Original article

Mapping the risk of exposure to Crimean-Congo haemorrhagic fever virus in the Iberian Peninsula using Eurasian wild boar (*Sus scrofa*) as a model

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ABSTRACT

Crimean-Congo haemorrhagic fever (CCHF) virus (CCHFV) is a tick-borne zoonotic pathogen that can cause a lethal haemorrhagic disease in humans. Although the virus appears to be endemically established in the Iberian Peninsula, CCHF is an emerging disease in Spain. Clinical signs of CCHFV infection are mainly manifested in humans, but the virus replicates in several animal species. Understanding the determinants of CCHFV exposure risk from animal models is essential to predicting high-risk exposure hotspots for public health action. With this objective in mind, we designed a cross-sectional study of Eurasian wild boar (*Sus scrofa*) in Spain and Portugal. The study analysed 5,291 sera collected between 2006 and 2022 from 90 wild boar populations with a specific double-antigen ELISA to estimate CCHFV serum prevalence and identify the main determinants of exposure probability. To do so, we statistically modelled exposure risk with host- and environment-related predictors and spatially projected it at a 10 × 10 km square resolution at the scale of the Iberian Peninsula to map foci of infection risk. Fifty-seven (63.3 %) of the 90 populations had at least one seropositive animal, with seroprevalence ranging from 0.0 to 88.2 %. Anti-CCHFV antibodies were found in 1,026 of 5,291 wild boar (19.4 %; 95 %

confidence interval: 18.3–20.5 %), with highest exposure rates in southwestern Iberia. The most relevant predictors of virus exposure risk were wild boar abundance, local rainfall regime, shrub cover, winter air temperature and soil temperature variation. The spatial projection of the best-fit model identified high-risk foci as occurring in most of western and southwestern Iberia and identified recently confirmed risk foci in eastern Spain. The results of the study demonstrate that serological surveys of CCHFV vector hosts are a powerful, robust and highly informative tool for public health authorities to take action to prevent human cases of CCHF in enzootic and emergency settings.

1. Introduction

The reported increase in the prevalence and incidence of tick-borne diseases is a major health concern worldwide (Aslam et al., 2023; Lantos et al., 2015; Vandekerckhove et al., 2021; Wondim et al., 2022). Ticks are the second leading arthropod vector of pathogens for humans and animals after mosquitoes (Jore et al., 2020), and are the group of arthropods that transmit most zoonotic pathogens to vertebrates in the northern hemisphere (Dennis and Hayes, 2002). In recent decades, populations of several tick species of medical and veterinary importance are increasing in Europe. Furthermore, their geographical ranges are expanding (Hartemink and Takken, 2016).

Crimean-Congo haemorrhagic fever virus (CCHFV) is an *Orthonairovirus* of the family *Nairoviridae*. It causes Crimean-Congo haemorrhagic fever (CCHF), a geographically widespread tick-borne human viral disease. Although the virus can infect many animal species (Spengler et al., 2016), clinical disease is primarily confined to humans (Bente et al., 2013). CCHFV can cause severe outbreaks of haemorrhagic fever with case fatality rates ranging from 5 to 40 % (Bente et al., 2013). Due to its epidemiological potential, the World Health Organization (WHO) listed CCHF as a priority disease for research and development (WHO, 2018) and it is also a priority pathogen in wildlife research in Europe (Gavier-Widen et al., 2023). Although the first case of CCHF was recognized in 1944 in the Crimean Peninsula (Hoogstraal, 1979), phylogenetic analyses suggest that the virus dates back more than 2500 years (Carroll et al., 2010). Humans can become infected by the bite of a CCHFV vector (the main source of transmission), by direct contact with a highly infected person during the acute phase of the infection or by contact with blood or tissues of viraemic animals (Ergonul, 2012). It is estimated that 90 % of human infections are subclinical, but some patients may develop the severe and often fatal haemorrhagic disease (Bente et al., 2013). In addition, surviving patients may experience a variety of health problems that may not resolve for up to a year (Ergonul, 2006). The general population has a low probability of exposure to this virus, mainly due to their urban lifestyle (Frías et al., 2022). However, farmers, slaughterhouse personnel, veterinarians, hunters and environmental officers are groups at higher risk of becoming infected with CCHFV (Spengler et al., 2019). As there is no vaccine or specific antiviral treatment against CCHF, prevention is the only measure to avoid new infections and clinical outcomes. Therefore, the identification of risk areas through new techniques and developments in statistical and mathematical modelling can help reduce exposure to CCHFV vectors and cases of CCHF (Hartemink and Takken, 2016).

