



Exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the endangered Iberian lynx (*Lynx pardinus*)

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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an emerging zoonotic virus of public and animal health concern, of which felids have been suggested as potential reservoirs. Although SARS-CoV-2 exposure has been detected in domestic and wild captive animals belonging to *Felidae* family, surveillance has not been carried out in free-ranging wild felids so far. The aim of the present study was to assess SARS-CoV-2 exposure in the Iberian lynx (*Lynx pardinus*), the most endangered felid in the world. Between 2019 and 2022, we conducted a seroepidemiological study of SARS-CoV-2 in 276 free-ranging and captive Iberian lynxes. Our results evidenced limited (0.4%; 95%CI: 0.0–1.1) but not negligible exposure to this emerging virus in this endangered felid species, increasing the SARS-CoV-2 host range. The circulation of this virus in wildlife evidences the need of integrated European wildlife monitoring.

1. Introduction

The high transmissibility of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) among humans allowed its rapid global distribution, representing a threat not only for public but also for

animal health (WHO, World Health Organization, 2022). During the last few years, the susceptibility to SARS-CoV-2 infection has been confirmed in an increasing number of domestic and wild animal species, of which felids have been suggested as potential reservoirs for this virus (Qiu et al., 2023). Of note, the affinity of SARS-CoV-2 to the ACE2, the

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main host cell receptor for the virus, of these species agree with the reports of natural SARS-CoV-2 transmission among big cats and clinical cases and/or pathological disorders due to viral infection evidenced in free-ranging and captive felid species, including Eurasian lynx (*Lynx lynx*) and Canada lynx (*Lynx canadensis*) (Grome et al., 2022; EFSA Panel on Animal Health and Welfare AHAW et al., 2023; Qiu et al., 2023). The Iberian lynx (*Lynx pardinus*) remains the most endangered felid species in the world and one of the most threatened carnivores in Europe (IUCN, International Union for Conservation of Nature's, 2022). Since 2000, when the population of this species was around 100 free-ranging individuals, *in situ* and *ex situ* conservation programs were launched, including captive breeding, reintroduction actions and health surveillance programs. Although the census has risen during the last two decades to over 1600 in 2022 (MITECO, 2022), the monitoring of pathogens that could be a threat for either or both free-ranging and captive Iberian lynx populations is still a key point in the conservation of this feline. Nevertheless, whether this endangered species has been in contact with SARS-CoV-2 is still unknown. Here, we aimed to assess SARS-CoV-2 exposure in free-ranging and captive populations of the Iberian lynx.

2. Material and methods

Routine health monitoring of the Iberian lynx population includes health evaluations of living individuals to perform hematology, biochemistry, proteinogram (García et al., 2010; Pastor et al., 2009) as well as serological and molecular analyses of the recognized as main pathogens for this species (Nájera et al., 2021). For this study, we analyzed serum samples taken from 276 animals within this routine health monitoring across the Iberian Peninsula between October 2019 and February 2023 (Table 1). Of them, 218 were free-ranging lynxes from the three major distribution areas of this species (central, south, and southwest Spain) (Fig. 1), and 58 were captive animals from breeding (BC1–BC3) and centers for the recovery of threatened species (CC1–CC3) within the Iberian lynx *ex situ* conservation program (Fig. 1). In addition, eleven of these 276 Iberian lynxes were longitudinally sampled (two to three samplings per animal) during the study period. During follow-up, the median (Q1–Q3) interval between consecutive samplings was 10 months (2–19).

The presence of antibodies against the Receptor Binding Domain (RBD) (Wuhan SARS-CoV-2 strain) and the nucleoprotein of SARS-CoV-2 was determined using two commercial multispecies ELISAs: NeutralISA® SARS-CoV-2 (Euroimmun, Germany) (ELISA-1) and ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA (IDvet, Grabels, France) (ELISA-2), respectively, following the manufacturer's instructions. Positive samples by either of these two ELISAs were tested by seroneutralization assays (SNT), as previously described by Fernández-Bellón et al. (2021). Sera with titers $\geq 1/20$ were considered positive. Only samples that tested positive to either of the two ELISAs

analyzed and to SNT were considered positive to anti-SARS-CoV-2 antibodies.

