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Paper

Evidence of Crimean-Congo hemorrhagic fever (CCHF) susceptibility among big felids in Namibia: leopards (Panthera pardus) and lions (Panthera leo)

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Abstract

Crimean-Congo hemorrhagic fever (CCHF) is a severe zoonotic disease caused by the Crimean-Congo hemorrhagic fever virus (CCHFV), primarily transmitted by *Hyalomma* ticks. The virus has been detected in a wide range of domestic and wild animals, contributing to its persistence in endemic regions. However, felids of the *Panthera* genus have not been previously investigated in this context. The present study aimed to assess the seroprevalence of CCHFV antibodies in leopards (*Panthera pardus*; n=250; 1997-2017) and lions (*Panthera leo*; n=7; 2023) in Namibia using a commercial ELISA kit. CCHFV antibodies were detected in 36/250 leopards (14.40%; 95% CI: 10.59-19.29) and 3/7 lions (42.86%; 95% CI: 15.82-74.95). No tested animals had a known history of CCHFV-related clinical signs. Statistical analyses did not reveal significant temporal trends in seroprevalence. The presence of CCHFV antibodies in large felids suggests past exposure but does not confirm a definitive role in viral transmission. Further virological studies, including molecular detection and investigations of carcasses are needed to determine whether these species serve as viral reservoirs or dead-end hosts. Given the zoonotic potential of CCHFV and the role of wildlife in its epidemiology, increased surveillance is warranted. From a One Health perspective, awareness among veterinarians, caretakers, and conservationists is crucial. Vector control measures in livestock should be reinforced to limit spillover events between domestic animals, wildlife, and humans, particularly in the face of climate-driven tick expansion and the geographical spread of CCHFV.

Keywords

Crimean-Congo hemorrhagic fever virus, Panthera, seroprevalence, zoonosis, wildlife reservoirs, Namibia, vector-borne diseases

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Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a severe zoonotic disease caused by the Crimean-Congo hemorrhagic fever virus (CCHFV), a member of the genus *Orthonairovirus* within the family *Nairoviridae* (Hawman & Feldmann, 2023). The disease poses a significant public health threat, particularly in endemic regions of Africa, the Balkans, the Middle East, and Asia. The increasing number of reported cases in Southern Europe suggests a progressive geographic expansion of both the virus and its vectors (Hawman & Feldmann, 2023). CCHFV is primarily transmitted to humans through the bite of infected *Hyalomma* ticks, although CCHFV has been isolated from other tick genera shown to be competent vectors, including *Amblyomma*, *Dermacentor*, *Haemaphysalis*, and *Rhipicephalus* (Celina et al., 2025; Lule et al., 2022; Jafari et al., 2022). Direct contact with the blood or tissues of infected animals is a recognized source of infection. The virus's ability to replicate and transmit in various hosts is considered a key factor contributing to its wide distribution and persistence in endemic regions. Domestic animals, including cattle, sheep, and goats, are important amplification hosts (Bente et al., 2013; Hawman & Feldmann, 2023). These animals often develop viremia without showing severe clinical signs, facilitating silent circulation and underdiagnosis of the virus. Seroprevalence studies have shown high rates of CCHFV antibodies in livestock, particularly in endemic regions (Bente et al., 2013), where *Hyalomma* ticks thrive. Therefore, significant outbreaks have been reported in Nigeria, South Africa, and Sudan (Hawman & Feldmann, 2023). CCHF represents a critical public health challenge in Africa, where the World Health Organization estimates that between 10,000 and 15,000 infections occur annually, primarily affecting rural populations engaged in livestock farming (Muzammil et al., 2024). The interaction between agricultural practices, livestock management, and exposure to ticks strongly influences the transmission dynamics of CCHF (Atim et al., 2022; Bente et al., 2013; Nabeth et al., 2004), making it an example of a One Health challenge. Public health initiatives aimed at raising awareness, improving surveillance, and implementing preventive measures are essential to mitigate the impact of this disease in affected regions. In Namibia, a high seroprevalence for CCHFV has been observed, reaching 62.50% in cattle and 45.50% in sheep flocks (Samkange et al., 2024). Recent evidence also suggests that CCHFV can infect companion animal species, including domestic animals like dogs (Atim et al., 2022) and, to a lesser extent, cats (de Villiers et al., 2025). While these animals often remain asymptomatic, they may act as potential reservoirs, posing a potential zoonotic transmission risk. A study in Uganda found higher seropositivity rates among dog owners compared to non-owners, supporting the plausibility of this risk (Atim et al., 2022). Wildlife also represents a significant reservoir population since several species from different orders have been shown to be susceptible. Viral presence has been documented in members of the order *Carnivora*, including both domestic and wild species (Table I).

