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Crimean-Congo Hemorrhagic Fever Virus Circulation in Wild European Rabbits, Portugal, 2018–2023

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Crimean-Congo hemorrhagic fever virus is considered a public health risk in southwestern Europe. We surveyed serum samples from 667 European rabbits across Portugal, a rabbit species known to host immature *Hyalomma lusitanicum* ticks. We found low levels of virus antibodies (>1%), with a localized cluster reaching 5.77% in southern populations.

Crimean-Congo hemorrhagic fever virus (CCHFV) is a highly pathogenic tickborne pathogen able to cause severe hemorrhagic fever that has a high case-fatality rate in humans (*1*). Since the virus' first detection in southwestern Europe in 2010 (*2*), CCHFV has emerged as a formidable public health risk. Reports from Spain have identified *Hyalomma lusitanicum* ticks as reservoirs and vectors of CCHFV (*2*–*4*) and have suggested circulation and maintenance of the virus at local levels to be related to animal abundance (*5*). Mammals infected by tick bites become viremic for 2–10 days and develop a persistent immune response (*4*), making serologic surveys an effective tool for monitoring CCHFV dynamics (*2*,*4*–*6*).

Wild lagomorphs, and particularly European rabbits (*Oryctolagus cuniculus*), are key hosts of immature stages of *H. lusitanicum* ticks (*7*) and are expected to play a critical role in CCHFV epidemiology (*8*). However, prior reports have not established clear evidence of natural exposure of lagomorphs to CCHFV in Europe (*8*). Our study aimed to fill this gap through a serologic survey of rabbit populations from Portugal.

During May 2018–December 2023, we sampled 667 wild rabbits across 20 sites throughout mainland Portugal (average 33.4 ± 46.1 [standard deviation] rabbits per site) (Figure). We selected 8 longitudinal sites on the basis of their high rabbit abundance.

Twelve cross-sectional sites included hunted rabbits from ongoing studies. Study sites consisted of mixed agro-forestry landscapes with variable rabbit and wild ungulate abundance. Samples (1 per animal) encompassed blood from live-captured rabbits in a longitudinal capture-recapture study (n = 472) and hunted rabbits (n = 195), including 71 dried blood spots (DBS). We collected blood samples by way of the saphenous vein (live rabbits) or the thoracic cavity (hunted rabbits) and centrifuged samples at 2,000 × *g* for 10 minutes to obtain serum. We collected blood from the thoracic or abdominal cavities as DBS in Whatman Protein Saver 903 cards (Cytiva, https://www.cytivalifesciences.com), dried at room temperature (2–6 weeks) before being shipped to the laboratory. We stored serum samples and DBS at −20°C prior to processing them for CCHFV detection using a commercial ELISA with recombinant purified CCHFV nucleoprotein (IDScreen, Double Antigen Multi species; https://www.innovative-diagnostics.com), following the manufacturer's instructions.

We performed a univariate analysis because of a low number of positive ELISA results. We evaluated associations between seropositivity and individual categorical variables (year, month, sex, and age class) using Fisher exact test. We considered a p value <0.05 to be significant. We conducted statistical analyses in R v4.3.1 (The R Project for Statistical Computing, https://www.r-project.org) and QGIS v3.38.2 (https://qgis.org) for mapping the results.

We detected 4 animals with CCHFV antibodies (n = 667), resulting in an overall prevalence of 0.60% (95% CI 0.23%–1.53%). We collected the 4 positive samples from 2 sites located 74 km apart in southern Portugal (Figure), within an area previously identified as highly active for the circulation of CCHFV in wildlife (*5*). Those samples encompassed a DBS from a rabbit in 2023 (data not available) and 3 serum samples from

