New insights in the role of non-human hosts in the origin and future of SARS-CoV-2



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Coronaviruses are animal viruses



| Genus/subgenus | Notable virus species | Hosts species | | |
|------------------|--|--|--|--|
| Alphacoronavirus | | | | |
| Colacovirus | Myl-CoV | Bats (Vespertilionidae) | | |
| Pedacovirus | PEDV | Bats (Vespertilionidae), pigs | | |
| Nyctacovirus | | Bats (Vespertilionidae) | | |
| Decacovirus | | Bats (Hipposideridae, Rhinolophidae) | | |
| Minunacovirus | | Bats (Miniopteridae) | | |
| Myotacovirus | | Bats (Vespertilionidae) | | |
| Duvinacovirus | HCoV-229E | Bats (Hipposideridae), dromedary camels, alpacas, humans | | |
| Setracovirus | HCoV-NL63 | Bats (Rhinonycteridae), humans | | |
| Rhinacovirus | SADS-CoV | Bats (Rhinolophidae), pigs | | |
| Luchacovirus | | Rodents (Muridae, Cricetidae) | | |
| Minacovirus | | Ferrets, minks | | |
| Tegacovirus | CCoV, FCoV, TGEV | Cats, dogs, pigs | | |
| Soracovirus | | Shrews (Suncus murinus) | | |
| Sunacovirus | | Shrews (Sorex araneus) | | |
| Betacoronavirus | | | | |
| Hibecovirus | | Bats (Hipposideridae) | | |
| Sarbecovirus | SARS-CoV, SARS-CoV-2 | Bats (Rhinolophidae), Malayan pangolins, carnivores (Canidae, Felidae, Mustelidae, Viverridae), humans | | |
| Nobecovirus | | Bats (Pteropodidae) | | |
| Merbecovirus | MERS-CoV | Bats (Vespertilionidae), dromedary camels, humans | | |
| Embecovirus | BCoV, CRCoV, HCoV-OC43, HCoV-HKU1, MCoV | Rodents (Muridae, Cricetidae), dogs, rabbits, cattle, horses, pigs, sable antelopes, dromedary camels, giraffes, humans | | |
| Deltacoronavirus | PorCoV-HKU15 | Birds, pigs | | |
| Gammacoronavirus | IBV | Birds, cetaceans | | |



Ruiz-Aravena et al., 2022 Nature Reviews Microbiology

Animal origins of coronaviruses





Cui et al., 2019. Nature Reviews Microbiology

Where do we stand on SARS-CoV-2 origins?







In 2020 we knew:

- evolution for spillover to humans occurred in horseshoe bats, approx. 40-70 years ago
- also (separate) evolutionary emergence in pangolins
- closest relative >1000km away from Wuhan







Boni et al 2020 Nature Microbiology doi.org/10.1038/s41564-020-0771-4



Now:

- More evidence corroborates evolutionary origins in horseshoe bats
- Closest relatives SARS-CoV-2 in Yunnan and Laos
- Wide geographic range SARS-CoV-2-related bat viruses



RESEARCH; S. LYTRAS ET AL., GENOME BIOLOGY AND EVOLUTION, 14, 2 (2022)



- Wide geographic range SARS-CoV-2related bat viruses
- Recombination history
- Gene flow of across
 wide geographic range





Question remains: How did SARS-CoV-2 end up in humans? and where?

Contact with dead or live animal? Which one? Where?













Laboratory escape accident?

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December 2019: early COVID cases centered around Huanan wet market





December 2019: early COVID cases centered around Huanan wet market

- New in depth studies (e.g. WHO Global Study of the Origins of SARS-CoV-2)
 - Two lineages from the startTwo spillovers?
 - Early cases from both lineages linked to Huanan and/or other markets
 - Wuhan Insitute of Virology outside of early epidemic zone



University of Antwerp I Evolutionary Ecology Group December 2019: early COVID cases centered around Huanan wet market

- Surveillance of SARS-CoV-2 in the environment and animal samples of the Huanan Seafood Market Gao et al., 2022 preprint
 - ➢ 0/437 animals

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- > 73/923 environmental samples
- 1/30 environmental sample other market
- Two early lineages present



Holmes et al., 2021 Cell









Coronaviruses in Wild Animals Sampled in and Around Wuhan in the Beginning of COVID-19 Emergence 3

