Original research

Occupational and environmental exposure to SARS-CoV-2 in and around infected mink farms

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ABSTRACT

Objective Unprecedented SARS-CoV-2 infections in farmed minks raised immediate concerns regarding transmission to humans and initiated intensive environmental investigations to assess occupational and environmental exposure.

Methods Air sampling was performed at infected Dutch mink farms, at farm premises and at nearby residential sites. A range of other environmental samples were collected from mink’s housing units, including bedding materials. SARS-CoV-2 RNA was analysed in all samples by quantitative PCR.

Results Inside the farms, considerable levels of SARS-CoV-2 RNA were found in airborne dust, especially in personal inhalable dust samples (approximately 1000–10 000 copies/m3). Most of the settling dust samples tested positive for SARS-CoV-2 RNA (82%, 75 of 92). SARS-CoV-2 RNA was not detected in outdoor air samples, except for those collected near the entrance of the most recently infected farm. Many samples of minks’ housing units and surfaces contained SARS-CoV-2 RNA.

Conclusions Infected mink farms can be highly contaminated with SARS-CoV-2 RNA. This warns of occupational exposure, which was substantiated by considerable SARS-CoV-2 RNA concentrations in personal air samples. Dispersion of SARS-CoV-2 to outdoor air was found to be limited and SARS-CoV-2 RNA was not detected in air samples collected beyond farm premises, implying a negligible risk of environmental exposure to nearby communities. Our occupational and environmental risk assessment is in line with whole genome sequencing analyses showing mink-to-human transmission among farm workers, but no indications of direct zoonotic transmission events to nearby communities.

INTRODUCTION

The COVID-19 pandemic has its grip on the world. After the initial animal-to-human jump, human-to-human transmission took off. Several incident cases of human-to-animal transmission have been reported,1 but large outbreaks among commercially kept animals were not reported until minks (Neovison vison) at Dutch fur farms were found to be infected in April 2020.2 Subsequently, SARS-CoV-2 outbreaks in mink farms occurred worldwide.3–5 and transmission experiments with ferrets6–8 show that SARS-CoV-2 is efficiently transmitted between minks and related species.

The occurrence of an unprecedented SARS-CoV-2 infection in farmed minks raised immediate concerns regarding human health, both for the farm worker population and for neighbouring residents, and in view of global health considering the potential
evolution of SARS-CoV-2 by large-scale animal passage. Therefore, a One Health approach was deployed to gain insight into the (genomic) epidemiology, sources and modes of transmission, and the associated human health risks. Using whole genome sequencing, evidence was provided of SARS-CoV-2 transmission from humans to minks, between minks, and from minks back to humans. Both in the Netherlands and Denmark, widespread transmission between farms occurred, with yet unknown mode of transmission.

In this paper, we describe intensive environmental investigations in and around the first SARS-CoV-2-infected mink farms notified in the Netherlands to better understand occupational and environmental health risks for workers and neighbouring residents. The study objectives were the following:

- To measure SARS-CoV-2 exposure by personal and stationary air sampling in mink farms.
- To study potential dispersion of SARS-CoV-2 to ambient environment by means of outdoor air sampling.
- To assess SARS-CoV-2 contamination of surfaces and materials sampled from minks’ housing units.

**METHODS**

**Investigated farms**

On 23 April, SARS-CoV-2 infection was established at the first Dutch farm, NB1, which has two separate locations (NB1A and NB1B, 115 m apart). Two days later, another farm tested positive, NB2, situated at a 14 km distance from NB1. Both NB1 and NB2 experienced increased mortality among minks, coinciding with respiratory signs starting in the first half of April. From 28 April onwards, environmental sampling started at both farms. On 6 May, two additional farms tested positive, NB3 and NB4. NB4, in contrast to the other farms, appeared to be more recently infected at the time of detection. Therefore, NB4 was included for environmental sampling from 13 May onwards (see online supplemental methods A for additional information on the outbreaks, including a timeline and background information on husbandry practices).