Order	Animal	Study
Carnivora	Genet (*Genetta genetta senegalensis*)	Chunikhin et al., 1969
	Pallas' cat (*Otocolobus manul*)	Smirnova et al., 1969
	Red fox (*Vulpes vulpes*)	Zarubinsky et al., 1975
	Dog (*Canis lupus familiaris*)	Shepherd et al., 1987
	Cat (*Felis catus*)	Shepherd et al., 1987
	Meerkat (*Suricata suricatta*)	Shepherd et al., 1987
	African wild dog (*Lycaon pictus*)	Burt et al., 1993

Table I. Carnivorous species reported positive for CCHFV in different studies.

Despite these findings, the currently available data are scarce and fragmented, as they are limited to a small number of studies and host species. The present study aimed to investigate the presence of CCHFV antibodies in Namibian leopards (*Panthera pardus*) and lions (*Panthera leo*), which are potentially exposed to infected prey and may therefore play a role in the epidemiology of CCHF. Generating such evidence is crucial to inform field workers (veterinarians, caretakers, trainers, hunters, etc.), about the associated risks and the importance of implementing appropriate hygiene precautions when handling these animals (Riccò et al., 2023).

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Matherials and methods

The blood samples were primarily collected by professional veterinarians from leopards and lions that had been trapped by farmers on private farmland. All the animals were released either back onto the farm where they were captured or onto other farms or private land that consented to receive them. Blood samples were collected during animal captures conducted across seven regions in Namibia, namely Erongo, Hardap, Karas, Khomas, Kunene, Omaheke, and Otjozondjupa. A total of 250 leopards were tested between 1997 and 2017, and seven lions were tested in 2023. Serum was separated by centrifuging blood samples at 2000 g for 10 minutes and thereafter stored at -20°C until processing. The serum was tested for the presence of CCHFV antibodies using a commercial enzyme-linked immunosorbent assay (ELISA; ID Screen® CCHF Double Antigen Multi-Species Enzyme-Linked Immunosorbent Assay, IDvet, Grabels, France) following the manufacturer's instructions. The ELISA was a double-antigen assay targeting IgM and IgG antibodies, with a declared specificity of 100.0% and a sensitivity of 98.9%. Although the ELISA was not validated for leopards and lions, the kit had previously been successfully validated in monkeys, camels, rats, ferrets, raccoon dogs, raccoons, foxes, hares, pigs, and humans (Sas et al., 2018). Detection frequency and relative confidence intervals (CI) were calculated with the Binomial Wilson score interval. The presence of temporal trends in the proportion of positive samples was assessed using the Cochran-Armitage test for trend and the Chi-square test to compare the distribution of positives across different years. For both tests, the statistical significance level was set at $p \leq 0.05$.

Results

Thirty-six out of 250 leopards (14.40%; 95% CI: 10.59-19.29) tested positive over the considered time period. A detailed year-by-year summary is provided in Table II.

