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Serosurveillance of Crimean-Congo hemorrhagic fever virus in zoo animals, Spain, 2007–2024

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ABSTRACT

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne zoonotic pathogen of significant public health concern worldwide. In Spain, CCHFV infection is considered an emerging and underdiagnosed disease. In this country, wildlife exhibits high levels of CCHFV exposure, and 20 autochthonous human cases, including six fatalities, have been officially reported in recent years. Zoos represent unique epidemiological interfaces, housing a high diversity of wildlife species in close contact with humans and serving as habitats for pathogens and tick communities. However, information on the role of captive wildlife inhabiting urban and peri-urban areas in the epidemiology of CCHFV remains limited. The aim of the present study was to evaluate the circulation of CCHFV in zoo-housed wildlife in Spain. From 2007 and 2024, serum samples from 956 zoo-housed mammals covering 173 species and 38 families were collected across 19 zoos and wildlife rescue centers in Spain through intermittent sampling. Anti-CCHFV antibodies were detected by ELISA in two white rhinoceroses (*Ceratotherium simum*) and one dromedary camel (*Camelus dromedarius*) (0.3 %; 95 % CI 0.0–0.7) sampled in the same zoo in central Spain. Virus neutralization test was performed on ELISA-positive samples, confirming the presence of specific neutralizing antibodies in one white rhinoceros. To the best of the authors' knowledge, this is the first CCHFV surveillance in zoo-housed animals worldwide. Our results suggest low and geographically localized seropositivity for CCHFV. Including CCHFV monitoring in surveillance programs in zoos could provide valuable insights into the virus's epidemiology in anthropogenic environments, particularly in high-risk areas.

1. Introduction

Orthonairovirus haemorrhagiae, commonly known as Crimean-Congo hemorrhagic fever virus (CCHFV; family *Nairoviridae*), is a globally distributed tick-borne zoonotic pathogen (Hawman and Feldmann, 2023). Due to its severe impact on public health, high mortality rate, challenges in control, and the absence of approved treatment or vaccines (Srivastava et al., 2024), the World Health Organization (WHO) has listed it as a priority pathogen for research (WHO, 2018). Although CCHFV infects a wide range of animal hosts, clinical disease primarily occurs in humans, who are typically infected through the bite of infected ticks (mainly *Hyalomma* spp.) (Nasirian, 2022; Hawman and Feldmann, 2023). Secondary transmission can occur through direct contact with the blood or tissues of infected animals (Nasirian, 2020).

The maintenance of competent tick populations is strongly influenced by wildlife hosts, and the existence of a wildlife-human interface markedly increases the risk of human infection with CCHFV (Spengler et al., 2016). Specifically, zoos facilitate close human-animal

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interactions that can support the persistence and spread of tick populations and tick-borne pathogens, potentially influencing the dynamic of CCHFV transmission in anthropized areas (Adler et al., 2011; Hrnková et al., 2021). Nevertheless, information is lacking on the role of captive wildlife inhabiting urban and periurban areas in the epidemiology of CCHFV.

Therefore, the aim of this study was to evaluate the large-scale circulation of CCHFV among captive wildlife in zoos and wildlife rescue centers (WRCs) in Spain, a country where Crimean-Congo hemorrhagic fever is considered an emerging and underdiagnosed disease in humans (Spengler and Bente, 2017; Monsalve Arteaga et al., 2021; Negredo et al., 2021; Lorenzo Juanes et al., 2023; Contreras-Ferro et al., 2024), with a high exposure level in wildlife (Espunyes et al., 2021; Baz-Flores et al., 2024) and where 20 human cases, including six fatalities, have been officially reported since 2013 (ECDC, 2025).

2. Material and methods

2.1. Study design and sampling collection

A total of 956 zoo-housed mammals, representing to 173 species and 38 families kept in 19 zoos and WRCs were opportunistically sampled across Spain between 2007 and 2024 (Supplementary table; Fig. 1).

Serum samples were obtained either from existing serum banks or from animals undergoing routine medical check-ups or surgical procedures during the study period and were stored at −20°C until further serologic analysis. This study did not require any additional blood extraction during the study period. When available, individual epidemiological data were gathered for each animal. The distribution of sampled animals according to order, age, sex, sampling period, and zoo/wildlife rescue center is summarized in Table 1.

2.2. Serological analysis

All serum samples were tested for antibodies against the nucleoprotein (N) of CCHFV using a commercial double-antigen multispecies ELISA Kit (IDvet Screen®, Grabels, France), according to the manufacturer's instructions.