**Design of environmental sampling**

Air sampling was performed by filter-based techniques whereby air is forced through a filter and particles present in the air, including potentially SARS-CoV-2-contaminated particles, are captured. To gain insight into potential differences related to particle size fraction, air sampling was performed in parallel to measure both particulate matter 10 (PM$_{10}$) and inhalable dust based on the European norm defined size fractions.

We conducted long-term air sampling at both farms’ premises and nearby residential sites to detect potential dispersion of SARS-CoV-2 to the outdoor environment. We also collected air samples and settling dust at the farms and sampled surfaces and materials from minks’ housing units. Simultaneous upwind and downwind sampling around the mink houses was performed to assess dispersion. For this part of the study, we visited each farm once per week. For the more recent outbreak at NB4, long-term outdoor air sampling was performed for 3 weeks, while sampling inside the farm was done once. On 3 June, the Dutch government decided to cull all minks at all infected farms. To gain insight into potentially remaining environmental contamination after culling, NB4 was revisited 14 days postculling to collect samples.

**Multiple-day outdoor air sampling**

At the infected farms, consecutive 3-day to 5-day sampling of outdoor air was performed at a central position within 10 m from the (open) wall of the farm. Sampling of PM$_{10}$ and inhalable dust was performed in parallel at a 1.50 m height (for technical details see online supplemental methods B). After the first measurement week at NB4, measurement equipment was installed at three additional locations (B, C, D) within short distances from the initial location (A), where positive samples had been detected (see online supplemental figure S1 for a map). At B, C and D, total suspended particles (undefined size fraction) were additionally sampled.

Three residential sites were included in repeated 7-day air sampling. Three consecutive measurements per site were performed in the vicinity of NB1 (1500 m) and NB2 (1200 m). In addition, a residential site in a mink-free area (>70 km from NB1 and NB2) was included as a background location.

**Sampling inside farm and downwind/upwind**

**Air sampling**

Six-hour stationary air sampling and 8-hour personal air sampling were performed for both PM$_{10}$ and inhalable dust in parallel (for technical details see online supplemental methods B and for pictures see online supplemental figure S2). Personal air samples were collected using portable pumps and sampling heads attached within the breathing zone of the fieldworker. Stationary air sampling was performed at a 1.50 m height at three spots distributed within the farm, which remained the same over time. The locations of the 6-hour air sampling outside the farm were based on the wind direction on the day of measurement. Upwind sampling was performed at a 50 m distance from the farm and downwind sampling at 10–20 m and 100 m distance.

**Settling dust sampling**

Settling dust sampling was performed using electrostatic dust fall collectors (EDCs), which are sterilised electrostatic cloths (polyester electrostatic cloth; Albert Heijn, Zaandam, The Netherlands) placed in a disposable holder. Therefore, NB4 was included for environmental sampling from 13 May onwards (see online supplemental methods A for additional information on the outbreaks, including a timeline and background information on husbandry practices).

EDCs were placed at 11 spots distributed throughout the farm. EDCs were placed in proximity (<0.40 m) of the minks by placing them on currently unused top layers of minks’ housing units (1.60 m height). At NB1, some EDCs could also be placed farther away from the minks (several metres) by placing these on hanging plates or on a stand positioned in an empty alley (see online supplemental methods B for details and online supplemental figure S2 for pictures). After 1 week, the exposed EDCs were collected and replaced by new EDCs.

**Sampling of minks’ housing units**

Per farm visit, a minimum of 10 minks’ housing units were sampled, including those of recently deceased minks (<2 days) and at least three alive minks (see online supplemental methods B for details and online supplemental figure S3 for pictures). In short, swipes were collected of the materials settled on the hard-board border at the front of the housing unit. Bedding materials, consisting of straw/hay, were collected from the night/next box. Food residues were scraped off the top of the cage, where minimally once a day fresh food is placed. Swabs were taken of the rim of the drinker cup. If present, faecal materials were collected from the cage, otherwise from the floor beneath the cage.

**Procedures and analyses**

For details on the procedures and analyses, see online supplemental methods C. In short, each fieldwork day, all samples were immediately stored after collection at 4°C and directly brought to a biosafety level (BSL)-2 laboratory, where samples...
were prepared for storage at −80°C on the same day. Samples were transported in batches on dry ice to another laboratory (BSL-2/3) for RNA extraction and quantitative PCR analyses for SARS-CoV-2. Virus isolation was attempted from air samples with a cycle threshold (Ct) value below 32.