CCHFV has been reported in more than 30 countries worldwide, including countries in Africa, Asia, the Middle East, and southern Europe. Its distribution range is highly associated with that of its main vectors and reservoirs, ticks of the genus *Hyalomma* (Messina et al., 2015). Although the geographic range of the virus appears to be increasing since the beginning of the 21st century, genetic analyses indicate that it has been circulating undetected in many places for more than 1000 years (Madison-Antenucci et al., 2020). In the Iberian Peninsula, the first evidence of autochthonous CCHFV transmission was reported in humans in Portugal in 1985 (Filipe et al., 1985). The first human case of CCHF was notified in west-central Spain in 2016 (Negredo et al., 2017), although the first non-lethal case was retrospectively dated to 2013 in 2020 (Negredo et al., 2021). Since then,

twelve cases have been reported in Spain with four victims (ECDC, 2022). No human clinical cases of CCHF have been reported in Portugal to date.

Hyalomma spp. ticks are considered the primary vectors of CCHFV (Messina et al., 2015), but some field and laboratory studies suggest that other tick species may also be responsible for virus circulation (Gargili et al., 2017). Once a *Hyalomma* tick acquires the virus by feeding on a viraemic host (Shepherd et al.,1991) or while co-feeding with infected ticks on a host, what has been demonstrated for *Hyalomma truncatum* ticks feeding together on small vertebrates such as guinea pigs and rabbits (Gonzalez et al., 1992; Gordon et al., 1993), it remains infected throughout its life (transstadial maintenance) and can also transmit the virus to its offspring (transovarial transmission) (Gonzalez et al., 1992). Animals infected with the virus show short periods of viraemia (~ 5 days) but no signs of disease (Messina et al., 2015). Although vertebrates mount short viraemias after infection by CCHFV, they play an important role in maintaining tick vector populations through blood feeding and allowing viraemic transmission of CCHFV to ticks (Gargili et al., 2017). The contribution of tick-to-tick transmission (co-feeding transmission) to the maintenance of CCHFV in nature is currently unclear. Whereas this potential mechanism was suggested to play an irrelevant role in some studies (Estrada-Peña et al., 2013a), others found it a relevant transmission mechanism together with transstadial and transovarial transmission to maintain CCHFV (Bhowmick et al., 2022). In the Iberian Peninsula, wild ungulates such as red deer (*Cervus elaphus*) and Eurasian wild boar (*Sus scrofa*) are the main hosts of adult *Hyalomma lusitanicum* ticks and can feed large burdens of them (Ruiz-Fons et al., 2006, 2013). They can also host the other *Hyalomma* species that is abundant in the Iberian Peninsula, namely *Hyalomma marginatum* (Pereira et al., 2018; Peralbo-Moreno et al., 2022). Thus, they may contribute to the amplification and spread of CCHFV, both indirectly by contributing to the maintenance of *Hyalomma* tick populations (Peralbo-Moreno et al., 2022) and directly by infecting ticks that feed on viraemic animals (Shepherd et al., 1991). Importantly, even low levels of viraemia in vertebrate hosts result in a humoral immune response (Spengler et al., 2016), so seroepidemiological studies could serve as an important tool to map the distribution of the virus and the potential for future outbreaks (Spengler et al., 2016). In the Iberian Peninsula, CCHFV has been detected in ticks in several areas (Cajimat et al., 2017; Moraga-Fernández et al., 2021; Negredo et al., 2019) and some studies show high regional seroprevalence in both wild animals and livestock (Cuadrado-Matías et al., 2022a, 2022b; MSCBS, 2019). Therefore, CCHFV appears to be endemically established in the Iberian Peninsula. The wild boar is a relevant host for *Hyalomma* spp. ticks in the Iberian Peninsula (Ruiz-Fons et al., 2006; Pereira et al., 2018; Peralbo-Moreno et al., 2022), it is widely distributed in the territory (Bencatel et al., 2019; Palomo et al., 2007) and its abundance is increasing substantially (Wehr, 2021). This ungulate is also present in a wide range of habitat types and their sightings in urban and suburban areas have been increasing in recent decades (Cahill et al., 2003), which also raises concerns about the risk they pose for the transmission of zoonotic pathogens in urban environments (Castillo-Contreras et al., 2021). Therefore, the hypothesis of our study was that they would be good indicators for the spatial distribution of CCHFV and could be useful for mapping the risk of exposure to the virus across the territory and better inform public health authorities about risk hotspots. Our objectives were

to focus on Eurasian wild boar to unravel CCHFV exposure patterns in the Iberian Peninsula, identify the main environmental determinants of exposure risk and use them to map the risk of virus transmission at the Iberian scale.