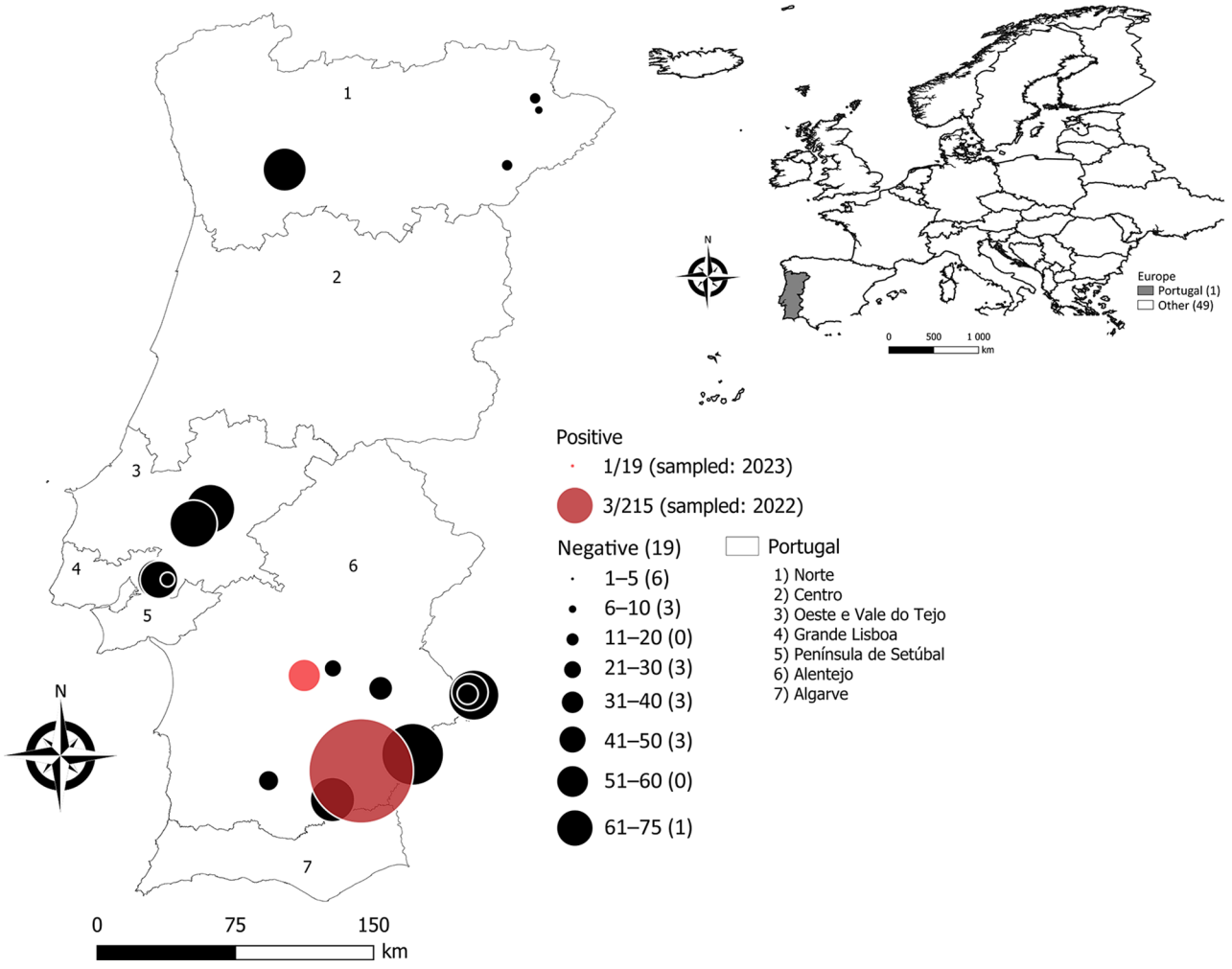

Figure. Study sites in Portugal, sample sizes, and locations of seropositive rabbits in study of Crimean-Congo hemorrhagic fever virus circulation in wild European rabbits, Portugal, 2018–2023. Inset map shows location of Portugal in Europe (gray shading).

Table. Univariate analysis of binomial serologic results in study of Crimean-Congo hemorrhagic fever virus circulation in wild European rabbits, Portugal

Category	Seropositive, no.	Sample size, no.	Prevalence, % (95% CI)	p value*
Sex				Referent
M	1	260	0.37 (0.07–2.15)	
F	2	289	0.71 (0.21–2.76)	
Unknown	1	122	0.82 (0.14–4.50)	
Age				**0.041**
Adult	0	326	0 (0–1.16)	
Subadult	0	104	0 (0–3.56)	
Juvenile	3	127	2.36 (0.81–6.72)	
Unknown	1	110	0.90 (0.16–4.97)	
Year				0.743
2018	0	60	0 (0–6.02)	
2019	0	51	0 (0–7.00)	
2020	0	59	0 (0–6.11)	
2021	0	127	0 (0–2.94)	
2022	3	195	1.54 (0.52–4.42)	
2023	1	175	0.57 (0.10–3.17)	
Month of sample collection				0.519
Jan–Apr	0	45	0 (0–7.87)	
May–Aug	3	299	1.00 (0.34–2.91)	
Sep–Dec	1	314	0.31 (0.06–1.78)	
Unknown	0	9	0 (0–29.91)	