Data processing and analyses were performed using RStudio (V3.6.3). For the purpose of quantification, viral load in positive samples was computed and expressed in number of copies per standard unit (see online supplemental table S1). Multivariable modelling was performed to explore the associations with determinants. Censored regression analyses (parametric survival on log-transformed outcomes due to otherwise skewed distribution) were performed on bedding material samples, swipes and EDCs. Estimates of associations were raised to the power of 10 to represent ratios in per standard unit (see online supplemental table S1). Multivariable modelling was performed to explore the associations with determinants. Censored regression analyses (parametric survival on log-transformed outcomes due to otherwise skewed distribution) were performed on bedding material samples, swipes and EDCs. Estimates of associations were raised to the power of 10 to represent ratios in per standard unit (see online supplemental table S1).

RESULTS

Air samples

Farms NB1A, NB1B and NB2

At the first farm visit, SARS-CoV-2 RNA was detected inside each farm in one out of three 6-hour inhalable dust samples (see table 1). At farm NB1B, personal sampling was also performed during the first visit, of which one of the two 8-hour inhalable dust samples tested positive. Quantification of these four positive active air samples showed concentrations ranging from 2.4×10^3 to 4.9×10^3 RNA copies/m^3 (Ct range: 35–36). All of the parallel collected PM_{10} samples inside the farm and all other inhalable dust and PM_{10} air samples inside or near the mink houses were negative.

SARS-CoV-2 RNA was detected in a high number of settling dust samples (75 out of 92, 82%; median virus load 7.4×10^4 copies/m^3). All EDCs deployed at the first farm visit were positive (Ct range: 25.1–34.6) at all three farms, with viral RNA loads ranging from 2.16×10^4 to 7.40×10^4 copies/m^3 per day sampled (see figure 1). At NB2, all EDCs deployed at the second and third farm visits were also all positive; at T2 (1 week after T1) at NB1A and NB1B, the percentage positives dropped to 73% and 80%, respectively, and further to 64% and 27% at T3. The results of the multivariable modelling (see online supplemental table S2) showed a significant (p<0.05) decrease in viral RNA load over time at all three farms (factor 2.7 weeks 2 vs 1, factor 5 weeks 3 vs 2). Furthermore viral RNA loads in EDCs placed in very close proximity (<0.40 m) to minks were on average 2.3 times higher than those farther away from animal cages (p<0.05).

Table 1 Overview of SARS-CoV-2 RNA in particulate matter 10 and inhalable dust samples collected at mink farms at a later phase of the ongoing SARS-CoV-2 outbreak

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>Date</th>
<th>Farm</th>
<th>6-hour stationary sampling inside farm</th>
<th>8-hour personal sampling inside farm</th>
<th>6-hour stationary sampling outside</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Inhalable dust</td>
<td>Particulate matter 10</td>
<td>Inhalable dust</td>
</tr>
<tr>
<td>1</td>
<td>28 April 2020</td>
<td>NB1</td>
<td>1/3</td>
<td>2.4×10^3</td>
<td>0/3</td>
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<tr>
<td>1</td>
<td>30 April 2020</td>
<td>NB2</td>
<td>1/3</td>
<td>4.9×10^3</td>
<td>0/3</td>
</tr>
<tr>
<td>1</td>
<td>2 May 2020</td>
<td>NB1B</td>
<td>1/3</td>
<td>3.8×10^3</td>
<td>0/3</td>
</tr>
<tr>
<td>2 and 3</td>
<td>5–16 May 2020</td>
<td>NB1A, NB1B, NB1B</td>
<td>0/18</td>
<td>Non-detects</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

All 4-day outdoor air samples collected between 28 April 2020 and 21 May 2020 at NB1A, NB1B and NB2 at their premises: SARS-CoV-2 RNA not detected. NA, not applicable.