2. Materials and methods

2.1. Survey design

We designed a retrospective cross-sectional study at the spatial scale of the Iberian Peninsula to estimate the risk of exposure of Eurasian wild boar to CCHFV. The Iberian Peninsula is a territory of 596,740 km^2, heterogeneous in climatic, ecosystemic, orographic and socioeconomic terms. For our purpose, we used the epidemiological population as the unit of study. In fenced private hunting grounds, we defined the epidemiological population as the set of wild boar inhabiting the hunting state. With this approach, we homogenised any possible effect on exposure caused by specific management measures to which wild boar were exposed to and which could lead to a lack of independence of observations. In the unfenced areas, we grouped the animals based on both their geographical proximity during the survey and the environmental similarity of the surveyed areas. This was done as they were under similar conditions of exposure to potential CCHFV vectors. We calculated the minimum number of samples per epidemiological unit needed to estimate antibody prevalence at previously known circulation rates in wild ungulates in Spain (25.4 %; Cuadrado-Matías et al., 2022a, 2022b; Espunyes et al., 2021) with a 95 % confidence level and an accepted error of 8 % (Sergeant, 2018). We took into account previous findings that seroprevalence in wild boar was substantially lower than that found in red deer (Cuadrado-Matías et al., 2022a). We selected serum samples from wild boar of all age classes collected between 2006 and 2022. Samples were collected from wild boar shot by hunters during commercial/social hunting events or after official population control events carried out by environmental rangers in protected areas. Sampling was carried out in accordance with Spanish and EU regulations. We did not require any ethical approval from the authorities because we did not deliberately shoot the animals for the study. Blood samples were transported to the laboratory and centrifuged at 10,000 x *g* for 5 min to obtain serum which was kept frozen at −20 °C until serological analyses. Seroprevalence data from the Basque Country (northern Spain) were included in another publication (Cevidanes et al., unpublished results).

2.2. Serological analyses

The presence of specific antibodies against CCHFV was determined using a commercial double antigen multispecies ELISA kit (IDScreen® CCHF Double Antigen Multispecies, IDVet, Grabels, France), following the manufacturer's indications and based on published protocols (Sas et al., 2018). This ELISA has a high sensitivity (98.9 %) and specificity (100 %) according to the manufacturers. The test has been previously used in multiple wild and domestic species (Cuadrado-Matías et al., 2022a; Dzikwi-Emennaa et al., 2022; Fanelli et al., 2022; Grech-Angelini et al., 2020; Mangombi et al., 2020) where specific antibodies have been demonstrated through viral neutralisation tests (Dr. Martin H. Groschup, personal communication).

2.3. Environmental predictors

We selected a set of predictors from the host population and environmental factors (Table 1) for statistical modelling to understand the drivers of variation in the probability of exposure to CCHFV in the wild boar studied. These predictors were rescaled to a UTM 10 × 10 km spatial resolution scale to cover the full range of study populations.

In terms of environmental factors, we considered two land cover variables as predictors of habitat, the area of forest and shrub cover, in the spatial unit of study. We selected these habitat variables because they have previously shown to be relevant drivers of *Hyalomma* spp. abundance (Valcarcel et al., 2020). Land use data were obtained from the CORINE Land Use/Land Cover database with a resolution of 250 m (EEA, 2000) and rescaled at the UTM 10 × 10 km square level. Eleven bioclimatic predictors were considered, four of them related to Land Surface Temperature (LST) and Normalised Difference Vegetation Index (NDVI), including annual mean NDVI, annual variance of NDVI (from monthly records), annual mean LST and annual variance of LST. These parameters were obtained at a 0.05° spatial resolution and monthly temporal resolution for the period 2000–2022 from the MODIS website (https://modis.gsfc.nasa.gov) and rescaled to the 10 × 10 km spatial unit level. These factors were considered potential predictors of CCHFV exposure risk due to their effects on local tick abundance (Estrada-Peña et al., 2013b; Peralbo-Moreno et al., 2022). In particular, NDVI was considered as a proxy for hydric stress experienced by ticks off the host, as it is an indicator of plant photosynthetic activity that is related to soil water availability (Benedetti and Rossini, 1993; Estrada-Peña, 1999). All other bioclimatic variables were obtained from the WorldClim 2 project database (https://worldclim.org/version2) with a spatial resolution of 1 × 1 km. We selected climate series of data to characterise climatic

Table 1
Set of explanatory predictors considered relevant to analyse the risk of exposure to Crimean-Congo haemorrhagic fever virus (CCHFV). Variables selected for modelling after the descriptive analysis are marked in bold italics.

Factor	Predictor	Description (unit of measure)	Average (range)
Host population	rdfav	Habitat favourability for red deer	3.85 (0.27–17.99)
	wbfav	**Habitat favourability for wild boar**	**2.78 (0.07–5.19)**
	fdfav	Habitat favourability for fallow deer	3.18 (0.32–6.73)
	roefav	Habitat favourability for roe deer	0.24 (0.00–0.79)
	catd	Cattle density (ind/ha)	0.04 (0.00–0.43)
	smd	Small ruminant density (ind/ha)	0.04 (0.00–0.98)
Habitat	***scr***	**Shrub cover (%)**	**25.50 (0.07–85.34)**
	for	Forests (%)	38.04 (0.00–94.31)
Bioclimatic	NDVI	Mean annual normalized difference vegetation index	0.55 (0.22–0.77)
	NDVIv	Annual normalized difference vegetation index variance	0.01 (0.00–0.05)
	LST	Mean annual land surface temperature (°C)	21.03 (13.22–28.43)
	LSTv	**Annual land surface temperature variance (°C)**	**85.51 (31.18–159.00)**
	tcold	**Average air temperature of the coldest annual quarter Dec-Feb (°C)**	**6.94 (−4.00–12.50)**
	twarm	Average air temperature of the warmest annual quarter Jun-Aug (°C)	20.85 (11.80–24.97)
	tseas	Air temperature seasonality (°C)	573.80 (379.40–760.70)
	pran	Average annual precipitation (mm)	737.50 (332.90–1619.30)
	pseas	**Precipitation seasonality (%)**	**40.45 (14.62–76.93)**
	pcold	Average precipitation of the coldest annual quarter Dec-Feb (mm)	230.66 (66.88–651.54)
	pwarm	Average precipitation of the warmest annual quarter Jun-Aug (mm)	104.94 (22.16–267.91)

