SARS-CoV-2 on mink farms in the Netherlands; a One Health investigation

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Summary

In 2020, 69 mink farms in the Netherlands were found to be infected with SARS-CoV-2 and subsequently culled. A One Health research consortium performed an extensive outbreak investigation, focusing on possible transmission routes and public health risks. Transmission via humans (owners, workers, farm advisors, suppliers), animals (mink, pets, wildlife) and the environment (manure, air, dust) were considered. Five different introductions from humans to the mink population were identified. Three of five introductions resulted in ongoing transmission between mink farms. SARS-CoV-2 infections were detected in feral cats and two dogs at mink farms, and in two escaped mink, but not in wildlife. In total, 102 people directly exposed on mink farms were infected with SARS-CoV-2. On the other hand, spill-over of the mink-related SARS-CoV-2 variant to the general population was limited. Some farm-to-farm transmissions could be explained by shared employees, but the mode of farm-to-farm transmission was unknown for the majority of cases.

Samenvatting

In 2020 zijn 69 Nederlandse nertsenbedrijven besmet verklaard met SARS-CoV-2 en geruimd. Een One Health-onderzoeksconsortium deed uitgebreid onderzoek naar mogelijke transmissieroutes en volksgezondheidsrisico's. Overdracht via mensen (eigenaren, medewerkers, erfbetreders), dieren (nertsen, huisdieren, dieren in het wild) en het milieu (mest, lucht, stof) werd onderzocht.

Er werden vijf aparte momenten van introductie van SARS-CoV-2 door mensen in de nertsenpopulatie geïdentificeerd. Drie van de vijf introducties resulteerden in verdergaande transmissie tussen nertsenbedrijven. SARS-CoV-2 RNA werd gedetecteerd bij zwerfkatten en twee honden op nertsenbedrijven en bij twee ontsnapte nertsen, maar niet bij dieren in het wild. SARS-CoV-2 werd bij 102 mensen op nertsenbedrijven vastgesteld, maar waarschijnlijk was de verspreiding van nertsgerelateerde SARS-CoV-2 naar de algemene bevolking beperkt. Hoewel transmissie tussen nertsenbedrijven soms kon worden verklaard door gedeelde werknemers, bleef de wijze van overdracht in de meeste gevallen onbekend. De multidisciplinaire aanpak was essentieel in het uitbraakonderzoek van dit nieuwe zoönotische virus en voor het ondersteunen van diergezondheids- en volksgezondheidsbeleid. Uiteindelijk besloot de Nederlandse regering het verbod op de pelsdierhouderij naar 2021 te vervroegen, vanwege het risico van ontwikkeling van een dierlijk SARS-CoV-2reservoir en mogelijke gevolgen voor de volksgezondheid van waargenomen mutaties in het SARS-CoV-2-genoom.

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On behalf of the 'One Health mink outbreak investigation consortium' (in addition to the authors): M. Augustijn (Royal GD), L. Begeman (Erasmus MC), R.W. Hakze-van der Honing (WBVR), R. Janssen (Dutch Mammal Society), T. Kuiken (Erasmus MC), M.P.G. Koopmans (Erasmus MC), P.A. Meijer (Utrecht University), B.B. Oude Munnink (Erasmus MC), W.H.M. van der Poel (WBVR), J. de Rond (Royal GD), R. Slaterus (Sovon), A.N. van der Spek (NVWA), J.A. Stegeman (Utrecht University) The multidisciplinary nature of the research consortium was essential in the outbreak investigation of this novel zoonotic virus. Research outcomes were used to inform animal health and public health policy makers. The decision of the Dutch government to ban fur farming in 2021, earlier than initially planned, was based on the risk of the development of an animal SARS-CoV-2 reservoir, as well as the possible public health impact of the mutations that were found in the SARS-CoV-2 genome after introduction into the mink population.

Introduction

The potential role of animals in the origin and spread of SARS-CoV-2 has been discussed since its discovery. Following reports of incidental SARS-CoV-2 infections in animals in several countries, the ministries of Agriculture, Nature and Food Quality (LNV) and of Public Health, Welfare and Sport (VWS) announced further research into SARS-CoV-2 in farmed animals and cats [1]. On 24 April 2020, the first SARS-CoV-2 outbreak in production animals was reported at a mink farm in the Netherlands [2] and more outbreaks on mink farms were reported in the subsequent months [3,4]. In total, mink on 69 out of 126 Dutch mink farms were diagnosed with SARS-CoV-2. All infected farms were located in a farm-dense area in the Southeastern part of the Netherlands. The SARS-CoV-2 outbreak in mink and associated humans was studied using a One Health approach, recognizing that human, animal and environmental health are closely linked and implementing a transdisciplinary approach. The aim was to gain timely insights in the ecology and spread of the virus, as well as in the impact on human and animal health.