Figure 1 Overview of viral load in settling dust samples per farm over time collected by means of electrostatic dust fall collectors (EDCs). Percentiles: 25th, 50th and 75th of the virus load detected in EDCs of 2.16E+04, 7.40E+04 and 3.68E+05, respectively. LOD, limit of detection.
samples were positive (see table 2). Concentrations measured in the personal air samples were higher compared with the stationary air samples, roughly $4 \times 10^4$ copies/m$^3$ (Ct: 31.7/31.8) compared with $7 \times 10^2$ to $2 \times 10^4$ copies/m$^3$ (Ct range: 33.0–34.4). Two out of six stationary PM$_{10}$ samples and both personal PM$_{10}$ samples were positive, with lower concentrations compared with the inhalable dust samples.

In 4-day outdoor air samples collected at the premises, SARS-CoV-2 RNA was detected at measurement location A, positioned within 1.5 m of the farm’s open entrance, but also at measurement spots B and C, positioned within 10 m from the farm’s open entrance. PM$_{10}$ samples collected at spot A were either negative or contained limited levels of SARS-CoV-2 RNA. Inhalable dust and total suspended particles samples contained levels of SARS-CoV-2 RNA ranging from $3 \times 10^2$ to $5 \times 10^3$ copies/m$^3$ (Ct range: 32.3–35.0). At location D, 20 m from the entrance, all samples were negative.

### Samples of minks’ housing units

**Preculling**

SARS-CoV-2 RNA was detected in all swipes of minks’ housing units (n=99, lowest Ct: 21.5, median virus load $6.0 \times 10^6$ copies). A high percentage of bedding materials (83%, 78 out of 94, lowest Ct: 15.9, median virus load $8.2 \times 10^5$ copies) were also positive, and to a lesser extent faecal materials (54%, 51 out of 95, lowest Ct: 24.6). Some swabs of the drinker cups and few food residues were positive (31%, 30 out of 97, lowest Ct: 24.8; 10%, 9 out of 90, lowest Ct 28.9, respectively). Significant differences in viral load in swipes and bedding materials were observed between farms (see table 3 and online supplemental figure S4). Viral load was estimated to be 7 times higher in swipes and 45 times higher in bedding materials at NB4 compared with NB1A and with even larger differences when compared with NB1B and NB2. Higher viral loads were observed in the bedding materials of the housing units belonging to recently deceased minks versus minks that were still alive. Significantly lower odds of detection in faecal materials and swabs of drinker cups were observed for sampling at later timepoints compared to the first timepoint (table 3). In swabs of drinker cups, higher odds of detection were also associated with recently deceased minks.

**Postculling**

Samples collected postculling at NB4 of the same housing units sampled preculling showed a clearly decreased detection of viral RNA. However, RNA was still measurable in 14% of the swipes and 21% of faecal materials, and especially in bedding materials (top layer 57%, bottom layer 85%). A large drop in viral load postculling versus preculling was observed in bedding materials’ top layer (factor 100 difference, p<0.001), and for the bottom layer this drop was considerably smaller (factor 10 difference, p<0.05) (see online supplemental table S3 and figure S5).

### Controls

None of the field blanks collected (minimally 1 per 10 samples) tested positive. Fieldworkers remained SARS-CoV-2-negative throughout the study; they always wore personal protective equipment (PPE), including full face mask.

### DISCUSSION

Air and surfaces at infected mink farms were found to be highly contaminated with SARS-CoV-2 RNA. Airborne inhalable dust contaminated with SARS-CoV-2 RNA was detected inside each investigated farm. Most settling dust samples tested positive, as well as many samples of minks’ housing units. In contrast to the high level of contamination detected inside the farm, no SARS-CoV-2 RNA or incidental low concentrations were detected in outdoor air. This raises caution for occupational health risks in infected farms and suggests a negligible role of SARS-CoV-2 dispersion by air in community transmission.
In the course of the outbreak, effective SARS-CoV-2 spread among minks was observed, as well as transmission between minks and farm workers. High levels of environmental contamination inside the farm suggest a potential role of environmental exposure in mink-to-mink and mink-to-human transmission. The intensity of animal handling by farm workers varies over the course of a year and is typically solely high in April to June, which is the period from end of gestation to weaning. Animal handling is a likely cause of exposure among farm workers; however, zoonotic transmission events were also indicated in months when no/little animal handling was performed. SARS-CoV-2-contaminated airborne particles of sizes smaller than 10 µm and larger were detected in the air within the breathing zone of fieldworkers who performed environmental sampling. Both smaller-sized and larger-sized airborne particles should be considered relevant to health as deposition of inhaled SARS-CoV-2-contaminated particles anywhere along the respiratory tract has the potential to initiate infection. Considerable RNA concentrations were measured in the air within fieldworkers’ breathing zone, which probably underestimate the exposure of actual farm workers as they do have direct contact with the animals. The observed high levels of contamination throughout the farms and the likelihood of a mink-to-human transmission, as demonstrated by whole genome sequencing research, called for implementation of improved biosecurity, hygienic measures and wearing of PPE by farm workers and was effectuated by the Dutch authorities as of May 2020.

