European Journal of Wildlife Research (2023) 69:33
https://doi.org/10.1007/s10344-023-01662-6

SHORT COMMUNICATION

Lack of SARS-CoV-2 RNA evidence in the lungs from wild European polecats (*Mustela putorius*) from Spain

Guillermo Carmona[1] · Tamara Burgos[2] · Rafael Barrientos[1] · Sara Martin-Garcia[2] · Clara Muñoz[3,4] · Marta Sánchez-Sánchez[3] · Javier Hernández-Hernández[1] · Carlos Palacín[5] · Pablo Quiles[1] · Alberto Moraga-Fernández[3] · Victor Bandeira[6] · Emilio Virgós[2] · Christian Gortázar[3] · Isabel G. Fernandez de Mera[3]

Received: 7 July 2022 / Revised: 22 February 2023 / Accepted: 2 March 2023 / Published online: 11 March 2023

Abstract

Data on SARS-CoV-2 infection in wildlife species is limited. The high prevalences found in mustelid species such as free-ranging American minks (*Neovison vison*) and domestic ferrets (*Mustela putorius furo*) justify the study of this virus in the closely related autochthonous free-ranging European polecat (*Mustela putorius*). We analysed lung samples from 48 roadkilled polecats collected when the human infection reached its highest levels in Spain (2020–2021). We did not detect infections by SARS-CoV-2; however, surveillance in wild carnivores and particularly in mustelids is still warranted, due to their susceptibility to this virus.

Keywords SARS-CoV-2 · European polecat · Coronavirus disease

Introduction

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) probably originated in bats and spread to humans, via a still unknown bridge host, caused more than 6.6 million deaths by January 2023 (Delahay et al. 2021; World Health Organization 2023). It is known that some human-associated mammals can be infected, such as dogs, cats (Sit et al. 2020; Newman et al. 2020), and hamsters (Hobbs and Reid 2021). Also, this virus has been isolated in apes and big cats living in zoos (Gillespie and Leendertz 2020; Wang et al. 2020), as well as in captive-bred carnivores in fur farms, like the American mink (*Neovison vison*) (Boklund et al. 2021). Experimental studies have demonstrated SARS-CoV-2 infection and transmission in other species like fruit bats (*Rousettus aegyptiacus*), deer mice (*Peromyscus maniculatus*) and tree shrews (*Tupaia belangeri*) (Hobbs and Reid 2021; Fagre et al. 2021; Mastutik et al. 2022). North American white-tailed deer (*Odocoileus virginianus*) may acquire SARS-CoV-2 and is the first wildlife species known to maintain the infection (Chandler et al. 2021). However, data on SARS-CoV-2 infection in many other free-living wildlife species is still scarce (Sharun et al. 2021; Delahay et al. 2021). Mustelids seem one of the most susceptible families, especially in those cases where animals live in close proximity groups and in contact with humans (e.g. American mink farms) (Delahay et al. 2021). Ferrets (*Mustela putorius furo*) may acquire the virus (Kim et al. 2020; Shi et al. 2020; Hobbs and Reid 2021), and natural infection has been recorded both in pet ferrets (Giner et al. 2021) and in hunting ones (Gortázar et al. 2021). Moreover, SARS-CoV-2 infection has also been detected in exotic free-ranging American

✉ Guillermo Carmona
guillecarmonacastresana@gmail.com

1 Road Ecology Lab, Department of Biodiversity, Ecology and Evolution, Faculty of Biology, Complutense University of Madrid, Madrid, Spain

2 Department of Biology and Geology, King Juan Carlos University, Madrid, Spain

3 Grupo SaBio, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM), Ciudad Real, Spain

4 Department of Animal Health, Faculty of Veterinary Sciences, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Murcia, Spain

5 Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain

6 Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal

mink populations (Aguiló-Gisbert et al. 2021). The virus has also been recorded in other native wild mustelids such as the Eurasian otter (*Lutra lutra*) (Padilla-Blanco et al. 2022). In the cases of domestic (ferrets) or farm individuals (American minks), it is thought that transmission occurs from person to animal, although the reverse is also likely to be true (Boklund et al. 2021). In wild conditions, transmission is more improbable due to the lower population densities and scarce contact with humans. Even so, it is possible that infected American minks can escape to the wild where they could spread the disease to native species. Some animals (i.e. hunting ferrets) could transmit the disease to free-living animals when employed by humans.