Samples testing positive by ELISA were subsequently analyzed by virus neutralization test (VNT) using the Kosova Hoti strain of CCHFV (genotype V) (Carroll et al., 2010; Bost et al., 2024), a well-characterized and cultivable strain representative of one of the CCHFV genotypes currently circulating in Spain and southern Europe. Briefly, each serum sample was tested in triplicate and serially diluted in Leibovitz-15 (L-15) medium starting from 1:4–1:32, then added to empty wells of a 96-well plate. The serial dilutions of sera were then mixed with approximately 150 plaque-forming units of CCHFV per well and incubated for 1 h at 37°C. To assess possible cytotoxic effects, a serum control without virus was added. After incubation, 100 µl of the serum-virus mixture was added to wells containing human adrenocortical carcinoma (SW13) cells, followed by 6-day incubation at 37°C. Wells were then examined for cytopathic effect (cpe). Neutralizing activity was defined as complete inhibition of cpe. The neutralization titer was calculated as the geometric mean of the three replicates showing neutralizing activity. Samples with titer > 1:8 were considered VNT-positive.

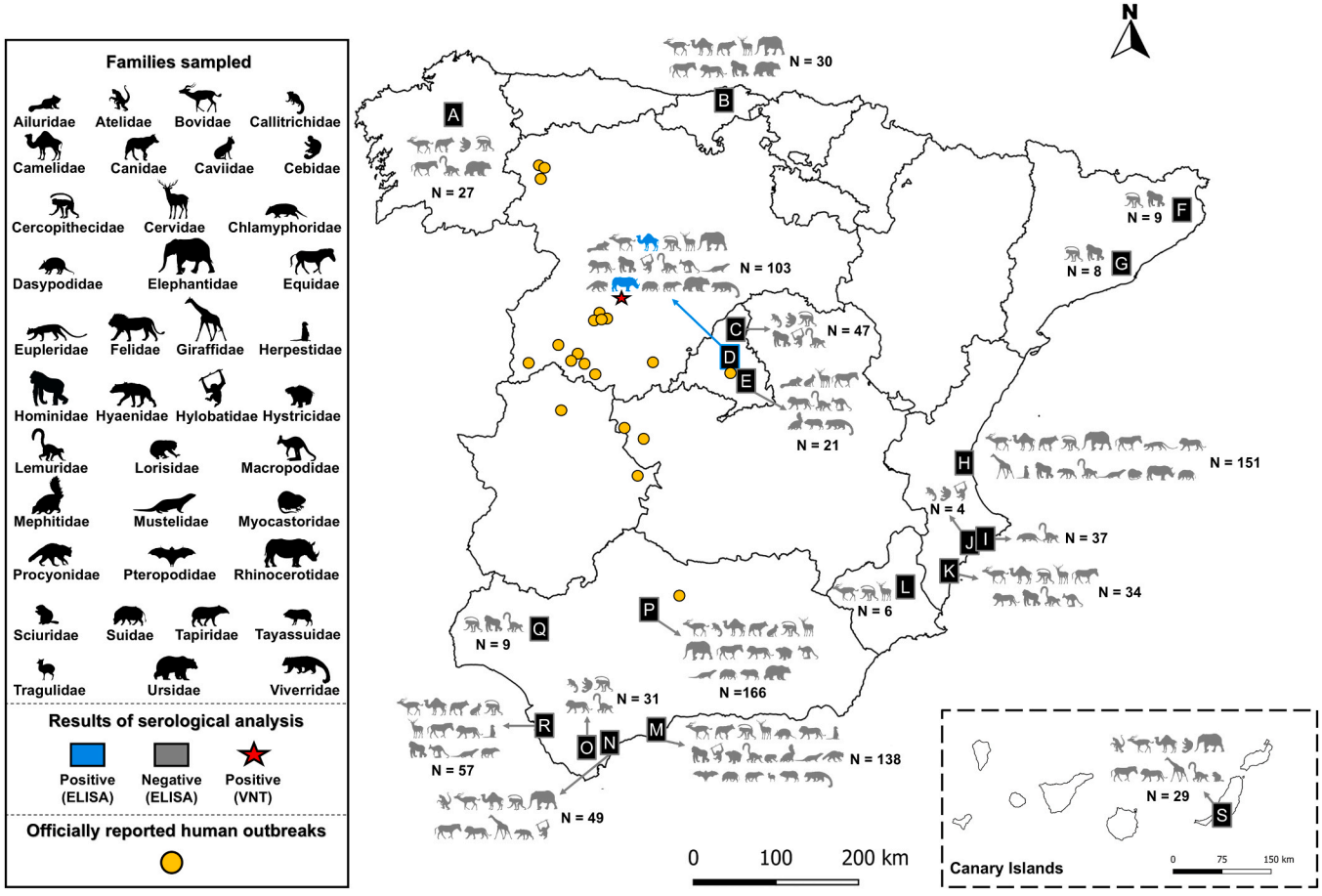

Fig. 1. Geographic distribution of sampled zoo-housed animals from 19 zoos and wildlife rescue centers (letters A–S), and human outbreaks officially reported in Spain.