3. Results and discussion

None of the Iberian lynx sera tested positive to ELISA-1 whereas sixteen individuals showed seropositivity by ELISA-2. Due to hemolysis or the limited volume available, only three ELISA-2-positive sera could be tested using SNT. One (0.4%; 95%CI: 0.0–1.1) sample analyzed by SNT was positive with high titres of neutralizing antibodies against SARS-CoV-2 (SNT₅₀: 518.1). The positive Iberian lynx to SNT was a free-ranging female sampled in December 2021 in a periurban area of southern Spain. This animal had no clinical signs compatible with infection by SARS-CoV-2. Of note, this animal tested positive for ELISA-2 but not for ELISA-1. A possible explanation for this result could be the high cut-off of the ELISA-1 in comparison with similar assays, which could lead to a loss of sensitivity, as previously described by Hofmann et al. (2022). Seropositivity to SARS-CoV-2 was not detected in any of the eleven longitudinally surveyed animals.

Although it was not possible to perform an alternative diagnostic technique to SNT to validate most of the ELISA-positive sera, the seropositivity found in an Iberian lynx confirms circulation of SARS-CoV-2 among free-ranging wildlife in the Iberian Peninsula. In this European region, previous studies detected viral RNA in other wild species, including feral American mink (*Neovison vison*) and Eurasian otter (*Lutra lutra*) (Aguiló-Gisbert et al., 2021; Padilla-Blanco et al., 2022), and anti-SARS-CoV-2 antibodies have been found in red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) populations (Encinas et al., 2023). Our results highlight the need of continued and integrated wildlife disease monitoring to prevent the spread of the virus in animal populations, the potential emergence of new variants and the possible spill-back to humans (WHO, World Health Organization, 2022). As examples, divergent lineages of SARS-CoV-2 in farmed mink and free-ranging white-tailed deer (*Odocoileus virginianus*) as well as zoonanthroponosis transmission events from these wild species have already been reported (Munnink et al., 2021; Pickering et al., 2022).

Contact with human being, white-tailed deer, Malayan tiger (*Panthera tigris jacksoni*) and American mink, has been evidenced as a source of SARS-CoV-2 transmission in wildlife (EFSA Panel on Animal Health and Welfare AHAW et al., 2023; Grome et al., 2022). Human-to-animal direct or indirect transmission have also been reported in previous studies conducted in captive and free-ranging wildlife (EFSA Panel on Animal Health and Welfare AHAW et al., 2023). In this context, the Iberian lynx is subjected to different *in situ* and *ex situ* conservation programs, in which close contact with humans does occur due to medical checkups, health surveillance programs or reintroduction actions (MITECO, 2022; Rivas, 2016). The seropositive animal was handled in April and May 2020. Although none of the field staff showed symptoms of COVID-19 and all followed strict biosecurity measures, including the use of face masks, during sampling, human-to-animal transmission cannot be ruled out. On the other hand, stray cats are frequent in the distribution area of the seropositive lynx (Lázaro et al., 2023) and both contacts and pathogen transmission between stray cats and the Iberian lynx are common (López et al., 2009; Nájera et al., 2019). The consumption of infected animals has also been suggested as a potential source of SARS-CoV-2 to wildlife. In this respect, the Iberian lynx has showed opportunistic scavenging behavior feeding on wild ruminant carcasses, such as red deer and fallow deer (González et al., 2023; Tobajas et al., 2023), and circulation of SARS-CoV-2 has been detected in these deer species from areas where the Iberian lynx is distributed (Encinas et al., 2023). Nevertheless, further studies are needed to elucidate the sources of SARS-CoV-2 infection in this endangered species.

Table 1
Distribution of the animals by categories.

Variable	Categories	No. of samples*	Relative frequency (%)
Age	Yearling	89	35.9
	Subadult	69	27.8
	Adult	73	29.4
	Senile	17	6.8
Sex	Female	116	42.8
	Male	155	57.2
Habitat status	Captivity	58	21.0
	Free ranging	218	79.0
Sampling year	2019	27	9.8
	2020	60	21.7
	2021	105	38.0
	2022	75	27.2
	2023	9	3.3