differences between the diversity of localities surveyed at the spatial scale of the Iberian Peninsula. Air temperature variables included the mean temperature of the coldest annual quarter of the year (December-February), the mean temperature of the warmest annual quarter (June-August) and temperature seasonality. We selected the first two predictors as possible limiting conditions for off-host tick survival caused by harsh winter (extreme cold) or summer (extreme heat) conditions that may modulate tick abundance. The third predictor of temperature was selected as a potential driver of the duration and intensity of the period of exophilic tick activity at the study sites. Although LST and air temperature may be correlated, their interrelationship is complex and can vary with multiple factors such as land cover type or geographical position (Cao et al., 2021). Since ticks spend most of their life on the ground, LST should be a better predictor for ticks than air temperature. However, Hubálek et al. (2003) found that soil temperature was an important predictor of abundance for some tick species, e.g. *Dermacentor reticulatus,* while for others, air temperature was the most relevant predictor. We chose to include predictors of both surface and air temperature to explore their effects on a virus transmitted mainly by *H. lusitanicum* ticks in our study context. Precipitation variables included mean annual precipitation, mean precipitation of the coldest annual quarter of the year (December-February), mean precipitation of the warmest annual quarter (June-August) and seasonality of precipitation. Precipitation-related parameters were considered potential indicators of general environmental humidity conditions at the study sites that modulate tick activity, mainly because high precipitation regimes in Mediterranean environments have been found to be associated with the high questing activity of *H. lusitanicum* ticks (Cuadrado-Matías et al., unpublished results), or with tick survival rates (Lees, 1946). To rescale predictors to the UTM 10 × 10 km square study unit, we calculated the mean value of the smaller spatial cells in which each climate parameter was available within each 10 × 10 km square unit for each predictor.

The demography of the hosts (wild and domestic ungulates) may be a relevant factor in predicting the risk of exposure to CCHFV of any susceptible vertebrate host. Indeed, it has previously been demonstrated to be an important factor shaping tick population density and dynamics (Cuadrado-Matías et al., 2022b; Peralbo-Moreno et al., 2022). Furthermore, tick density is a highly relevant predictor of the risk of exposure to CCHFV (Cuadrado-Matías et al., 2022a). Two demographic parameters of domestic ungulates (cattle and small ruminant head density) and four population parameters of wild ungulates (environmental favourability index for red deer, wild boar, roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*)) were selected. Census data used to estimate livestock parameters were obtained from the 2022 national census conducted by the Spanish Institute of Statistics (https://www.ine.es) and the 2019 national census conducted by the Portuguese Institute of Statistics (https://www.ine.pt), both at regional spatial scale and at veterinary unit level. The veterinary unit data were downscaled to the smallest UTM grids of 10 × 10 km. For this purpose, cattle and small ruminant densities were first calculated for each regional unit and then the weighted mean was calculated for each UTM square. On the other hand, for wild ungulate demographic parameters, we chose to estimate species abundance using environmental favourability indices estimated for different wild ungulate species (Illanas et al., 2022), as no large-scale wild ungulate density/abundance data are available for the Iberian Peninsula.

2.4. Risk factor analysis and risk mapping

All continuous predictors were standardised to reduce variability in measurement scales using the 'scale' function of the R statistical software. An initial exploration of the data based on Zuur et al. (2010) allowed us to check for imbalances in the data, outliers, explore response-predictor relationships and analyse multicollinearity between predictors. To study the relationship between continuous predictors, we constructed the Spearman correlation matrix using the 'chart

correlation' function of the R package 'PerformanceAnalytics'. We removed predictors that exhibited strong correlations ($r \geq |0.7|$) within each set of highly correlated predictors, excluding them from the following steps. Within each set of highly correlated predictors, we selected for modelling only those that showed the lowest AIC values in the bivariate generalised mixed-effects models performed as detailed below. We then analysed the influence of the selected predictors in individual risk of CCHFV exposure (antibody positive/negative; $N = 5291$) using logistic regression (generalised linear mixed-effects models). The random effects included in the model were the UTM 10 × 10 km square survey unit and the epidemiological population from which samples were drawn. Models were built using a manual forward stepwise procedure to reduce the Akaike information criterion (AIC; Burnham and Anderson, 2002) in which, at each step, we selected the model with the lowest AIC and retained the predictor(s) included in the model. We explored the structure of the model residuals using different functions of the R package 'DHARMa'. The spatial autocorrelation of the residuals of the best-fit model were tested using the 'testSpatialAutocorrelation' function of the R package 'DHARMa'. Finally, to check the predictive ability of the model, a κ-fold internal cross-validation was performed. For this, the database was divided into five groups (κ = 5) and the 'cross_validate' function of the R package 'cvms' was used (Jeyaraman et al., 2019). Finally, the best-fit model was spatially projected at a UTM spatial resolution of 10 × 10 km to map the risk probability and identify CCHFV transmission hotspots for the Iberian Peninsula using the 'predict' function of the 'car' package in R environment (Fox and Weisberg, 2018).