Table 1
Epidemiological variables collected from zoo-housed animals sampled in Spain.

Variable	Categories	No. Positives/No. analyzed (%)[a]
Order	Artiodactyla	1/327 (0.3 %)
	Carnivora	0/157 (0.0 %)
	Chiroptera	0/1 (0.0 %)
	Cingulata	0/3 (0.0 %)
	Diprotodontia	0/11 (0.0 %)
	Perissodactyla	2/39 (5.1 %)
	Primates	0/363 (0.0 %)
	Proboscidea	0/23 (0.0 %)
	Rodentia	0/32 (0.0 %)
Age	Adult	3/381 (0.8 %)
	Subadult	0/9 (0.0 %)
	Juvenile	0/90 (0.0 %)
Sex	Female	2/390 (0.5 %)
	Male	1/390 (0.3 %)
Sampling period	2007–2013	0/238 (0.0 %)
	2014–2017	2/328 (0.6 %)
	2018–2020	1/258 (0.4 %)
	2021–2024	0/132 (0.0 %)
Zoo/Wildlife Rescue Center	A	0/27 (0.0 %)
	B	0/30 (0.0 %)
	C	0/47 (0.0 %)
	D	3/103 (2.9 %)
	E	0/21 (0.0 %)
	F	0/9 (0.0 %)
	G	0/8 (0.0 %)
	H	0/151 (0.0 %)
	I	0/37 (0.0 %)
	J	0/4 (0.0 %)
	K	0/34 (0.0 %)
	L	0/6 (0.0 %)
	M	0/138 (0.0 %)
	N	0/49 (0.0 %)
	O	0/31 (0.0 %)
	P	0/166 (0.0 %)
	Q	0/9 (0.0 %)
	R	0/57 (0.0 %)
	S	0/29 (0.0 %)

[a] Missing values omitted.

3. Results and discussion

To the authors' knowledge, this is the first CCHFV surveillance carried out in zoo-housed animals on a global scale. Antibodies against CCHFV nucleoprotein were detected in three (0.3 %; 95 % CI 0.0–0.7) of the 956 specimens tested by ELISA (Table 1; Supplementary table; Fig. 1), specifically two white rhinoceroses (*Ceratotherium simum*) sampled in 2016–2017 and one dromedary camel (*Camelus dromedarius*) sampled in 2020.

Of the ELISA-seropositive individuals, specific neutralizing antibodies were confirmed by VNT (titer 1:13) in one of the white rhinoceroses. All three seropositive ungulates were sampled at the same zoo (zoo D; Table 1; Supplementary table; Fig. 1). The white rhinoceroses were an adult male and female housed at the same facility, both of which arrived from South Africa in 1971. The ELISA-positive dromedary camel was an adult female that arrived from Algeria in 2008. At the time of sampling, the rhinoceroses and the camel were in non-adjacent enclosures located approximately 250 m apart. Antibodies against CCHFV have been previously reported in free-ranging white rhinoceroses as well as in dromedary camels across various African countries, particularly in endemic regions (Nasirian, 2019; Temur et al., 2021; Celina et al., 2025).

Data on the kinetics of anti-CCHFV antibodies remain scarce. IgG antibodies have been shown to persist for at least eight years post-infection in humans (Vasmehjani et al., 2024), whereas an experimental study in African wild micromammals reported antibody levels pealing at 14–21 days, followed by a decline by 28–35 days post-infection (Shepherd et al., 1989). Although prior CCHFV exposure in their countries of origin (Temur et al., 2021; Temani et al., 2025), and the possibility of long-term antibody persistence cannot be entirely ruled out, our findings suggest that the seropositive ungulates in this study may have been exposed to the virus in Spain. It should be noted that zoo D is located within a large natural reserve (1535 ha) near Madrid (central Spain), which hosts a high diversity of wildlife species, including lagomorphs, artiodactyls, and wild birds. This biodiversity may contribute to the maintenance of competent tick populations and the circulation of CCHFV (Moraga-Fernández et al., 2020; Baz-Flores et al., 2024; Celina et al., 2025). Given Spain's location along major migratory flyways, the potential role of migratory birds in the dispersal of infected ticks from Africa to Europe warrants consideration (Palomar et al., 2013; Estrada-Peña et al., 2021; Kiwan et al., 2024). Based on this information, we hypothesize that the seropositive animals detected in our study could have been infected by ticks inhabiting the adjacent natural area. Nevertheless, although the exclusive detection of seropositive animals at this site suggests a potential localized epidemiological risk, further ecological and competent vector studies from the area are needed to provide stronger evidence for this association.

