of Crimean-Congo hemorrhagic fever virus), in: *Review*, 2023, <https://doi.org/10.21203/rs.3.rs-2581237/v1>.
- [34] A. Estrada-Peña, A.D. Mihalca, T.N. Petney (Eds.), *Ticks of Europe and North Africa: A Guide to Species Identification*, Springer International Publishing, 2018. <https://doi.org/10.1007/978-3-319-63760-0>.
- [35] L. Varela-Castro, C. Zuddas, N. Ortega, E. Serrano, J. Salinas, J. Castellà, R. Castillo-Contreras, J. Carvalho, S. Lavín, G. Mentaberre, On the possible role of ticks in the eco-epidemiology of *Coxiella burnetii* in a Mediterranean ecosystem, *Ticks Tick Borne Dis.* 9 (2018) 687–694, <https://doi.org/10.1016/j.ttbdis.2018.02.014>.
- [36] A. Estrada-Peña, L. Jameson, J. Medlock, Z. Vatanever, F. Tishkova, Unraveling the ecological complexities of tick-associated Crimean-Congo hemorrhagic fever virus transmission: a gap analysis for the Western Palearctic, *Vector Borne Zoonotic Dis.* 12 (2012) 743–752, <https://doi.org/10.1089/vbz.2011.0767>.
- [37] J.R. Spengler, A. Estrada-Peña, A.R. Garrison, C. Schmaljohn, C.F. Spiropoulou, É. Bergeron, D.A. Bente, A chronological review of experimental infection studies of the role of wild animals and livestock in the maintenance and transmission of Crimean-Congo hemorrhagic fever virus, *Antivir. Res.* 135 (2016) 31–47, <https://doi.org/10.1016/j.antiviral.2016.09.013>.
- [38] A. Estrada-Peña, J. de la Fuente, The ecology of ticks and epidemiology of tick-borne viral diseases, *Antivir. Res.* 108 (2014) 104–128, <https://doi.org/10.1016/j.antiviral.2014.05.016>.
- [39] M. Labuda, V. Danielova, L.D. Jones, P.A. Nuttall, Amplification of tick-borne encephalitis virus infection during co-feeding of ticks, *Med. Vet. Entomol.* 7 (1993) 339–342, <https://doi.org/10.1111/j.1365-2915.1993.tb00702.x>.
- [40] S.E. Randolph, Transmission of tick-borne pathogens between co-feeding ticks: Milan Labuda's enduring paradigm, *Ticks Tick Borne Dis.* 2 (2011) 179–182, <https://doi.org/10.1016/j.ttbdis.2011.07.004>.
- [41] R. Castillo-Contreras, L. Magen, R. Birtles, L. Varela-Castro, J.L. Hall, C. Conejero, X.F. Aguilar, A. Colom-Cadena, S. Lavín, G. Mentaberre, J.R. López-Olvera, Ticks on wild boar in the metropolitan area of Barcelona (Spain) are infested with spotted fever group rickettsiae, *Transbound. Emerg. Dis.* 69 (2022) e82–e95, <https://doi.org/10.1111/tbed.14268>.
- [42] C. Pradera, A. Estrada-Peña, *Hyalomma lusitanicum* (Acari: Ixodidae) como potencial problema de salud pública en el área de Barcelona, *Butlletí de la Institució Catalana d'Història Natural* 86 (2022) 111–116.
- [43] F. Valcárcel, J. González, M.G. González, M. Sánchez, J.M. Tercero, L. Elhachimi, J. D. Carbonell, A.S. Olmeda, Comparative ecology of *Hyalomma lusitanicum* and *Hyalomma marginatum* Koch, 1844 (Acarina: ixodidae), *Insects* 11 (2020) 303, <https://doi.org/10.3390/insects11050303>.
- [44] F.J. Márquez, Rickettsiae in ticks from wild ungulates of Sierra Nevada and Doñana national parks (Spain), *Clin. Microbiol. Infect.* 15 (2009) 227–229, <https://doi.org/10.1111/j.1469-0691.2008.02148.x>.
- [45] R. Calero-Bernal, A. García-Moreno, D. González-Barrio, J.M. Nieto-Rodríguez, P. Fernández-Llario, J.H. de Mendoza, M.Á. Habela, Infectious diseases surveillance of the Iberian ibex (*Capra pyrenaica victoriae*) in Western Spain: health and conservation implications, *Galemys* 32 (2020) 13–20.
- [46] A.J. Shepherd, R. Swanepoel, A.J. Cornel, O. Mathee, Experimental studies on the replication and transmission of Crimean-Congo hemorrhagic fever virus in some African tick species, *Am. J. Trop. Med. Hyg.* 40 (1989) 326–331.
- [47] O. Paye, D. Fontenille, J. Thonnou, J.P. Gonzalez, J.P. Cornet, J.L. Camicas, Experimental transmission of Crimean-Congo hemorrhagic fever virus by *Rhipicephalus evertsi* (Acarina: Ixodidae), *Bull. Soc. Pathol. Exot.* 92 (1990) 143–147.