3. Results

The minimum number of samples required per epidemiological unit was 66 individuals. Since the study was conducted with serum samples collected in the framework of different research projects with different objectives and from different research groups throughout the Iberian Peninsula, we had to adapt the sample selection to the available serum banks. Thus, we finally analysed 5291 serum samples collected between 2006 and 2022 from 90 populations, 83 in Spain and seven in Portugal (Fig. 1) instead of the 5940 samples (90 × 66) initially considered necessary during the design of the cross-sectional study.

Serological analyses identified 1026 wild boar sera with antibodies against CCHFV (1026/5291; 19.4 %, 95 % exact confidence interval [CI]: 18.3–20.5 %). Fifty-seven of the 90 populations (63.3 %) had at least one positive sample, with positive samples widely distributed in the Iberian Peninsula (Fig. 2). Antibody prevalence values in the populations ranged from 0.0 to 88.2 %. The highest antibody prevalence ($\geq$ 30 %) was observed in wild boar populations in the southwest of the Iberian Peninsula (Fig. 2).

The best-fit model included five of the predictors considered, including environmental favourability for wild boar, shrub cover, annual variance of LST, mean temperature of the coldest annual quarter and seasonality of rainfall (Table 2). A statistically significant positive relationship was observed between these predictors and the risk of individual wild boar exposure to CCHFV (Table 3). We observed a correct distribution and dispersion of the residuals, and no spatial autocorrelation was found. Cross-validation analysis showed that the model had a balanced accuracy of 0.799, with a sensitivity of 66.7 % and a specificity of 93.0 %. The discriminatory power of the model was also good (AUC = 0.922). The spatial projection of the best-fit model showed that the risk of CCHFV transmission is expected to be highest in western and southwestern Spain and in large regions of southern and eastern Portugal. The areas with the lowest predicted risk of virus transmission were located in the northern half of peninsular Spain (Fig. 3).

4. Discussion

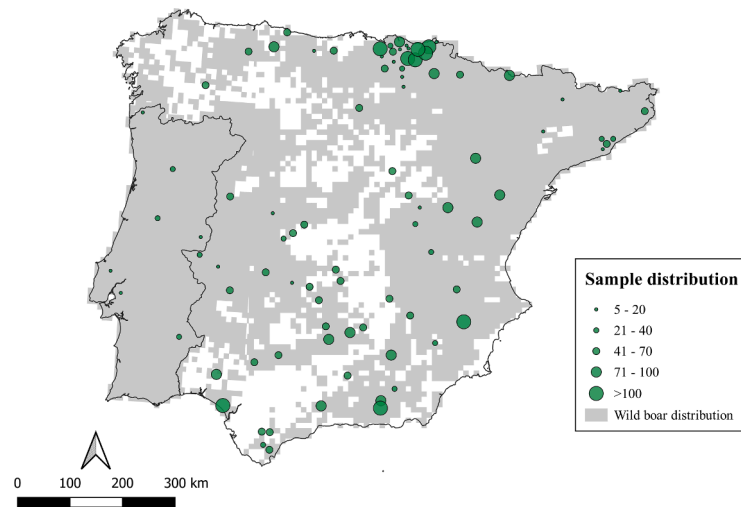CCHFV appears to be endemically established in the Iberian

Fig. 1. Spatial location of the surveyed wild boar populations and local sample size in relation to wild boar distribution, at the UTM 10 × 10 km square unit level, in the Iberian Peninsula (Bencatel et al., 2019; Palomo et al., 2007).
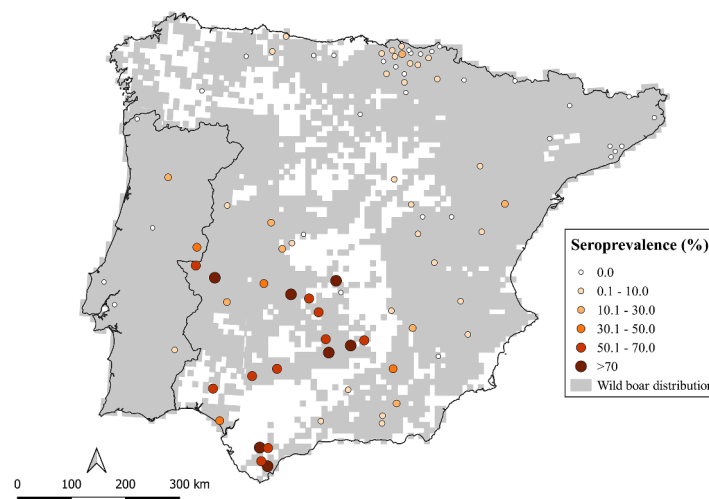

Fig. 2. Results of the serological survey of Crimean-Congo haemorrhagic fever virus (CCHFV) per epidemiological population (see the text) in relation with wild boar spatial distribution, at the UTM 10 × 10 km square unit level, in the Iberian Peninsula (Bencatel et al., 2019; Palomo et al., 2007).

