Investigations into the potential role of free-ranging wildlife as a reservoir of SARS-CoV-2 in Switzerland

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Aim of the study
The objective of this study was to determine whether in the course of the pandemic SARS-CoV-2 spread to free-ranging Swiss wildlife, focusing on species known to be susceptible or living in urban areas. Our research aimed to explore the possibility of SARS-CoV-2 spillovers in the human-domestic-wildlife interface in Switzerland.

Material and methods
Between 2020 and 2023, the Institute for Fish and Wildlife Health (FIWI) conducted postmortem examinations on 176 animals (wild felids, canids, and mustelids), collecting samples such as oronasal swabs (n=165), rectal swabs (n=163), lung tissue (n=160), and blood (n=168). An additional 535 animals, including red foxes, European pine and stone martens, European badgers, and European polecats, had blood samples collected by local partners like hunting authorities and wildlife rescue centers in 23 Swiss cantons and Liechtenstein. FIWI-veterinarians involved in Eurasian lynx and European wildcat captures collected an additional 46 blood samples, 44 oropharyngeal swabs, and 44 rectal swabs from live animals. In total, 748 blood samples underwent serological analyses, while molecular analyses included oronasal, oropharyngeal, and rectal swabs, as well as lung tissue if available, from 211 animals.
Serological analyses were conducted at the Clinical Laboratory (Vetsuisse Faculty, UZH) to evaluate the presence of antibodies in the blood samples indicating prior exposure to SARS-CoV-2. Enzyme-linked immunosorbent assay (ELISA) tests using specific viral antigens (RBD, S1, Omicron S1) were used to detect binding antibodies. The validation of the detection of binding antibodies was conducted through indirect immunofluorescence tests (iIFT) at the Friedrich-Loeffler-Institut (FLI). Surrogate Virus Neutralization Test (sVNT) and Pseudotype-Based Virus Neutralization Assay (PVNA) were performed at the UZH and the University of Glasgow, respectively, to assess neutralizing activity against SARS-CoV-2 in collected samples. The PVNA tests allowed the testing of neutralizing antibodies against various variants of SARS-CoV-2. Viral RNA detection was performed using SARS-CoV-2 RT-qPCR assays at the UZH.

Results and significance

Among the 446 fox blood samples (targeted number: 500), 69 animals tested seroreactive in at least one of the ELISA tests (15.5% suspect positive; 95% CI of 12.4% to 19.1%). Of these, a total of 13 samples were confirmed through iIFT, sVNT or PVNA, showing a prevalence of confirmed infected animals of 2.9% (with a 95% CI of 1.7% to 4.9%).

In the case of lynx, out of 92 blood samples (targeted number: 70), 3 samples tested seroreactive in at least one ELISA test, with 2 confirmed through the IIFT, showing a prevalence of 2.2% (with a 95% CI of 0.6% to 7.6%). Among these two confirmed lynx, one lynx sample was seroreactive in sVNT, indicating the presence of neutralizing antibodies. We targeted to include 400 samples from mustelids, however, received only 147 due to low submission rates. One polecat (1/10) and one stone marten (1/51) were tested seroreactive in one ELISA but could not be confirmed by IIFT or by sVNT (suspect positive). Furthermore, six wildcats (6/24) and three wolves (3/41) were tested seroreactive in one ELISA but could not be confirmed by IIFT or by sVNT (suspect positive). Owing to the protracted CITES approval timeline and the complex customs clearance procedures for samples of protected species in the UK, some PVNA test results are still pending.

No viral RNA was detected in any of the samples tested by RT-qPCR (N=211), including 209 oronasal swabs, 206 rectal swabs and 161 lung tissue samples.

In conclusion, no evidence of active infection (viral RNA) was detected in any of the animals tested at the timepoint of sampling. The serological analysis confirmed past infections in approximately 2% to 3% of free-ranging Swiss red foxes and Eurasian lynx. The investigation into potential neutralizing activity against different variants is in progress (PVNA, University of Glasgow).

There are no discernible geographical or temporal patterns, suggesting multiple sporadic spillover events. Factors such as proximity to human settlements, contact with human waste, feeding behavior and shared habitats with domestic species susceptible for the infection - such as domestic cats and dogs - are potential influencers on the transmission pathways. This underscores the importance of monitoring infection transmission and dynamics using a One Health approach and respecting protective measures when managing wildlife.

Presentations, poster, publication, and public relations work

Presentations:

- “Studies on the potential role of wildlife as a reservoir of SARS-CoV-2 in Switzerland” GEEFSM 2022, Cordoba
- “SARS-CoV-2 in Swiss wildlife” Science@Lunch 2022, Vetsuisse Faculty, Bern
- “Investigations into the potential role of wild animals as a reservoir of SARS-CoV-2 in Switzerland” Wildlife Disease Association Conference 2023, Athens
- “Investigations into the potential role of wild animals as a reservoir of SARS-CoV-2 in Switzerland” Fachtagung für Biotechnologie BAFU 2023, Bern

Poster:

- “Investigations into the potential role of wild animals as a reservoir of SARS-CoV-2 in Switzerland”, DIP Retreat 2021, Münchenwiler

Publication/doctoral thesis (Juliette Kuhn) in preparation:

- “Investigations into the potential role of wild animals as a reservoir of SARS-CoV-2 in Switzerland”

Public relations work:

- ''Jetzt werden Wildtiere auf Corona getestet'' NZZ am Sonntag, 05.12.2021
- ''Le Covid est traqué dans la faune suisse'' Tribune de Genève, 09/12/2021

- ''Les facultés vétérinaires de Berne et Zürich testent l'exposition des animaux sauvages au coronavirus'' RTS, 12.12.2021
- ''Omikron-Maus: Beunruhigende Entdeckung im Labor'' SRF, 02.02.2022
- ''Das Wild wird auf Corona getestet'' Südostschweiz (Graubünden), 04.02.2022

Project 1.21.15

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