Airborne SARS-CoV-2 RNA could be a result of direct shedding into the air by an infectious mink (eg, via sneezing, breathing) and/or indirectly via shedding into the environment and subsequently becoming airborne of stirred up particles (eg, dust particles arising from the bedding material). SARS-CoV-2 experiments with ferrets showed transmission via respiratory droplets, aerosols and/or fomites between ferrets placed apart at 10 cm and >1 m. We performed stationary air sampling at a fixed distance of 30 cm from the nearest minks and personal air sampling naturally at varying distances of up to as close as 10 cm, and thus we could have detected direct shedding of respiratory droplets and/or aerosols. Co-occurrence of indirect routes of airborne spread of contaminated particles is also highly likely, given high levels of contamination observed in matrices easily becoming airborne, such as bedding materials, and presence of airborne dust-generating events (eg, uncontrolled air/wind flows, and movements of animals, food carts and humans). Visual inspections of dust that had settled on EDCs over the course of a week confirmed the high level of dust inside these farms. SARS-CoV-2 RNA loads were high in settling dust samples even at distances of several metres from the animals.

Environmental RNA load is the net result of shedding rates by SARS-CoV-2 infectious minks and processes such as degradation or removal/cleaning. Seroprevalence among minks at the investigated farms was high, indicating many had been infectious at some point in time. The rate of RNA decay is influenced by factors such as temperature and humidity, chemical exposure (eg, reactive oxygen species, alkylating agents) and radiation exposure (eg, ultraviolet radiation), and depending on the environmental matrix more or less decay may take place. SARS-CoV-2 RNA was still detectable in the environment 2 weeks after all animals were culled, similar to what has been observed in an outbreak investigation performed on a cruise ship. Farm cleaning is performed only sporadically; the last time the minks’ housing units were cleaned was months before the outbreak started and thus the results were not affected by a cleaning regimen. Proper cleaning/disinfecting of such a contaminated environment is difficult, as also clearly shown by the remaining SARS-CoV-2 RNA loads. These results, in light of the current many unknowns to actually characterise infection risk, warn of extended precaution in the period after culling.

These unprecedented SARS-CoV-2 infections in farmed minks raised immediate concerns regarding public health, which resulted in government-imposed enclosure of the area around the farms awaiting results of outdoor air investigations. In contrast to the high indoor environmental contamination, none of the 54 outdoor air samples collected at NB1A, NB1B and NB2 tested positive, implying that SARS-CoV-2 RNA was either not present in outdoor air, or if present then in very low concentrations (limit of detection ~10 copies/m³ of air). At NB4, the farm in a more acute phase of the SARS-CoV-2 outbreak, SARS-CoV-2 RNA was detected in outdoor air. Results clearly showed notable RNA concentrations in the air near the open entrance (<1.5 m) and a considerable drop in concentrations several metres further. Outside the farm premises, at a distance of 20 m from the minks, no RNA was detected. Outdoor air SARS-CoV-2 RNA concentrations in PM₁₀ were lower compared with inhalable
Exposure assessment