The aim of our work was to search SARS-CoV-2 infections in autochthonous free-ranging European polecats (*Mustela putorius*). This is a worth studying species for several reasons: (i) they are the wild ancestor of the ferret, so both species could show similar susceptibilities to infection by SARS-CoV-2; (ii) it usually forages on rabbits in their burrows (e.g., Barrientos and Bolonio 2009); thus, it could be in contact with hunting ferrets; and finally, (iii) interactions among human-origin individuals from species established in the wild such as American mink or ferrets and wild mustelid species like polecat are frequent (Costa et al. 2013; Barrientos 2015).

Methods

We analysed lung samples from 48 roadkilled polecats collected in the period 2020–2021 in seven Spanish provinces: Ávila (2 individuals), Burgos (4), Jaén (8), Madrid (13), Palencia (1), Toledo (7) and Valladolid (13). Forty-one of them were fresh animals (less than 24–48 h since dead), and seven were in varying degrees of rot. Once collected, they were stored in sealed labelled bags and carried to a freezer. The carcasses were kept at − 20 °C until necropsy. Sample extraction was performed, while the animal was still frozen to avoid RNA degradation. Once extracted, the lungs were refrozen at − 20 °C and stored in zip bags. For each animal, viral RNA was extracted from different lung lobes using the NucleoSpin RNA Virus kit (Macherey–Nagel, Düren, Germany) following the manufacturer's instructions. Molecular detection of SARS-CoV-2 RNA was performed by real-time RT-PCR using the FDA EUA 2019-nCoV CDC kit (IDT, Belgium), based on N1 and N2 gene targets to detect SARS-CoV-2. Real-time RT-PCR was carried out using the SuperScript III Platinum One-Step qRT-PCR Kit (Thermo Fisher, MA, USA), following the manufacturer's protocol. Final volume of RT-PCR reaction was 15 µl including 5 µl of RNA extracted. Nuclease-free water was used as negative control and a synthetic SARS-CoV-2 control (IDT, Belgium) as positive control. PCR reactions were carried out in a CFX96 Touch Real-Time PCR Detection System Thermal Cycler (BioRad, Berkeley, USA).

Results and discussion

We did not detect infections by SARS-CoV-2 in the lungs of 48 wild European polecats collected. While sample size was necessarily limited, it represents the largest polecat sample tested so far for SARS-CoV-2 RNA (EFSA et al. 2021; Davoust et al. 2022). As mutually non-exclusive explanations, we suggest that (i) the jump from humans to free-living European polecats is unlikely (Delahay et al. 2021); (ii) to date, SARS-CoV-2 in free-ranging carnivores shows low prevalence (see Aguiló-Gisbert et al. 2021; Padilla-Blanco et al. 2022), which makes the infection by other coexisting species difficult; (iii) the solitary habits of our study species (Lodé 1996; Marcelli et al. 2003) reduce intraspecific encounters and, consequently, potential infections. On the other hand, we are aware that the quality of the analysed samples was not the optimal to detect viral RNA, since the ideal procedure is to preserve samples at − 80 °C to avoid RNA degradation and to avoid repeated freeze–thaw cycles. In addition, sampled individuals were found road-killed, although in most cases they were fresh animals. Also, we acknowledge that we did not sample other, less common tissues like small intestine from where SARS-CoV-2 could also be isolated or blood for antibody detection.