Containment measures

After the first two outbreaks (24 and 25 April 2020), mink farm owners and their veterinarians were obligated to notify respiratory signs and increased mortality in mink to the Netherlands Food and Consumer Product Safety Authority (NVWA). In May, it became clear that an employee of one of the infected mink farms possibly acquired COVID-19 through contact with infected mink. From 19 May onwards, SARS-CoV-2 was officially assigned as a notifiable infectious animal disease and strict biosecurity and biocontainment measures were implemented on all mink farms [5]. These initially (figure 1, HP1) included a ban on mink and manure transport, visitor restrictions and an advice to use personal protective equipment (PPE) and avoid working with mink in case of symptoms. Also, mink farmers were asked to prevent any contact between their mink and other animals, and to take measures to prevent cats, dogs and other animals leaving farm premises [4,5].

Mink farming in the Netherlands

Before the SARS-CoV-2 outbreaks, 4.5 million American mink (Neovison vison) were bred yearly on 126 Dutch farms, employing 1,200 full-time and 400 part-time individuals. Mink are the most common animal species farmed for fur production. Most of the world's farmed fur is produced in Europe, with the Netherlands ranking third after Denmark and Poland. Mink are kept in adjoining wire mesh cages with a nest box and bedding, often placed in open sheds (long roofs on piles without sidewalls) or halls. The feed is a liquid mixture of slaughter and fish waste which is placed on top of the wired cage. Adults are solitarily housed until females give birth in April and May. After weaning, the offspring is kept in small groups until most of them are euthanized at the end of the year to remove their pelts. In the same period the breeding stock for the next year (season) is selected.

From 3 June 2020 onwards, all mink of infected farms were culled [6]. From 9 June (figure 1, HP2) triage of workers and visitors was required and contacts of Covid-19 patients were not allowed to work with minks unless practically unfeasible, in which case surgical (IIR) face masks were required [7]. In July (figure 1, HP3), following increasing numbers of SARS-CoV-2 infections in employees, non-medical face masks and protective eyewear became mandatory on all mink farms and medical FFP2 face masks on farms that were

suspected to be infected and during culling. Furthermore, when one of the workers tested SARS-CoV-2 positive, NVWA had to be notified [7]. In September (figure 1, HP4), employees of infected mink farms were advised to get tested and restrictions on exchange of personnel between farms were put into effect [8]. For pelting on non-infected farms, additional hygiene measures (figure 1, HP5), including mandatory use of FFP2 face masks were implemented.

Figure 1. Overview of SARS-CoV-2 outbreaks on mink farms per week in 2020, in relation to implementation of the most important control measures and the mink farm cycle.



The numbered blue arrows point to implementation or adaptations of hygiene measures for people entering the mink farm (referred to as Hygiene Protocols (HP) 1-5) and orange arrows to other control and monitoring measures described in the text. Not = Covid-19 inminks assigned as notifiable infectious animal disease; EW = Early Warning; ZS = upscaling of Zoonosis Structure; S1 & S2: first and second serological screening. Below the graph: important mink farming periods, associated with intensive mink-human contact and additional personnel (note that the mating period (March) is not shown). Vaccinations were targeted at mink-related diseases (not SARS-CoV-2). Pelting continued until the beginning of December.

The ban on fur farming was enforced on 8 January 2021 instead of 2024, and currently no farmed mink are left in the Netherlands.

Clinical signs and surveillance

Mink

Clinical signs of SARS-CoV-2 infection in mink differed between animals and between farms, but most common signs were increased mortality, reduced feed intake, decreased activity and respiratory signs such as coughing and sneezing, eye and nasal discharge and dyspnea. At post-mortem, extensive pneumonia could be found in some mink. Mortality was slightly increased compared to the expected mink mortality [4,9]. Extensive random sampling on a selection of farms showed that fewer than 10 percent of SARS-CoV-2 ribonucleic acid (RNA) positive minks showed clinical signs, indicating that subclinical infections were common. An Early Warning (figure 1, EW) system was implemented on 25 May, which included mandatory weekly screening of recently dead minks on all farms. All dead minks, with a maximum of five (increased to 50 by the end of August) were tested weekly using naso-pharyngeal swabs by polymerase chain reaction (PCR) targeting the SARS-CoV-2 E gene [10]. A mandatory serological screening (figure 1, S1) started at the end of May and was carried out again in Sep-