dust/total suspended particles. Considering natural dispersion, large particles, especially >30 µm, deposit quickly and typically do not reach distances farther than tens of metres.\textsuperscript{22, 23} Particles sized 10 µm or smaller are able to disperse further, but given the measured concentrations and the observed strong reduction over short distances it is unlikely that a potential risk of infection exists beyond farm premises. Furthermore, various outdoor environmental factors unfavourable to viruses, such as ultraviolet radiation, must be considered. Consequently, the direct environmental health risk to passers-by or neighbouring residents of infected farms is expected to be negligible. This substantiates findings by whole genome sequencing\textsuperscript{1} indicating no spillover to people living in the surroundings. Sequences of large particles, especially >30 µm, do not reach distances farther than tens of metres.\textsuperscript{22, 23} Particles as the sampling itself stresses the pathogens (to a more or lesser extent but inappropriate for viability assessment. To actually assess viability of a pathogen when in the air is extremely challenging as the sampling itself stresses the pathogens (to a more or lesser extent depending on the technique).\textsuperscript{25, 26} Nevertheless, research performed in experimental settings\textsuperscript{27, 28} and in a hospital situation\textsuperscript{29} was able to identify viable SARS-CoV-2 in air samples. In aerosols generated under laboratory conditions, SARS-CoV-2 remained stable for several hours (<16 hours).\textsuperscript{27, 28} These findings support the potential risk of infection due to transmission via air. Another limitation is the unknown recovery efficiency of SARS-CoV-2 RNA from the environmental samples. A recent study by Minich et al\textsuperscript{10} on SARS-CoV-2 RNA recovery from swabs reported overall good recovery rates, and many factors were found to play a role (including swab type, storage solution, molecular processing), but especially the sampling itself. In our study, these factors remained the same throughout the study period, including collection of samples, which was strictly performed according to protocol by the same fieldworker, preventing potential differential errors. Absolute SARS-CoV-2 RNA levels in our study will be more or less underestimated depending on recovery efficiency. More experimental research on aspects such as viability and recovery of SARS-CoV-2 while in the environment is warranted to address important knowledge gaps, eventually enabling risk characterisation of SARS-CoV-2 environmental contamination.

In conclusion, infected mink farms can be highly contaminated with SARS-CoV-2 RNA in airborne dust, on surfaces and in various other environmental matrices. Dispersion of SARS-CoV-2 to outdoor air was limited, which implies a negligible risk of environmental exposure to neighbouring residents.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The Medical Research Ethics Committee (MREC) Utrecht confirmed that the Medical Research Involving Human Subjects Act (WMO) does not apply to (personal) air sampling and therefore an official approval of this study by the MREC Utrecht was not required under the WMO (protocol 20-385/C, reference number WAGM/mb/20/021975).

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Supplemental Information accompanying manuscript

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<table>
<thead>
<tr>
<th>Section</th>
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<tbody>
<tr>
<td>Supplemental Methods</td>
<td>Part A, Part B, Part C</td>
</tr>
<tr>
<td>Supplemental Tables</td>
<td>Table S1, Table S2, Table S3</td>
</tr>
<tr>
<td>Supplemental Figures</td>
<td>Figure S1, Figure S2, Figure S3, Figure S4, Figure S5</td>
</tr>
</tbody>
</table>
Supplemental Methods

Supplemental Methods A. Background information on investigated farms

The first SARS-CoV-2 infected mink farms were investigated thoroughly including sampling of animals, humans and the environment. These farms were all situated in the region of the Netherlands with the highest mink farm density, the eastern part of the province of Noord-Brabant (NB). On April 23rd, SARS-CoV-2 infection was established in the first farm, NB1, which has two separate locations, NB1A and NB1B, which are 115m apart. Two days later another farm was diagnosed positive, NB2, situated at 14 km distance from NB1. For NB1 as well as NB2, the source of the outbreak amongst minks was traced back to an SARS-CoV-2 infected farmer/worker at the specific farm. Results of whole-genome sequencing research indicated the virus strain at NB2 to be different from NB1, thus underlining the occurrence of two separate anthropozoonotic transmission events2,9. Both NB1 and NB2 experienced increased mortality amongst the minks coinciding with respiratory signs starting in the first half of April but, as it was unprecedented, initially SARS-CoV-2 infection was not suspected. Serological findings suggested, based on a random subset of minks tested, many minks to already have seroconverted by the time of diagnosis, indicating the outbreak to be indeed circulating for a while. This was confirmed by sequencing research showing considerable viral genomic diversity at the time of diagnosis. Animal investigations performed from the moment of diagnosis onwards, indicated the number of infected minks to be decreasing over time thus substantiating that both NB1 and NB2 were in a later phase of the outbreak when detected. From 28th of April onwards, environmental sampling started at and around NB1 and NB2. On May 6th, SARS-CoV-2 infection was detected at two more farms, NB3 and NB4. NB4 was, in contrast to the other farms, at the time of diagnosis in a more early phase of the outbreak based on disease history, serology and sequence diversity. Therefore, NB4 was included for environmental sampling from May 13th onwards, while NB3 was not included. For a detailed description on the course of the outbreak in the farmed minks, clinical/pathological findings, and genomic epidemiology see Oreshkova et al2, Molenaar et al10, and Oude Munnink et al9.