However, our negative results do not discard the possibility of polecat and/or other carnivore mustelid species and populations to be affected and/or potentially act as reservoirs. Consequently, SARS-CoV-2 surveillance is still necessary in carnivores, and particularly in mustelids, due to their known susceptibility.

References

Aguiló-Gisbert J, Padilla-Blanco M, Lizana V, Maiques E, Muñoz-Baquero M, Chillida-Martínez E, Cardells J (2021) Rubio-Guerri C (2021) First description of SARS-CoV-2 infection in two feral American mink (*Neovison vison*) caught in the wild. Animals 11:1422. https://doi.org/10.3390/ani11051422

Barrientos R, Bolonio L (2009) The presence of rabbits adjacent to road increases polecat road mortality. Biodivers Conserv 18:405–418. https://doi.org/10.1007/s10531-008-9499-9

Barrientos R (2015) Adult sex-ratio distortion in the native European polecat is related to the expansion of the invasive American mink. Biol Cons 186:28–34. https://doi.org/10.1016/j.biocon.2015.02.030

Boklund A, Gortazar C, Pasquali P, Roberts H, Nielsen SS, Stahl K, Stegeman A, Baldinelli F, Broglia A, Van Der Stede Y, Adlhoch C, Alm E, Melidou A, Mirinaviciute G (2021) European Food Safety Authority and European Centre for Disease Prevention and Control