Table 2

Akaike information criterion (AIC) values of the models selected in each step of the forward procedure. *Random effects refer to the null model with random effects (UTM 10 × 10 km squares and epidemiological populations).

Step	Variables	AIC
0	Random*	2936.2
1	Random + pseas	2880.6
2	Random + pseas + wbfav	2864.8
3	Random + pseas + wbfav + LSTv	2862.0
4	Random + pseas + wbfav + LSTv + tcold	2860.9
5	Random + pseas + wbfav + LSTv + tcold + scr	2858.9

Table 3

Output of the generalized linear mixed-effects model performed to analyse the risk of exposure to Crimean-Congo haemorrhagic fever virus. The table shows predictors (see Table 1), their estimates and associated standard errors (SE), the statics (z) and the p-value (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

Predictor	Estimate	SE	z	*p*
Intercept	−3.2600	0.2245	−14.518	***
pseas	1.0684	0.3126	3.418	***
wbfav	0.9307	0.1988	4.681	***
LSTv	0.6346	0.2104	3.016	**
tcold	0.5999	0.2718	2.207	*
scr	0.5072	0.2516	2.015	*

Peninsula, at least in its western and southwestern regions (Cuadrado-Matías et al., 2022b; Moraga-Fernández et al., 2021). This study confirms virus circulation, at least since 2006, amongst Iberian wild boar populations at levels much lower than those found in Iberian red deer populations analysed with the same ELISA test at local (76.1 %; Cuadrado-Matías et al., 2022a) and national (72.3 %; Cuadrado-Matías et al., 2021) spatial scales. The individual seroprevalence found in our study (19.4 %) is also lower than the antibody prevalence found in red deer in a previous study in the Iberian Peninsula (25.4 %) using a

slightly less sensitive competitive ELISA, but the spatial distribution pattern of the virus is very similar (Cuadrado-Matías et al., 2022b). The highest seroprevalence was documented in southwestern peninsular Spain. Differences in seroprevalence between wild ungulate species were shown in a previous study carried out in Doñana National Park, southern Spain (Cuadrado-Matías et al., 2022a), and may be caused by the observed higher burdens of *H. lusitanicum* ticks, one of the main vectors of the virus in the Iberian Peninsula, in red deer
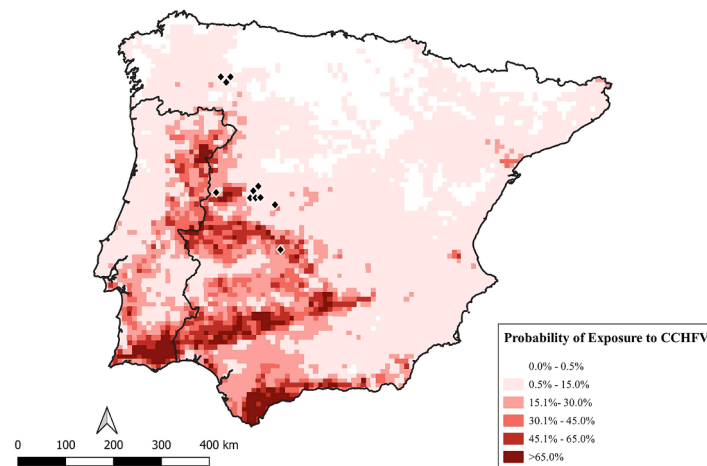
Fig. 3. Spatial projection of the Crimean-Congo haemorrhagic fever (CCHF) virus exposure risk model to the Iberian Peninsula. The model was projected at a UTM 10 × 10 km spatial scale resolution. The location of notification of primary human cases of CCHF reported in Spain (until July 2022) is shown with diamonds.

(Peralbo-Moreno et al., 2022). A higher seroprevalence in red deer has also been detected in northern Spain (Basque Country), where there is no record of *H. lusitanicum* ticks and only a few specimens of *H. marginatum* ticks have been found on domestic ungulates (Cevidanes et al., unpublished results). Focusing on Portugal, we found seropositive samples in the north, east-central and south of the country, but the highest seroprevalence was observed in the wild boar population near the province of "Cáceres" (southwestern Spain), where CCHFV-infected *H. lusitanicum* ticks were detected in 2010 (Estrada-Peña et al., 2012) and where we found a high prevalence of infection in fed ticks (Moraga-Fernández et al., 2021). This agrees with the recent results of a study on sheep in Portugal (Mesquita et al., 2022) where the authors confirm the circulation of the virus in the vicinity of the city of "Évora" which is also close to the province of "Cáceres".