- [48] S.A. Lule, R. Gibb, D. Kizito, G. Nakanjako, J. Mutyaba, S. Balinandi, L. Owen, K. E. Jones, I. Abubakar, J.J. Lutwama, N. Field, Widespread exposure to Crimean-Congo haemorrhagic fever in Uganda might be driven by transmission from *Rhipicephalus* ticks: evidence from cross-sectional and modelling studies, *J. Inf. Secur.* 85 (2022) 683–692, <https://doi.org/10.1016/j.jinf.2022.09.016>.
- [49] A. Papa, M. Marklewitz, S. Paraskevopoulou, A.R. Garrison, S.V. Alkhovsky, T. Avšič-Županc, D.A. Bente, É. Bergeron, F. Burt, N. Di Paola, K. Ergüyan, History and classification of Aigai virus (formerly Crimean-Congo haemorrhagic fever virus genotype VI), *J. Gen. Virol.* 103 (2022), 001734, <https://doi.org/10.1099/jgv.0.001734>.
- [50] A. Papa, A. Kontana, K. Tsioka, I. Chaligiannis, S. Sotiraki, Molecular detection of Crimean-Congo hemorrhagic fever virus in ticks, Greece, 2012–2014, *Parasitol. Res.* 116 (2017) 3057–3063, <https://doi.org/10.1007/s00436-017-5616-6>.
- [51] F. Ruiz-Fons, I.G. Fernández-de-Mera, P. Acevedo, U. Höfle, J. Vicente, J. de la Fuente, C. Gortazar, Ixodid ticks parasitizing Iberian red deer (*Cervus elaphus hispanicus*) and European wild boar (*Sus scrofa*) from Spain: geographical and temporal distribution, *Vet. Parasitol.* 140 (2006) 133–142, <https://doi.org/10.1016/j.vetpar.2006.03.033>.
- [52] A. Peralbo-Moreno, S. Baz-Flores, R. Cuadrado-Matías, P. Barroso, R. Triguero-Ocaña, S. Jiménez-Ruiz, C. Herraiz, C. Ruiz-Rodríguez, P. Acevedo, F. Ruiz-Fons, Environmental factors driving fine-scale ixodid tick abundance patterns, *Sci. Total Environ.* 853 (2022), 158633, <https://doi.org/10.1016/j.scitotenv.2022.158633>.
- [53] A. Ortuño, M. Quesada, S. López, J. Miret, N. Cardenosa, J. Castilla, E. Anton, F. Segura, Prevalence of *Rickettsia slovaca* in *Dermacentor marginatus* ticks removed from wild boar (*Sus scrofa*) in northeastern Spain, *Ann. N. Y. Acad. Sci.* 1078 (2006) 324–327, <https://doi.org/10.1196/annals.1374.061>.
- [54] A. Ortuño, M. Quesada, S. Lopez-Claessens, J. Castilla, I. Sanfeliu, E. Anton, F. Segura-Porta, The role of wild boar (*Sus scrofa*) in the eco-epidemiology of *R. slovaca* in northeastern Spain, *Vector Borne Zoonotic Dis* 7 (2007) 59–64, <https://doi.org/10.1089/vbz.2006.0576>.
- [55] S. Grech-Angelini, F. Stachurski, R. Lancelot, J. Boissier, J.F. Allienne, S. Marco, O. Maestrini, G. Uilenberg, Ticks (Acari: Ixodidae) infesting cattle and some other domestic and wild hosts on the French Mediterranean island of Corsica, *Parasit. Vectors* 9 (2016) 1, <https://doi.org/10.1186/s13071-016-1876-8>.
- [56] N.V. Tsapko, A.S. Volynkina, A.Y. Evchenko, Y.V. Lisitskaya, L.I. Shaposhnikova, Detection of Crimean-Congo hemorrhagic fever virus in ticks collected from South Russia, *Ticks Tick Borne Dis.* 13 (2020), 101890, <https://doi.org/10.1016/j.ttbdis.2021.101890>.
- [57] A. Estrada-Peña, J. de la Fuente, The ecology of ticks and epidemiology of tick-borne viral diseases, *Antivir. Res.* 108 (2014) 104–128, <https://doi.org/10.1016/j.antiviral.2014.05.016>.
- [58] A. Estrada-Peña, J.S. Gray, O. Kahl, R.S. Lane, A.M. Nijhof, Research on the ecology of ticks and tick-borne pathogens—methodological principles and caveats, *Front. Cell. Infect. Microbiol.* 3 (2013), <https://doi.org/10.3389/fcimb.2013.00029>.
- [59] A. Schulz, Y. Barry, F. Stoek, A. Ba, J. Schulz, M.L. Haki, M.A. Sas, B.A. Dombia, P. Kirkland, M.Y. Bah, M. Eiden, M.H. Groschup, Crimean-Congo hemorrhagic fever virus antibody prevalence in Mauritanian livestock (cattle, goats, sheep and camels) is stratified by the animal's age, *PLoS Negl. Trop. Dis.* 15 (2021), e0009228, <https://doi.org/10.1371/journal.pntd.0009228>.