Year	Tested	Positive	Seroprevalence (%)	95% CI
1997	23	5	21.74	9.66-41.90
1998	14	4	28.57	11.72-54.65
1999	20	0	0.00	0.00-16.11
2000	37	4	10.81	4.29-24.71
2001	35	5	14.29	6.26-29.38
2002	13	2	15.38	4.33-42.24
2003	16	1	6.25	1.11-28.33
2004	14	4	28.57	11.72-54.65
2005	20	4	20.00	8.07-41.60
2006	9	0	0.00	0.00-29.92
2007	10	0	0.00	0.00-27.75
2008	5	0	0.00	0.00-43.45
2009	19	4	21.05	8.51-43.33
2010	5	1	20.00	3.62-62.45
2014	2	1	50.00	9.45-90.55
2015	3	0	0.00	0.00-56.15
2016	3	1	33.33	6.15-79.23
2017	2	0	0.00	0.00-65.76
Total	**250**	**36**	**14.40**	**10.59-19.29**

Table II. Number of leopards tested and CCHFV-Positive individuals in Namibia over time, 1997–2017.

A relevant variability was observed, ranging from 0% (in 1999, 2006, 2007, 2008, 2015, and 2017) to 50% (in 2014). However, the Cochran-Armitage test did not show a significant trend over time. Similarly, the Chi-square test did not

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find statistically significant differences between the proportions of positives in the different years, both when analysed individually and when grouped in 5-year blocks.

Among the 250 leopards tested between 1997 and 2017, the location was known for 192 individuals. The sampling covered seven regions of Namibia. The majority of the samples were collected in Otjozondjupa, Khomas, Erongo, and Kunene (Table III).

Region	Tested	Positive	Seroprevalence (%)	95% CI
Erongo	16	4	25.00	10.18-49.50
Hardap	1	0	0.00	0.00-79.35
Karas	1	0	0.00	0.00-79.35
Khomas	30	2	6.67	1.85-21.32
Kunene	10	0	0.00	0.00-27.75
Omaheke	1	0	0.00	0.00-79.35
Otjozondjupa	133	23	17.29	11.81-24.61

Table III. Number of leopards tested and CCHFV-Positive individuals across different regions of Namibia.

Three out of seven lions (42.86%; 95% CI: 15.82-74.95), sampled in 2023, tested positive. None of the animals showed clinical signs of CCHF.

Discussion

Leopard plays an important role in the Namibian ecological niche, with an estimated population of 14,154 individuals in 2011 (Stein et al., 2011). Until 2011, 240 leopards were tested, and 34 tested positive. The seroprevalence was approximately 14.17%, with a 95% confidence interval of 10.32–19.14%. Considering the lower bound of the interval, this suggests that, in 2011, around 1,400 individuals may have been seropositive.

Except for a serological study conducted in livestock in the Omaheke region (Samkange et al., 2024) and in dogs and cats in 8 regions (de Villiers et al., 2025), no information is available about CCHF infection in other species in Namibia. The study of de Villiers and colleagues, performed on dogs and cats in 2022, covered the same regions investigated in the present study, with the exception of the Kavango East region. The data, in Table IV, reveal a different distribution and prevalence (de Villiers et al., 2025).

Region	Seroprevalence in leopards (%)	Seroprevalence in dogs (%)(De Villiers et al., 2025)	Seroprevalence in cats (%)(De Villiers et al., 2025)
Erongo	25.00	0.00	0.00
Hardap	0.00	38.30	8.82
Karas	0.00	2.38	0.00
Khomas	6.67	10.00	0.00
Kunene	0.00	29.79	12.50
Omaheke	0.00	6.38	0.00
Otjozondjupa	17.29	4.25	0.00

Table IV. Comparison of CCHFV seroprevalence among leopards, dogs, and cats across different regions of Namibia.

Interestingly, regions like Hardap and Kunene, where a high prevalence was observed in companion animals, were characterized by a seronegative leopard population, while the opposite was observed in Erongo. This evidence, although potentially affected by the limited sample size in certain regions, may suggest the influence of different risk

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factors. These findings also support the hypothesis that virus circulation in domestic and wild populations may follow distinct ecological and epidemiological patterns. Nevertheless, the combination of both studies contributes to a broader understanding of CCHFV circulation in Namibia (Figure 1).