*By Fisher exact test. Bold indicates significance (p<0.05).

rabbits captured alive in 2022 (1 female and 1 male in June, 1 female in July, all juveniles). None of the seropositive rabbits were later recaptured. In this location, longitudinal monitoring of the same population identified a sudden increase of seroprevalence between 2 consecutive years, ranging from 0 (0/86 rabbits) in 2021 to 5.77% (95% CI 1.98–15.64%; 3/52 rabbits;) in 2022 (Table), before dropping again to undetectable levels in 2023 (0/78 rabbits). We analyzed 143 rabbits in 9 other sites from Portugal collected in 2022 without detecting any seropositivity, suggesting that CCHFV circulation was very localized. Given the rabbit density data obtained by capture-recapture methods in that same population (peak densities of 3.0–3.8 rabbits/hectare in 2021–2023) (*9*), we estimated the captured rabbits to represent 40%–75% of the total population yearly. Therefore, we inferred that the probability of CCHFV being undetected in 2021 and 2023 ranged from 1% to 10%. (https://epitools.ausvet.com.au/freedomss). Overall, our data suggest that CCHFV circulation in rabbit populations in Portugal was highly localized in space and time, with a sudden increase in a specific southern location (Mértola) in 2022.

Our results highlight the need for further studies to understand the ecologic and epidemiologic role of wild rabbits in the dynamics of *Hyalomma* tick populations and CCHFV circulation. Considering the preference of immature *H. lusitanicum* ticks for lagomorphs in the Iberian Peninsula, we anticipated the detected exposure of rabbits to CCHFV (*7*). Nevertheless, previous surveys in wild rabbit populations from areas of suspected active circulation in Spain (*5*) did not detect any evidence of CCHFV exposure. Given our results of 10% between-cluster prevalence and 5% within-cluster seroprevalence, we estimated that aiming for 95% cluster sensitivity, a minimum sample of 31 rabbits per cluster would be required to detect exposure to CCHFV in areas of active viral circulation (https://epitools.ausvet.com.au/twostagefreedomsstwo). Therefore, when planning future surveys in wild rabbits, we recommend implementing a 2-stage cluster sampling approach (*10*) to better detect spatiotemporally clustered CCHFV antibodies.

The live capture protocol was approved by CIBIO Animal Welfare committee (ORBEA/2023_01) and performed under the nature conservation authority licenses (08/2019, 1/2020, 197/2020, 23/2021, and 574/2022), according to Portuguese and European legislation. No animals were killed for the purpose of this study.

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Increased Rates of *Purpureocillium lilacinum* Mold among Laboratory Culture Results, United States

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Purpureocillium lilacinum, a common environmental mold and bionematicide, can cause human infections. At a major US commercial laboratory during March 2019–February 2025, *P. lilacinum* culture rates increased; rates were highest in the South Atlantic and Pacific states. Nonculture-based diagnostic tools such as microscopy may help identify and confirm clinical infection earlier.

Purpureocillium lilacinum (formerly *Paecilomyces lilacinus*) is a naturally occurring filamentous fungus that is common in the environment, particularly in soil and decaying vegetation (*1*). Two strains of *P. lilacinum* registered in 2005 and 2021 are used as agricultural bionematicides in the United States (*2*,*3*). The fungus rarely causes human infection but can cause hyalohyphomycosis, an infection with varied clinical soft-tissue, ocular, or pulmonary manifestations. Infection most frequently affects immunocompromised persons but can also occur in immunocompetent persons. Mortality rates can reach 20% (*1*).

P. lilacinum infection is clinically indistinguishable from other mold infections, and the organism resembles other molds on cytology, histopathology, and culture, potentially leading to misidentification and to delayed or inappropriate treatment (*1*). *P. lilacinum* is intrinsically resistant to amphotericin B and can be correlated with poorer treatment outcomes (*1*,*4*).

Worldwide, clinical characteristics and outcomes of 101 *P. lilacinum* infections have been described, 31 of which were from the United States, but epidemiology of the infection in the United States is poorly understood (*1*). We explored *P. lilacinum* culture data from a large national commercial laboratory to describe this organism in the United States.