The fact that nearly all animals tested in our study yielded negative results suggests that the ELISA does not exhibit non-specific reactivity with serum samples from a wide range of animal species. This observation is particularly relevant in the case of camels, given previous studies in several African countries have reported high seroprevalence rates (89.7–97.0 %) in this species using the same assay (Bouaicha et al., 2021; El Ghassem et al., 2023; Degui et al., 2024). Of note, only one of the 19 dromedary camels tested positive by ELISA (Supplementary table), further supporting the specificity of the assay.

Although false-positive ELISA results cannot be entirely ruled out, the discrepancies between ELISA and VNT results in the two VNT-negative animals may reflect the strain-specificity of the neutralizing activity assessed by the VNT, which targeted antibodies against the Kosova Hoti strain of CCHFV (genotype V) (Carroll et al., 2010). While several studies have confirmed the circulation of four CCHFV genotypes in various tick species across Spain (I, III, IV and V) (Moraga-Fernández et al., 2020; Sánchez-Seco et al., 2021), the specific genotype distribution in ticks within and around the zoos included in this study remain unknown. Consequently, these VNT-negative individuals may have been exposed to genotypes other than V. An alternative explanation could be a weak neutralizing response in these animals, resulting in VNT titers below the detection threshold, as previously reported in domestic ungulates (Bost et al., 2024).

The seroprevalence detected in the present study suggests that zoo-housed wildlife in Spain have a lower level of exposure to CCHFV compared with free-ranging wildlife, particularly wild ungulate species. Higher seroprevalence values have been detected in red deer (*Cervus elaphus*) (25.4–76.1 %) (Cuadrado-Matías et al., 2022a, 2022b), Iberian ibex (*Capra pyrenaica*) (68.0–100 %) (Espunyes et al., 2021; Carrera-Faja et al., 2022) and wild boar (*Sus scrofa*) (15.4–39.7 %) (Carrera-Faja et al., 2022; Baz-Flores et al., 2024; Frías et al., 2024) across various regions of Spain. Notably, individuals of these species were included in our study and all tested seronegative.

Differences observed between zoo-housed and free-ranging wildlife may be attributed to several factors: (I) routine external deworming of zoo animals, as well as cleaning and disinfestation procedures implemented in captive settings; (II) the fact that enclosed zoo facilities limit tick exposure; (III) ecological differences between free-ranging and captive animals, including habitat use and movement patterns; and (IV) variation in sampling years among studies, which may reflect temporal fluctuations in virus circulation and tick activity.

This study has some limitations that should be considered. First, the opportunistic nature of the sampling, based on available serum banks and routine veterinary procedures, may have introduced biases in temporal coverage, species representation, and seasonal tick activity. Second, validation of the commercial ELISA used in this study across all tested species is lacking, potentially allowing cross-reactivity. Finally, using of a single CCHFV genotype in VNT assays may limit the detection

of genotype-specific antibodies. Therefore, future studies including multiple genotypes could enhance the sensitivity of serological confirmation.

4. Conclusions

Our findings suggest low and geographically localized seropositivity for CCHFV among zoo-housed wildlife in Spain during the study period. These results indicate that captive wildlife could have a role in the virus's epidemiology in this country, but appear to pose only a minimal risk of infection to humans in close contact with them, particularly zoo staff. Implementing CCHFV monitoring in surveillance programs in zoo-housed wildlife may enhance understanding of the virus's epidemiology in urban and periurban areas.

Ethical approval

Samples were obtained from serum banks or from animals subjected to rutinary health programs, surgical interventions or medical check-ups during the study period. No ethical approval was necessary.

CRediT authorship contribution statement

Ricardo Navarro-López: Writing – review & editing, Validation, Methodology. **Benjamin Gutjahr:** Writing – review & editing, Validation, Methodology, Investigation. **Eva Martínez-Nevado:** Writing – review & editing, Validation, Methodology. **Kerstin Fischer:** Writing – review & editing, Validation, Methodology, Investigation. **Ignacio García-Bocanegra:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **David Cano-Terriza:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Data curation, Conceptualization. **Mario Torro:** Writing – review & editing, Validation, Methodology. **Martin H. Groschup:** Writing – review & editing, Validation, Methodology, Investigation. **Moisés Gonzálvez:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Data curation, Conceptualization. **Adrián Beato-Benítez:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetmic.2025.110787.

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