Wen Wang, Jun-Hua Tian, Xiao Chen, Rui-Xue Hu, Xian-Dan Lin, Yuan-Yuan Pei, Jia-Xin Lv, Jiao-Jiao Zheng, Fa-Hui Dai, Zhi-Gang Song, Yan-Mei Chen, Yong-Zhen Zhang Author Notes











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| amily | Species | | Wuhan | Xiaogan | Jingmen | Total |
|----------------|--------------|---------------|-------|---------|---------|--------|
| eaf nosed bats | Hipposideros | armiger | - | - | 0/107 | 0/107 |
| | Hipposideros | pratti | - | 0/3 | - | 0/3 |
| lorseshoe bats | Rhinolophus | pusillus | - | 7/86 | - | 7/86 |
| | | | | | | |
| | Rhinolophus | ferrumequinum | - | 0/4 | - | 0/4 |
| | Rhinolophus | pearsonii | - | 0/2 | - | 0/2 |
| | Rhinolophus | sinicus | - | 2/8 | - | 2/8 |
| | Rhinolophus | sp. | - | 0/51 | - | 0/51 |
| /esper bats | Myotis | chinensis | - | 0/2 | - | 0/2 |
| | Myotis | petax | - | 0/4 | - | 0/4 |
| | Myotis | sp. | - | 0/67 | - | 0/67 |
| Carnivores | Nyctereutes | procyonoides | 8/15 | - | - | 8/15 |
| Deer | Muntiacus | reevesi | 0/3 | - | - | 0/3 |
| ∕lustelids | Arctonyx | collaris | 0/3 | - | - | 0/3 |
| | Mustela | sibirica | 0/7 | - | - | 0/7 |
| otal | | | 8/28 | 9/227 | 0/107 | 17/362 |

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- CoV in Wuhan bats not direct progenitor tot SARS-CoV-2
- But confirms SARS-CoV-2-related viruse widespread in horseshoe bats eastern Asia







Canine alphacoronavirus

> 53.3%

- CoV spread easily
- superspreader conditions
- Closest to UK dog CoV

Novel Canine Coronavirus Isolated from a Hospitalized Patient With Pneumonia in East Malaysia @

Anastasia N Vlasova, Annika Diaz, Debasu Damtie, Leshan Xiu, Teck-Hock Toh, Jeffrey Soon-Yit Lee, Linda J Saif, Gregory C Gray 🐱 🛛 Author Notes

Clinical Infectious Diseases, Volume 74, Issue 3, 1 February 2022, Pages 446–454, https://doi.org/10.1093/cid/ciab456 Published: 20 May 2021 Article history ▼



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Most likely scenario





Pekar et al., 2022 preprint Zenodo

New animal hosts for SARS-CoV-2?



Mammal Review

Perspective 🙃 Open Access 💿 🚺

Risk of human-to-wildlife transmission of SARS-CoV-2

Sophie Gryseels 🕱, Luc De Bruyn 🕱, Ralf Gyselings 🕿, Sébastien Calvignac-Spencer 🕿, Fabian H. Leendertz 🕿, Herwig Leirs 🕿

First published: 06 October 2020 | https://doi.org/10.1111/mam.12225 | Citations: 8



BOF-COVID project: Avoiding a local wildlife reservoir of SARS-CoV-2 in Belgium.

check susceptibility wild mammals via ACE2 genotyping

































photos: zoogdiervereniging.nl

prof. dr. Yann

Sterckx



dr. Lieselotte Van Bockstal



dr. Luc De Bruyn

First step in infection: molecular binding to ACE2 protein



Casalino, Amaro Lab, U.C. San Diego. https://www.nytimes.com/interactive/2020/health/coronavirus-unveiled.html

Fig: Yann Sterckx

- 63 relevant mammal species in Belgium
 - Wild, domestic, feral, invasive
- 46 species with samples/carcasses in our or collaborators collections
- 1 stranded minke whale
- Remaining 16 ACE2 sequence avaiable in Genbank



Measure binding strength
 computational protein models of biochemical interactions









Biological capacity to infect many different mammal species: broad host range



yes

no

N۸

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New animal hosts for SARS-CoV-2

Pets



- sporadic infections in dogs, cats, ferrets and hamsters
- serosurveys cats and dogs suggest quite widespread infection
- one cat-to-human transmission recorded (Sila et al, 2022 EID).