The output of the best-fit model shows the relevance of abiotic factors as modulators of the risk of exposure to CCFHV. Indeed, bioclimatic predictors can define the spatial distribution of the expected most relevant vector of CCHFV for wild boar, *H. lusitanicum*, at small (Peralbo-Moreno et al., 2022) and large (Williams et al., 2015) spatial scales. *Hyalomma lusitanicum* population densities, which are highly relevant predictors of CCHFV exposure risk (Cuadrado-Matías et al., 2022a), are also modulated by host abundance (Peralbo-Moreno et al., 2022). Seasonality of rainfall was a relevant predictor with a positive effect on the risk of exposure to the virus. The regions of the Iberian Peninsula showing the highest rainfall seasonality are mainly located in the southwest (Supplementary Fig. 1). In fact, these areas are highly environmentally favourable for *H. lusitanicum* ticks (Williams et al., 2015). Very hot and dry summers are characteristic of southwestern Iberia, but this region also benefits from highly seasonal abundant rainfall regimes in autumn-winter and, eventually, spring (www.aemet.es) that result in a higher seasonality of precipitation than in drier and wetter Iberian regions (Supplementary Fig. 1). Several studies have demonstrated that high soil moisture has a positive effect on ticks (Estrada-Peña, 2008) and, specifically, on *H. lusitanicum* (Peralbo-Moreno et al., 2022) survival and activity. A previous study on CCHFV exposure of red deer across the Iberian Peninsula also found a positive effect of the soil moisture indicator, NDVI, on the risk of exposure (Cuadrado-Matías et al., 2022b). This expected effect of the NDVI might have been masked in our model due to higher NDVI values in northern Spain (Supplementary Fig. 2), where exposure to the virus was extremely low. *Hyalomma lusitanicum* ticks are more adapted to dry and hot environments than other European tick species (Valcarcel et al., 2020), but within their distribution range, their abundance is higher in foci where soil moisture is higher (Peralbo-Moreno et al., 2022). Thus, regions with high seasonality of rainfall may provide the soil moisture conditions necessary

for *H. lusitanicum* ticks to survive for longer periods than in drier regions of continental Spain (e.g., east central, and north-eastern Spain), promoting higher *H. lusitanicum* densities and driving the risk of CCHFV exposure of wild boar. Meanwhile, the high seasonality of temperatures, which positively influences the risk of exposure of wild boar to CCHFV, may favour appropriate time windows (in spring and summer) for the development of the different stages of *H. lusitanicum*. Soil temperature variations are greatest in central and west-central Iberia (Supplementary Fig. 3) while milder winter temperatures are observed in large parts of southwestern Iberia (Supplementary Fig. 4). The increased risk of exposure of wild boar to CCHFV, expected to be driven mainly by *H. lusitanicum* (Cuadrado-Matías et al., 2022a, 2022b), due to seasonal changes in relatively warm Mediterranean climates (Supplementary Fig. 4) means that colder winter temperatures may limit the distribution and abundance of *H. lusitanicum* ticks (see Williams et al., 2015). The observed increased risk of virus exposure with increasing winter temperatures and large variations in annual LST support this reasoning. Thus, areas favourable to CCHFV appear to be characterised by a combination of large seasonal variations in rainfall, marked seasonal changes in temperature and milder winter temperatures, which most likely define the climatic niche of *H. lusitanicum* in Iberia (Valcarcel et al., 2020). This tick species appears to depend on a balance between soil moisture for tick survival and activity and temperature for development based on our findings on the risk of exposure to CCHFV and the predominant role it is expected to play in virus transmission.

A biotic factor that proved to be an important driver of the likelihood of exposure to CCHFV is the habitat structure, specifically the proportion of the soil covered by shrub. Shrub is one of the preferred habitats for *H. lusitanicum* and *H. marginatum* ticks (Valcarcel et al., 2020), but also for some of their hosts. Wild boar and red deer positively select shrubland, especially during autumn-winter, because it provides them with more shelter and, eventually, food than other habitats (Laguna et al., 2021a; 2021b).

The best-fit model also captured the importance of environmental favourability for wild boar on the risk of exposure to the virus. Unlike Cuadrado-Matías et al. (2022b) who relied on a wild boar favourability index estimated from presence/absence of the species (Acevedo et al., 2010) to model the risk of exposure to CCHFV in red deer, our index was based on hunting bags. Hunting bags are a better proxy for abundance than records of presence (Illanas et al., 2022). Thus, while Cuadrado-Matías et al. (2022b) found a negative effect of the Iberian-scale wild boar favourability index on the risk of exposure to CCHFV, we found a positive effect. Although wild boar host lower tick burdens of *H. lusitanicum* than red deer in Iberia, this tick species accounts for the highest frequency of ticks parasitising wild boar in areas where it is

present (Ruiz-Fons et al., 2006; Peralbo-Moreno et al., 2022). Thus, as expected, the abundance of this wild ungulate contributes to the maintenance of abundant *H. lusitanicum* tick populations and contributes to the risk of exposure to CCHFV.

