

CORONAVIRUS

Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans

Bas B. Oude Munnink^{1,*}, Reina S. Sikkema¹, David F. Nieuwenhuijse¹, Robert Jan Molenaar², Emmanuelle Munger¹, Richard Molenkamp¹, Arco van der Spek³, Paulien Tolsma⁴, Ariene Rietveld⁵, Miranda Brouwer⁵, Noortje Bouwmeester-Vincken⁶, Frank Harders⁷, Renate Hakze-van der Honing⁷, Marjolein C. A. Wegdam-Blans⁸, Ruth J. Bouwstra², Corine GeurtsvanKessel¹, Annemiek A. van der Eijk¹, Francisca C. Velkers⁹, Lidwien A. M. Smit¹⁰, Arjan Stegeman⁹, Wim H. M. van der Poel⁷, Marion P. G. Koopmans¹

Animal experiments have shown that nonhuman primates, cats, ferrets, hamsters, rabbits, and bats can be infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In addition, SARS-CoV-2 RNA has been detected in felids, mink, and dogs in the field. Here, we describe an in-depth investigation using whole-genome sequencing of outbreaks on 16 mink farms and the humans living or working on these farms. We conclude that the virus was initially introduced by humans and has since evolved, most likely reflecting widespread circulation among mink in the beginning of the infection period, several weeks before detection. Despite enhanced biosecurity, early warning surveillance, and immediate culling of animals in affected farms, transmission occurred between mink farms in three large transmission clusters with unknown modes of transmission. **Of the tested mink farm residents, employees, and/or individuals with whom they had been in contact, 68% had evidence of SARS-CoV-2 infection.** Individuals for which whole genomes were available were shown to have been infected with strains with an animal sequence signature, providing evidence of animal-to-human transmission of SARS-CoV-2 within mink farms.

In late December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the cause of a viral pneumonia outbreak, possibly related to a seafood and live animal market in Wuhan, China (1). Since then, SARS-CoV-2 has spread across the world. By 8 October 2020, more than 36.1 million people had been infected with SARS-CoV-2, resulting in more than 1 million deaths (2). In the Netherlands, more than 155,000 infections have been confirmed, more than 6500 SARS-CoV-2-related deaths have been reported, and nonpharmaceutical interventions have been put into place to prevent further spread of SARS-CoV-2 (3).

In view of the similarities of the new virus and SARS-CoV-1, a zoonotic origin of the outbreak was suspected and linked to a Wuhan fresh market where various animals—including fish, shellfish, poultry, wild birds, and exotic animals—were sold. However, other cases with onset well before the period correlated with the Wuhan market-associated cluster were

observed, which suggests the possibility of other sources (4). Although closely related coronaviruses found in bats (5, 6) and pangolins (7, 8) have the greatest sequence identity to SARS-CoV-2, the most likely divergence of SARS-CoV-2 from the most closely related bat sequence is estimated to have occurred between 1948 and 1982 (9). Therefore, the animal reservoir(s) of SARS-CoV-2 is(are) yet to be identified.

Similarly to SARS-CoV-1, SARS-CoV-2 binds to the host angiotensin-converting enzyme 2 (ACE2) receptor. On the basis of ACE2 similarities, a range of different animals have been used as models. Experimental infections in dogs (10), cats (10–13), ferrets (10, 14), hamsters (15, 16), rhesus macaques (17), tree shrews (18), cynomolgus macaques (19), African green monkeys (20), common marmosets (21), rabbits (22), and fruit bats (23) have shown that these species are susceptible to SARS-CoV-2, and experimentally infected cats, tree shrews, hamsters, and ferrets could also transmit the virus. By contrast, experimental infection of pigs and several poultry species with SARS-CoV-2 proved to be unsuccessful (10, 23, 24). SARS-CoV-2 has also sporadically been identified in naturally infected animals. In the United States and Hong Kong, SARS-CoV-2 RNA has been detected in dogs (25). In the Netherlands, France, Hong Kong, Belgium, Spain, and the United States, cats have tested positive for SARS-CoV-2 by reverse transcription polymerase chain reaction (RT-PCR) (26–30). Furthermore, SARS-CoV-2 has been detected in four tigers and three lions in a zoo in New York