The Dutch 'Zoonoses Structure'

The National 'Zoonoses Structure' is an integrated human-veterinary risk analysis structure according to which the collaboration between the Dutch Minister of Health, Welfare and Sport (VWS) and Minister of Agriculture, Nature and Food Quality (LNV) is organized in case of a crisis due to a zoonosis. In this structure, VWS has the leading role, but both Ministers have their own responsibilities with respect to the control and implementation of measures. tember (figure 1, S2). Blood on filter paper of 60 mink farm was obtained by toenail clipping and was tested for SARS-CoV-2 antibodies using an in-house (GD Animal Health) indirect ELISA validated for mink samples.

All mink farms suspected to be infected, based on reporting of clinical signs, EW or serological screening, were visited for official confirmational sampling. Thirty-two farms were found positive after notification of clinical signs to the NVWA and 37 farms after testing positive in EW or serological screening. Only one farm did not report any clinical sign (and no PCR positive nasal swabs were found).

Human

Symptoms of SARS-CoV-2 infected human individuals on mink farms varied from asymptomatic to mild respiratory symptoms, fever and/or loss of smell, one employee was admitted to the intensive care unit due to COVID-19.

The municipal health service (GGD) visited the first 16 infected farms for extensive human contact tracing,

PCR testing and serum collection of people with possible exposure to infected mink [6]. Subsequent infected farms were not visited and owners, employees and farm visitors were expected to visit a regional SARS-CoV-2 testing facility in case of any symptoms indicative of COVID-19, in line with the national SARS-CoV-2 testing and surveillance policy. When people tested positive, contact tracing was executed. From 5 August onwards, testing policy was changed to allow for testing of personnel without clinical signs [11,12]. A total of 102 owners, family members and employees at 42 of the 69 farms (61 percent) tested SARS-CoV-2 positive.

To determine whether infections with mink strains of persons living near mink farms had occurred, a screening of a random selection of stored respiratory samples of COVID-19 patients in the same postal code region as the infected mink farms was executed between March 4 and April 29 [4] and between October 15 and November 15, using whole genome sequencing. In the first screening, no sequences (34 sequences) were found that clustered with the mink sequences. In the second screening, one sequence was found (189 sequences). The identified individual reported not having had any contact with mink farms, nor with people who lived or worked on mink farms. Two other sequences of individuals without any con-.

nection to mink (collected in July and October), clustering with mink sequences, were found in the regular SARS-CoV-2 genomic surveillance by RIVM and ErasmusMC. After November 2020, no minkrelated sequences were found in the general Dutch population, indicating that there was no ongoing spread between humans of these mink variants.

Epidemiology

An epidemiological investigation was performed, for which information on several potential veterinary and human contacts and farm characteristics were collected. Moreover, human and mink samples with sufficiently high viral loads were sequenced to obtain their genetic code. Despite increasingly strict hygienic measures from May onwards and quick detection and culling of infected farms, new outbreaks occurred up to 20 November 2020 (figure 1).

Phylogenetic analyses of mink sequences indicated that the first introductions into mink farms had occurred several weeks before the detection of the first positive farms, and that these sequences were linked to human viruses circulating in the Netherlands at that time. In total, five different introductions from humans into mink farms were identified [6]. For three introductions (later referred to as 'clusters'), ongoing transmission between mink farms was seen, and one cluster was found in all outbreaks from September onwards (cluster A).

Viruses from different clusters were found in neighboring farms and in farms located further away from each other. Geographical clustering was increasingly found from August onwards and for the largest cluster A we indeed determined that nearby farms had a higher risk of infection. Epidemiological investigation showed that many farms shared the same veterinarian or feed supplier, but no unambiguous veterinary service company contacts were found between farms within the different virus clusters explaining the farm-to-farm spread. Only in a limited number of farms, transmission could be linked to shared personnel. Despite extensive analyses, other modes of transmission explaining the farm-to-farm spread were not found.

All human samples that could be sequenced (n = 57) contained mink strains [6]. All sequences of human workers were identical to the sequence cluster found at the mink farm where they worked, except for one

case where the human worker assisted during culling on another infected farm. In most cases (38/42 farms, 2 farms unknown), the date of the first positive human test result was in the same week or after the mink on the farm tested positive, making mink-to-human transmission the most likely scenario.