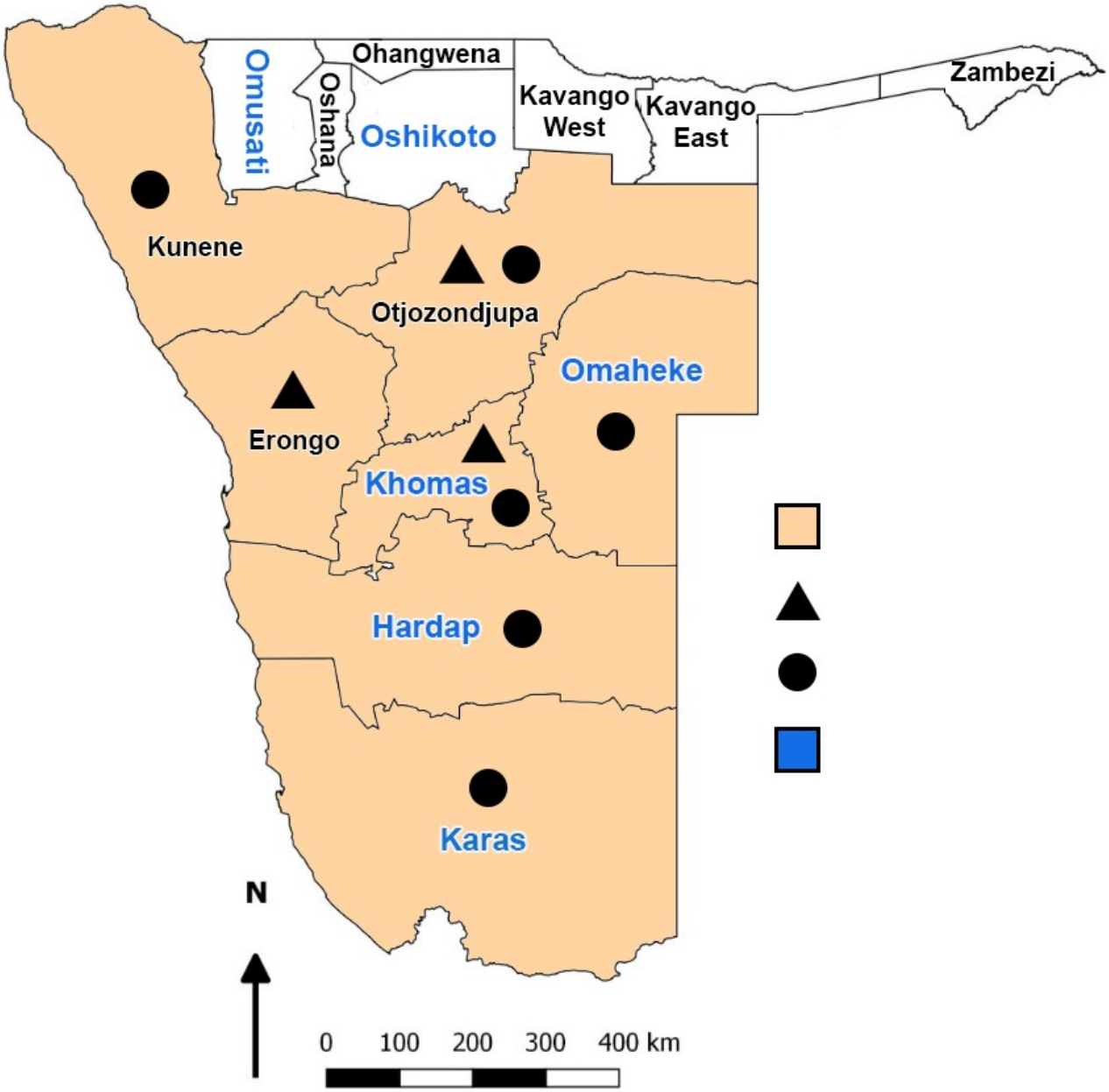

Figure 1. Map of Namibia showing the regions from which samples were collected. Triangles indicate locations where leopard-positive cases were detected. Circles indicate regions where positive domestic animal cases were found (de Villiers et al., 2025). The name of the regions where human cases had been reported are colored in blue (Ministry of Health and Social Services, 2023; de Villiers et al., 2025; ProMED).