Zoo animals

lions tigers pumas snow leopards fishing cats binturongs coatimundis hyenas otters gorillas Eurasian and Canadian lynx giant anteater West Indian manatee black-tailed marmoset



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Coronavirus: Tiger at Bronx Zoo tests positive for Covid-19 f







Otters at Georgia Aquarium test positive for coronavirus

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By Rachel Trent, CNN () Updated 1709 GMT (0109 HKT) April 19, 2021







Captive g In-Sync Exotics' Cougar Tests Positive for COVID-19

Read our COVID-19 research and news

The cougar along with three tigers tested positive for COVID-19









Zoo animals

lions tigers pumas snow leopards fishing cats binturongs coatimundis hyenas otters gorillas **Eurasian and Canadian lynx** giant anteater West Indian manatee black-tailed marmoset hippopotamus

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cience



vr 03 dec 2021 () 17:10

Nijlpaarden Hermien en Imani van ZOO Antwerpen besmet met corona: "Loopneus, maar ze stellen het goed"



The Georgia Aquarium said it tested its Asian small-clawed otters after they showed symptoms.



Read our COVID-19 research and news

D-19







Zoo animal deaths

Second Lion In Indian Zoo Dies Of Covid-19 And 10 More Still Being Treated For Infection

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Siladitya Ray Forbes Staff Covering breaking news and tech policy stories at Forbes. Jun 17, 2021, 09:12am EDT

Updated Jun 17, 2021, 09:12am EDT

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(►)

TOPLINE A second lion at India's Valandur zoo died Wednesday after a Covid-19 infection, zoo authorities announced, while 10 other lions continue to be treated for the disease, suggesting that big cats in zoological facilities may be more vulnerable to the coronavirus than previously thought.



A zoo's three 'beloved' snow leopards die of covid-19

By <u>Reis Thebault</u> November 14, 2021 at 8:00 p.m. EST



A snow leopard sits in its enclosure at New York's Central Park Zoo in February. Big cats in zoos across the country coronavirus. (Cindy Ord/Getty Images) MOST RE

Snow leopard at Illinois zoo dies after contracting Covid-19

Rilu, 11, began showing symptoms in November and the CDC says most animal Covid infections come from contact with humans



Snow leopards are one of a number of mammal species susceptible to coronavirus. Ramil, above, contracted Covid-19 at San Diego zoo. Photograph: AP



Zoo surveillance







- sampling animal faeces from Zoo Antwerp (47 species) and Zoo Planckendael (67 species)
 - September, October, December 2020
 - July 2021 (Alpha)
 - December 2021 (Delta, after Hippo infection)
- PCR tests for SARS-CoV-2 and general coronaviruses
- no infections detected

Tine Cooreman Léa Joffrin Erik Verheyen Herwig Leirs



Farm and bred animals



- Infected hamsters imported from Europe to Hong Kong
- Transmission in pet shops
- Transmission to humans
- Onwards human-to-human transmission



Yen et al., 2022 The Lancet



Farm and bred animals

Mink farms



infections observed in 11 countries massive culling of (infected) farms

very fast transmission among mink in farm

human-mink-human transmission





Oude Munnink et al. 2021 Science



- Mink-adapted evolution in mink
 - mutations not crucial for mink-infection
 - no faster/slower evolution
- Genetic substitutions transmitted to humans
- No impact on human epidemic / evolution



Zoo and farm animals

- close contact with people
- > easy transmission in dense, indoor populations

Notable absence of SARS-CoV-2 infection in livestock and other farmed animals

- > cattle, pigs, poultry, llamas, rabbits, ...
- sanitation procedures?
- > unnoticed?
- > not susceptible?







SARS-CoV-2 Exposure in Escaped Mink, Utah, USA

Susan A. Shriner, Jeremy W. Ellis, J. Jeffrey Root, Annette Roug, Scott R. Stopak, Gerald W. Wiscomb, Jared R. Zierenberg, Hon S. Ip,¹ Mia K. Torchetti,¹ Thomas J. DeLiberto¹

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DOI: https://doi.org/10.3201/eid2703.204444

In August 2020, outbreaks of coronavirus disease were confirmed on mink farms in Utah, USA. We surveyed mammals captured on and around farms for evidence of infection or exposure. Free-ranging mink, presumed domestic escapees, exhibited high antibody titers, suggesting a potential severe acute respiratory syndrome coronavirus 2 transmission pathway to native wildlife.