The aim of our model was to better understand what factors determine the risk of exposure of wild boar to CCHFV. We also aimed to predict the areas of highest risk of exposure in the Iberian Peninsula as a tool for disease prevention in humans. We found the highest predicted exposure risk in the southwest of the Iberian Peninsula, in agreement with findings in red deer (Cuadrado-Matías et al., 2022b). However, slight differences in the extent of risk areas were observed between these studies, most likely because the models captured the different spatial distribution patterns of red deer and wild boar in the Iberian Peninsula. Indeed, our wild boar model predicted a higher risk for the southern coast of Spain than the red deer model because red deer are absent in this area while wild boar are abundant (Supplementary Fig. 5). A recent CCHFV serological study in Andalusia (southern Spain) also found that the risk of exposure to the virus extended to some coastal areas of this region (Baz-Flores et al., unpublished results). Taken together, these findings show that a combination of serological studies in different hosts involved in the CCHFV life cycle would allow more accurate predictions of the actual risk of infection especially in areas of marginal risk. Outside the core distribution of *H. lusitanicum*, the model predicted an increased risk in limited foci in northeastern Spain, which is consistent with our crude findings and with findings in Iberian ibex (*Capra pyrenaica*) (Espunyes et al., 2021). Our model also predicts a risk area in eastern Spain, where a recent study also found antibodies against CCHFV in wild ungulates (Carrera-Faja et al., 2022). These findings indicate that, even with the limitations observed for marginal areas, the wild boar model captures with remarkable accuracy the spatial distribution of CCHFV in Iberia. This model may constitute an important tool for the prevention of human cases of CCHF in the Iberian Peninsula. Our findings show how the predicted high-risk exposure hotspots extend into southern Portugal and reach regions close to the highly humanised surroundings of Lisbon. The model also predicted high-risk areas in northwest Portugal that extend beyond the northern limits of the high-risk areas predicted for Spain. This prediction contrasts with recent findings in a sheep study in Portugal (Mesquita et al., 2022), but small domestic ruminants appear to play a limited role in CCHFV ecology (Schuster et al., 2017). This is probably because small domestic ruminants tend to have lower *Hyalomma* spp. tick burdens (Camicas et al., 1990). Further research is required to confirm the presence and circulation rates of CCHFV in Portugal to confirm or refute our predictions.

5. Conclusions

Modelling the risk of CCHFV exposure using Eurasian wild boar, despite not being the main host of *H. lusitanicum* ticks in Iberia, is a useful tool for mapping CCHFV infection risk and inform Spanish and Portuguese health authorities on where to focus preventive and monitoring actions. Future research on the prevalence of the virus in tick populations and on exposure rates of wild and domestic CCHFV hosts will be needed to more accurately predict and map the risks of virus transmission, especially in marginally favourable regions, and to better understand the complex ecology of this virus.

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Ethics approval

The study was conducted on wild boar that were shot by hunters during commercial hunting events or by environment agents as part of population control measures. Therefore, they were not shot deliberately for this study and no ethical permission was required to collect the samples.

CRediT authorship contribution statement

Sara Baz-Flores: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Cesar Herraiz:** Formal analysis, Investigation, Writing – review & editing. **Alfonso Peralbo-Moreno:** Formal analysis, Investigation, Writing – review & editing. **Marta Barral:** Investigation, Writing – review & editing. **Mari Cruz Arnal:** Investigation, Writing – review & editing. **Ana Balseiro:** Investigation, Writing – review & editing. **David Cano-Terriza:** Investigation, Writing – review & editing. **Sabrina Castro-Scholten:** Investigation, Writing – review & editing. **Aitor Cevidanes:** Investigation, Writing – review & editing. **Alazne Conde-Lizarralde:** Investigation, Writing – review & editing. **Raúl Cuadrado-Matías:** Investigation, Writing – review & editing. **Fernando Escribano:** Investigation, Writing – review & editing. **Daniel Fernández de Luco:** Investigation, Writing – review & editing. **Luis Eusebio Fidalgo:** Investigation, Writing – review & editing. **Javier Hermoso-de Mendoza:** Investigation, Writing – review & editing. **Paulino Fandos:** Investigation, Writing – review & editing. **Félix Gómez-Guillamón:** Investigation, Writing – review & editing. **José E. Granados:** Investigation, Writing – review & editing. **Débora Jiménez-Martín:** Investigation, Writing – review & editing. **Jorge R. López-Olvera:** Investigation, Writing – review & editing. **Inés Martín:** Investigation, Writing – review & editing. **Remigio Martínez:** Investigation, Writing – review & editing. **Gregorio Mentaberre:** Investigation, Writing – review & editing. **Ignacio García-Bocanegra:** Investigation, Writing – review & editing. **Francisco Ruiz-Fons:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Resources.

Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2023.102281.

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