(31). In Italy, the Netherlands, and Wuhan, China, antibodies to SARS-CoV-2 have been detected in cats (29, 32, 33). Recently, SARS-CoV-2 was detected in farmed mink (*Neovison vison*), resulting in signs of respiratory disease and increased mortality (29, 34).

In response to the outbreaks in mink farms, the Dutch national response system for zoonotic diseases was activated. Although the public health risk of exposure to animals with SARS-CoV-2 was determined to be low, increased awareness of animals' possible involvement in the COVID-19 epidemic was needed. Therefore, from 20 May 2020 onward, mink farmers, veterinarians, and laboratories were obliged to report symptoms in mink (family Mustelidae) to the Netherlands Food and Consumer Product Safety Authority (NFCPSA), and an extensive surveillance system was established (35).

Whole-genome sequencing (WGS) can be used to monitor the emergence and spread of pathogens (36–39). As part of the surveillance effort in the Netherlands, more than 1750 SARS-CoV-2 viruses have been sequenced to date from patients from different parts of the country (40). Here, we describe an in-depth investigation into the SARS-CoV-2 outbreak in mink farms and mink farm employees in the Netherlands, combining epidemiological information, surveillance data, and WGS on the human–animal interface.

SARS-CoV-2 was first diagnosed on two mink farms (designated NB1 and NB2) in the Netherlands on 23 and 25 April 2020, respectively. After the initial detection of SARS-CoV-2 on these farms, a thorough investigation was initiated to identify potential transmission routes and to perform an environmental and occupational risk assessment. Here, we describe the results of the investigation of the first 16 SARS-CoV-2-affected mink farms by combining SARS-CoV-2 diagnostics, WGS, and in-depth interviews.

Owners and employees of the 16 mink farms with SARS-CoV-2-positive animals were included in the contact tracing investigation by the Netherlands Municipal Health Services and were tested according to national protocol. Ninety-seven individuals were tested by either serological assays and/or RT-PCR. Forty-three of 88 (49%) upper respiratory tract samples tested positive by RT-PCR, whereas 38 of 75 (51%) serum samples tested positive for SARS-CoV-2-specific antibodies. In total, 66 of 97 (68%) tested individuals had evidence for SARS-CoV-2 infection (Table 1).

During the interview on 28 April, four of five employees from NB1 reported that they had experienced respiratory symptoms before the outbreak was detected in minks, but none of them had been tested for SARS-CoV-2. Their symptom-onset dates ranged from 1 April to 9 May. For 16 of the mink (sampled on 28 April)

¹Department of Viroscience, Erasmus MC, WHO Collaborating Centre for Arbovirus and Viral Hemorrhagic Fever Reference and Research, Rotterdam, Netherlands. ²Royal GD, Deventer, Netherlands. ³Netherlands Food and Consumer Product Safety Authority (NVWA), Utrecht, Netherlands. ⁴Municipal Health Services GGD Brabant-Zuidoost, Eindhoven, Netherlands. ⁵Municipal Health Services GGD Hart voor Brabant, 's-Hertogenbosch, Netherlands. ⁶Municipal Health Services GGD Limburg-Noord, Venlo, Netherlands. ⁷Wageningen Bioveterinary Research, Lelystad, Netherlands. ⁸Stichting PAMM, Veldhoven, Netherlands. ⁹Division of Farm Animal Health, Department of Population Health Sciences, Utrecht University, Utrecht, Netherlands. ¹⁰Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, Netherlands. *Corresponding author: Email: b.oudemunnink@erasmusmc.nl