Scientific Opinion on the monitoring of SARS-CoV-2 infection in mustelids. EFSA 19:645. https://doi.org/10.2903/j.efsa.2021.6459
Chandler JC, Bevins SN, Ellis JW, Linder TJ, Tell RM, Jenkins-Moore M, Shriner SA (2021) SARS-CoV-2 exposure in wild white-tailed deer (Odocoileus virginianus). Proc Natl Acad Sci 118. https://doi.org/10.1073/pnas.2114828118
Costa M, Fernandes C, Birks JDS, Kitchener AC, Santos-Reis M, Bruford MW (2013) The genetic legacy of the 19th-century decline of the British polecat: evidence for extensive introgression from feral ferrets. Mol Ecol 22:5130–5147. https://doi.org/10.1111/mec.12456
Davoust B, Guérin P, Orain N, Fligny C, Flirden F, Fenollar F, Edouard S (2022) Evidence of antibodies against SARS-CoV-2 in wild mustelids from Brittany (France). bioRxiv. Preprint. https://doi.org/10.1101/2022.01.20.477038
Delahay RJ, de la Fuente J, Smith GC, Sharun K, Snary EL, Flores Girón L, Nziza J, Fooks AR, Brookes SM, Lean FZX, Gortazar C (2021) Assessing the risks of SARS-CoV-2 in wildlife. One Health Outlook 3:1–14. https://doi.org/10.1186/s42522-021-00039-6
European Food Safety Authority and European Centre for Disease Prevention and Control, Boklund A, Gortázar C, Pasquali P, Roberts H, Nielsen SS, Mirinaviciute G (2021) Monitoring of SARS-CoV-2 infection in mustelids. EFSA 19. https://doi.org/10.2903/j.efsa.2021.6459
Fagre A, Lewis J, Eckley M, Zhan S, Rocha SM, Sexton NR, Schountz T (2021) SARS-CoV-2 infection, neuropathogenesis and transmission among deer mice: Implications for spillback to New World rodents. PLoS Pathogens 17. https://doi.org/10.1371/journal.ppat.1009585
Gillespie TR, Leendertz FH (2020) COVID-19: protect great apes during human pandemics. Nature 579(7800):497. https://doi.org/10.1038/d41586-020-00859-y. PMID: 32210385
Giner J, Villanueva-Saz S, Tobajas AP, Pérez MD, González A, Verde M, Yzuel A, García-García A, Taleb V, Lira-Navarrete E, Hurtado-Guerrero R, Pardo J, Santiago L, Paño JR, Ruíz H, Lacasta D, Fernández A (2021) SARS-CoV-2 seroprevalence in household domestic ferrets (*Mustela putorius furo*). Animals 11:667. https://doi.org/10.3390/ani11030667
Gortázar C, Barroso-Arévalo S, Ferreras-Colino E, Isla J, Fuente G, Rivera B, Domínguez Rodríguez L, de la Fuente J, Sánchez-Vizcaíno J (2021) Natural SARS-CoV-2 infection in kept ferrets. Spain Emerg Infect Dis 27:1994–1996. https://doi.org/10.3201/eid2707.210096
Hobbs EC, Reid TJ (2021) Animals and SARS-CoV-2: Species susceptibility and viral transmission in experimental and natural conditions, and the potential implications for community transmission. Transbound Emerg Dis 68:1850–1867. https://doi.org/10.1111/tbed.13885
Kim YI, Kim SG, Kim SM, Kim EH, Park SJ, Yu KM, Chang JH, Kim EJ, Lee S, Casel MAB, Um J, Song MS, Jeong HW, Lai VD, Kim Y, Chin BS, Park JS, Chung KH, Foo SS, Choi YK (2020) Infection and rapid transmission of SARSCoV-2 in ferrets. Cell Host Microbe 27
Lodé T (1996) Conspecific tolerance and sexual segregation in the use of space and habitats in the European polecat. Acta Theriol 41:171–176
Marcelli M, Fusillo R, Boitani L (2003) Sexual segregation in the activity patterns of European polecats (*Mustela putorius*). J Zool 261:249–255. https://doi.org/10.1017/S0952836903004151
Mastutik G, Rohman A, I'tishom R, Ruiz-Arrondo I, de Blas I (2022) Experimental and natural infections of severe acute respiratory syndrome-related coronavirus 2 in pets and wild and farm animals. Vet World 15:565. https://doi.org/10.14202/vetworld.2022.565-589
Newman A, Smith D, Ghai RR, Wallace RM, Torchetti MK, Loiacono C, Behravesh CB (2020) First reported cases of SARS-CoV-2 infection in companion animals—New York, March–April 2020. Morb Mortal Wkly Rep 69:710. https://doi.org/10.15585/mmwr.mm6923e3
Padilla-Blanco M, Aguiló-Gisbert J, Rubio V, Lizana V, Chillida-Martínez E, Cardells J, Maiques E, Rubio-Guerri C (2022) The finding of the severe acute respiratory syndrome coronavirus (SARS-CoV-2) in a wild Eurasian river otter (*Lutra lutra*) highlights the need for viral surveillance in wild mustelids. Front Vet Sci 9. https://doi.org/10.3389/fvets.2022.826991
Sharun K, Dhama K, Pawde AM, Gortázar C, Tiwari R, Bonilla-Aldana DK, Attia YA (2021) SARS-CoV-2 in animals: potential for unknown reservoir hosts and public health implications. Vet Qtly 41:181–201. https://doi.org/10.1080/01652176.2021.1921311
Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, Liu R, He X, Shuai L, Sun Z, Zhao Y, Liu P, Liang L, Cui P, Wang J, Zhang X, Guan Y, Tan W, Wu G, Bu Z (2020) Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. Science eabb7015. https://doi.org/10.1126/science.abb7015
Sit TH, Brackman CJ, Ip SM, Tam KW, Law PY, To EM, Peiris M (2020) Infection of dogs with SARS-CoV-2. Nature 586:776–778. https://doi.org/10.1038/s41586-020-2334-5
Wang L, Mitchell PK, Calle PP, Bartlett SL, McAloose D, Killian ML, Torchetti MK (2020) Complete genome sequence of SARS-CoV-2 in a tiger from a US zoological collection. Microbiol Resour Announc 9:e00468–20. https://doi.org/10.1128/MRA.00468-20
World Health Organisation (2023) Coronavirus disease (COVID-19) dashboard. https://covid19.who.int/. Accessed 10 Jan 2023