As for human cases, several have been recorded in Namibia from 2016 to the present, particularly in the regions of Omaheke, Karas, Omusati, and Oshikoto. However, the latter two were not included in our study (Ministry of Health & Social Services, 2023). Previously, in 2002 and 2010, confirmed cases were reported in Khomas (ProMED). In the study of de Villiers, cases were also found in the Hardap region (de Villiers et al., 2025). For Omaheke, Hardap, Karas and Kunene regions the data are too limited to state that the virus is not circulating among leopards or more generally in wildlife. Interestingly, the Otjozondjupa region, involved in the circulation of the virus in both wildlife and domestic animals, has not recorded cases of CCHF in humans so far.

While the detection of CCHFV antibodies in several wildlife species is well-documented; the infection of felids of the

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Panthera genus has never been previously reported. Thus the present findings provide evidence that both leopards and lions can be exposed to CCHFV infection, potentially expanding the known host range of the virus. Unfortunately, the level of viremia or its duration could not be investigated, as the serological survey only allows the assessment of prior exposure. Therefore, whether leopards and lions in Namibia serve as reservoirs for viral propagation or are dead-end hosts remains an open question. Molecular testing could provide valuable information. However, studies conducted on sheep have shown that viremia may last up to nine days (Gonzales et al., 1998). Therefore, given this short timeframe, detecting positive individuals is unlikely in the absence of clinical signs. Conversely, the development of CCHFV antibodies occurs promptly, at least in sheep, with IgM appearing 6–7 days after experimental infection and IgG the following day (Gonzales et al., 1998; Wilson et al., 1991). The serological assay implemented in this study, despite the aforementioned limitations, remains a valuable tool for monitoring CCHFV circulation and exposure risk, especially given that viral shedding may occur even in the presence of antibodies. Current evidence further underscores the need for field workers (e.g., veterinarians, zookeepers) to exercise caution during their professional activities. Since tick infestations represent a significant risk of CCHFV infection, vector control measures in livestock and wild ruminants are essential to reduce infections that can spread from wildlife to domestic animals and, consequently, to humans (Bente et al., 2013). The absence of clinical signs in all tested animals suggests past exposure with a favourable outcome. However, the clinical relevance of this infection cannot be excluded, as fatal cases would have inevitably been missed by a serological investigation alone. Therefore, further surveillance studies are necessary to obtain virological evidence, including investigations of carcasses from deceased animals.

Conclusion

The present study results suggest that additional wildlife species may undergo seroconversion following exposure to CCHFV. While their specific role in the epidemiology of CCHFV in Namibia remains uncertain, it merits further investigation. Although the direct contacts among wild felids and humans are limited, these populations might represent a potential risk for other wild and domestic populations, potentially contributing to tick and virus maintenance. In contrast to wild ruminants, predator species may acquire infection not only via vector-borne transmission but also through direct contact with infected prey. Therefore, they could serve as valuable sentinels to indirectly monitor infection trends, especially in wildlife populations.

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Ethical approval

Ethical approval was not required for the studies involving animals in accordance with the local legislation and institutional requirements because sampled material originated from the archived sample biobanks.

Conflict of interest

The authors declare that they have no conflict of interest.

Author Contributions

Conceptualization: Umberto Molini; Methodology: Giovanni Franzo; Formal analysis: Umberto Molini, Karen Codling, Mark Jago, Leandra van Zyl, Siegfried Khaiseb, Giovanni Franzo; Investigation: Frank Busch; Writing original draft preparation: Umberto Molini, Giovanni Franzo; Writing, review and editing: Gloria Plebani, Siegfried Khaiseb, Frank Busch, Klaas Dietze, Sascha Knauf, Tetyana Petrova; Supervision: Sascha Knauf; Funding acquisition: Klaas Dietze.

All authors have read and agreed to the published version of the manuscript.

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