* Missing values excluded

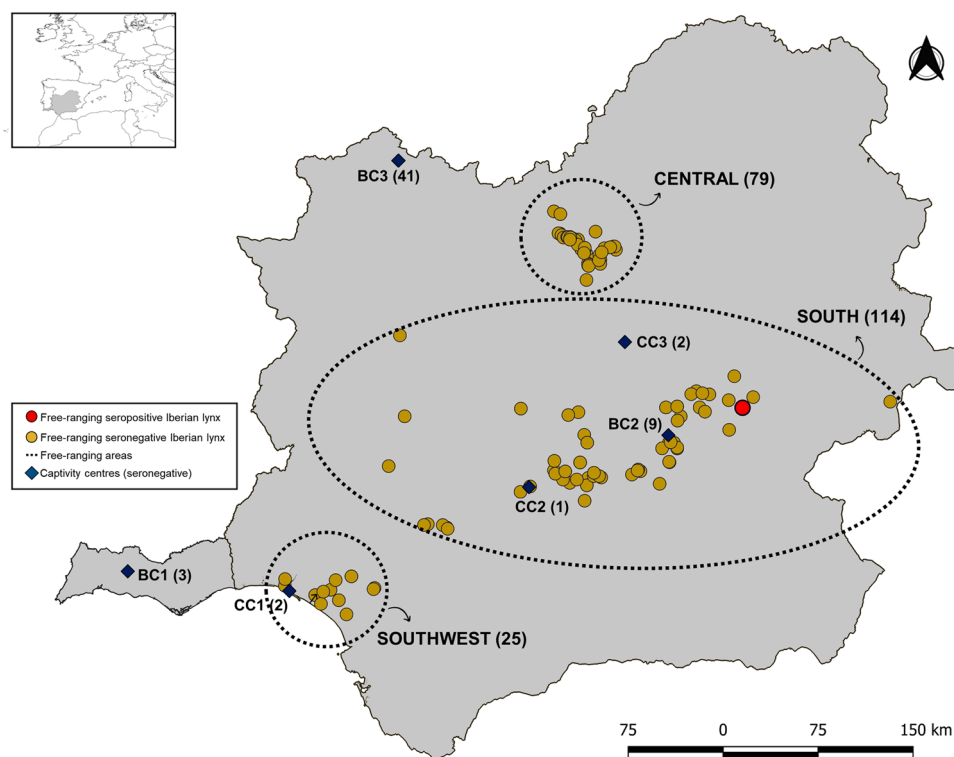


Fig. 1. Geographical distribution of Iberian lynxes sampled in the Iberian Peninsula. Total number of animals analyzed in each free-range area and captivity center are shown in parentheses. The abbreviations 'BC' and 'CC' refer to breeding centers and conservation centers, respectively.

4. Conclusions

We found a limited but not negligible exposure to SARS-CoV-2 in the endangered Iberian lynx. The detection of anti- SARS-CoV-2 antibodies in this species increases the range of animal species susceptible to this emerging virus. Our results suggest a limited risk of transmission from this endangered felid to other sympatric species and *vice versa* so far. However, surveillance programs should be implemented in sympatric wild and domestic species to assess SARS-CoV-2 circulation in Iberian Mediterranean ecosystems.

Ethical statement

This study did not involve purposeful killing of animals. Samples included in this study were taken from serum banks or from animals subjected to medical check-ups, health programs or surgical interventions during the study period. Samples from Iberian lynx were collected by authorized veterinarians and animal keepers following routine procedures with alive and dead individuals before the design of this study, in compliance with the Ethical Principles in Animal Research. Thus, ethical approval by an Institutional Animal Care and Use Committee was not deemed necessary.

CRediT authorship contribution statement

del Rey Teresa: Data curation, Methodology, Resources, Writing – review & editing. **Zorrilla Irene:** Data curation, Methodology, Resources, Supervision, Writing – review & editing. **González Moisés:** Formal analysis, Investigation, Software, Writing – review & editing. **Paniagua Jorge:** Formal analysis, Supervision, Validation, Visualization, Writing – review & editing. **García-Bocanegra Ignacio:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing, Project administration. **Cano-Terriza David:** Conceptualization, Investigation, Resources, Supervision,

Writing – review & editing, Project administration. **Salcedo Javier:** Resources, Validation, Visualization, Writing – review & editing. **Caballero-Gómez Javier:** Data curation, Investigation, Writing – original draft, Writing – review & editing, Formal analysis. **Vergara-Alert Júlia:** Methodology, Resources, Supervision, Validation, Writing – review & editing. **Segalés Joaquim:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **Fernández-Bastit Leira:** Investigation, Methodology, Visualization, Writing – review & editing. **Montoya-Oliver Juan I.:** Resources, Supervision, Validation, Writing – review & editing. **Nájera Fernando:** Investigation, Resources, Supervision, Writing – review & editing.

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.

Data Availability

The data that support the findings of this study are available from the authors upon reasonable request.

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