Air and environmental examination

To evaluate environmental and occupational health risks. SARS-CoV-2 contamination in environmental samples from three mink farms was investigated [13]. Considerable levels of SARS-CoV-2 RNA (~1,000 to 10,000 copies per m³) were detected in air sampled in each of the farms. Dust samples, surfaces of mink cages, and bedding material were highly contaminated with SARS-CoV-2 RNA. Lower viral loads were found in fecal samples, feed leftovers, and swabs from the drinking water supply. Two weeks after all mink were culled, environmental sampling was repeated at one farm. SARS-CoV-2 RNA was still detectable in a substantial proportion of samples, especially in bedding material. Infectious virus was not detected. Outdoor air samples from the farm premises and at nearby locations (max. 1.5 km) all tested negative, apart from a few samples less than 10 meters from the entrance of one of the mink halls. Because of the negligible spread of the virus to the surrounding environment, the precautionary closure of roads for cvclists and pedestrians within 400 meters of the farms implemented after detection of the first two infected mink farms, was lifted on 15 May 2020.

Wildlife and domestic animals surrounding mink farms

Free-living animals (wildlife) may spread the virus by mechanical transmission (passive transfer of the virus, by means of a vector) or by excreting the virus at another farm once infected. Based on possible susceptibility for SARS-CoV-2 infection and abundance in the region with infected mink farms, mustelids (Mustelidae include mink but also weasels, badgers, ferrets, martens, amongst others), foxes, cats and bats were selected for further investigation. In addition, birds were studied to assess their potential role in mechanical virus transmission between farms. The open housing system of mink allowed access of birds, most free-ranging mustelids, foxes and cats into the farms. If a farm is accessed, mustelids, foxes, cats and multiple bird species are likely to have direct expo-



Left: Courtesy: Nederlandse Federatie van Edelpelsdierenhouders (NFE) Right: Courtesy Jan de Rond (Royal GD)

sure to mink, their food or the litter underneath the cages.

SARS-CoV-2 RNA and/or antibodies were found in twelve (89 tested) feral cats and two dogs (13 tested) on 10 mink farms [14]. In addition, two escaped mink from infected farms tested PCR positive. Samples of free-living wild animals in the region with SARS-CoV-2-positive mink farms (50 carcasses and 755 feces samples from mustelids, foxes and bats) did not contain SARS-CoV-2 RNA. Therefore, it is unlikely that large-scale circulation of SARS-CoV-2 in free-living animals has occurred, and their role in SARS-CoV-2 transmission is probably negligible. The possibility of farm-to-farm transmission of SARS-CoV-2 by feral cats and escaped mink could not be excluded, but was also considered low.

Conclusions

Despite extensive research into possible routes of transmission and stricter measures in the course of the epidemic, control of SARS-CoV-2 spread between farms proved to be challenging. However, spill-over of mink viruses to the general human population remained limited. Also in other countries with SARS-CoV-2 outbreaks, such as Denmark, community spread did not appear to occur, although sufficiently detailed phylogenetic analyses of human and mink sequences have not always been executed. Despite extensive outbreak investigations in several affected countries, critical gaps in knowledge remain regarding the exact modes of transmission. To date, large scale SARS-CoV-2 infections in other farmed animals have not been found, in line with infection experiments showing limited susceptibility to SARS-CoV-2 infection.

The decision to cull infected mink farms and to move the end date of fur farming forward to 2021 was taken after careful consideration of the risks for human and animal health. After introduction into mink populations, multiple changes in the virus genome were seen that can possibly influence virus transmissibility and antibody recognition. Moreover, continued circulation may lead to the establishment of a reservoir in mink, from which SARS-CoV-2 could be reintroduced into the human population. This is especially a concern because transmission could not be stopped with strict measures. With recent indications that the novel SARS-CoV-2 variants may have different host ranges [15], future animal surveillance remains imperative to prevent introduction and spread of SARS-CoV-2 in other animal populations.

Investigating transmission routes of novel zoonotic

pathogens such as SARS-CoV-2 requires intensive surveillance, information collection and contact tracing in the human, animal and environmental domain. As shown in the SARS-CoV-2 outbreak on mink farms, data collection and real-time exchange of information between human and veterinary institutes can be hampered by privacy legislation, high workload and other limitations. SARS-CoV-2 on mink farms is one example of an outbreak of a zoonotic pathogen in the Netherlands, preceded by avian influenza and Q-fever amongst others. Emergence and spread of novel viruses, involving animal reservoirs is expected to happen again in the future. Lessons learnt in this outbreak provide a good opportunity to make further improvements to prepare for future outbreaks that require a 'One Health' approach.

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