Article

First Description of SARS-CoV-2 Infection in Two Feral American Mink (*Neovison vison*) Caught in the Wild

Jordi Aguiló-Gisbert ^{1,†}[®], Miguel Padilla-Blanco ^{2,†}, Victor Lizana ^{1,3}[®], Elisa Maiques ⁴, Marta Muñoz-Baquero ¹, Eva Chillida-Martínez ¹, Jesús Cardells ^{1,3,*} and Consuelo Rubio-Guerri ^{2,*}

frontiers in Veterinary Science

BRIEF RESEARCH REPORT published: 31 March 2022 doi: 10.3389/fvets.2022.826991

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

OPEN ACCESS

Miguel Padilla-Blanco^{1†}, Jordi Aguiló-Gisbert^{2†}, Vicente Rubio³, Víctor Lizana^{2,4}, Eva Chillida-Martínez², Jesús Cardells^{2,4}, Elisa Maiques^{5*} and Consuelo Rubio-Guerri^{1*}

White-tailed deer

- Multiple introductions across North America (Canada and USA).
- Both Delta and Omicron
- Very high prevalences in wild deer populations.
- Extensive evolution
 - mutations not crucial for deerinfection
 - no faster/slower evolution
- Deer-to-human transmission

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Not (yet) observed:

- evolutionary divergence of completely different variant in wildlife
 - immune escape?
 - (some evidence for little immune escape in SARS-CoV-2 evolved in Danish mink)
 - better transmissibility in humans (than competing human variants)

SARS-CoV-2: respiratory virus but excreted in faeces too...

Belgium begins using wastewater to predict future coronavirus flare-ups

Monday, 05 October 2020

Credit: Belga

viral RNA found in sewer water also in rats living in the sewers?

Centers for Disease Control and Prevention CDC 24/7: Saving Lives, Protecting People™

EMERGING INFECTIOUS DISEASES[®]

EID Journal > Volume 28 > Number 2—February 2022 > Main Article

Volume 28, Number 2—February 2022

Research Letter

Surveillance of Rodent Pests for SARS-CoV-2 and Other Coronaviruses, Hong Kong

Elliott F. Miot¹, Brian M. Worthington¹, Kar Hon Ng¹, Lucy de Guilhem de Lataillade¹, Mac P. Pierce, Yunshi Liao, Ronald Ko, Marcus H. Shum, William Y. Cheung, Edward C. Holmes, Kathy S. Leung, Huachen Zhu, Leo L. Poon, Malik J. Peiris, Yi Guan, Gabriel M. Leung, Joseph T. Wu , and Tommy T. Lam

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<u>Cite This Article</u>

Abstract

We report surveillance conducted in 217 pestiferous rodents in Hong Kong for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We did not detect SARS-CoV-2 RNA but identified 1 seropositive rodent, suggesting exposure to a virus antigenically similar to SARS-CoV-2. Potential exposure of urban rodents to SARS-CoV-2 cannot be ruled out.

SARS-CoV-2 surveillance in Norway rats (*Rattus norvegicus*) Antwerp sewer system, Belgium

- November-December 2020 \rightarrow Original Wuhan strain
 - Colombo et al., 2021 Transbound Emerg Dis.
- April 2021 → Alpha
- February 2022 → Omicron
 - Possible positive feces?

Lieselotte Van Bockstal Yann Sterckx Peter Delputte Valeria Colombo Vincent Sluydts Joachim Mariën Kevin Ariën **Tine Cooreman** Natalie Van Houtte Francis Vercammen Léa Joffrin **Tine Cooreman** Tanmay Dharmadhikari Luc De Bruyn Muriel Vervaeke **Kristof Baert** Goedele Verbeylen Thierry Jauniaux Nathalie Charbonnel et al.

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Good example of clear animal spillover origins: Middle East Respiratory Virus (MERS)

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Dudas et al., 2017 *eLife*

MERS deeper evolutionary history more difficult to reconstruct