Minks kept at NB1, NB2 and NB4 were housed in wire netting cages placed in halls. Halls are naturally ventilated via both large openings in the roof as well as walls which are only (partially) closed in the winter months. Cages are arranged in long single rows, on one side separated by a narrow manure conveyer belt and on the other side by a feeding alley (see figure S2 and S3 for pictures). In the front of each cage is a sleep/nest box which contains bedding material. Solid boards are attached onto the sides of the cage to prevent direct contact between minks in bordering cages. Per cage, one adult mink is housed and if present kits are kept with their mothers. Whelping takes place once a year in the period end of April/beginning of May. The population before whelping consisted of: 8971 females and 24 males at NB1A, 2923 females and 1699 males at NB1B, 7500 females and 90 males at NB2, and 10300 females and 242 males at NB4.
52  **Timeline of the outbreaks at the mink farms and environmental sampling**

![Timeline diagram showing outbreaks and environmental sampling]

- **NB1A, NB1B, NB2**
  - Farm visit: T1, T2, T3
  - Continuous outdoor air sampling: 28/04 until 21/05

- **NB4**
  - Farm visit: 19/05
  - Continuous outdoor air sampling: 13/05 until 02/06

- **Residential sites (N=3)**
  - Continuous outdoor air sampling: 30/04 until 21/05

54  **Note.** T1: NB1A 28/04, NB2 30/04, NB1B 02/05
55  T2: NB1A 05/05, NB2 07/05, NB1B 09/05
56  T3: NB1A 12/05, NB2 14/05, NB1B 16/05
57  Mortality is expressed in percentage of naturally deceased minks per week.
58  GD Animal Health is acknowledged for providing the data on mortality
Supplemental Methods B. Additional information on sampling

Technical details on air sampling (see figure S2 for pictures)

Air sampling was performed by means of filter-based techniques using teflon filters (Pall Corporation, Ann Arbor, USA). Air sampling was performed in parallel to measure both Particulate Matter 10 (PM_{10}) and inhalable dust based on the by European norm (EN) defined size fractions\textsuperscript{12,13}. PM_{10} is defined by particles of a nominal aerodynamic diameter of 10 µm or less, particles of this size or smaller can penetrate the tracheobronchial regions of the airways. Inhalable dust covers all particles that may enter the nose and mouth thus including small particles (PM_{10}) but also larger particles (inhalable criterion: 100% penetration for particles <10µm, dropping to 50% for 100µm particles, no median cut-off aerodynamic diameter).

Outdoor air sampling

Outdoor air sampling involved continuous multi-day sampling in order to measure high volume of air per sample as levels in the outdoor environment were not expected to be high. Multiple-day sampling of PM_{10} was performed by means of Harvard impactors (Air Diagnostics and Engineering Inc., Naples, ME, USA) which selectively sample PM_{10} by trapping larger particles on an impaction plate. Harvard impactors were connected to self-designed air pumps with critical orifices calibrated at a flow of 10.0 l/min (optimal flow rate for this sampling head type). The air flow was checked before and after sampling using a calibrated rotameter. Sampling duration was typically 4 days (hence 57.6 m³ air sampled), minimally 3 days (43.2 m³ air) and maximally 5 days (72 m³ air). To enable sampling of total suspended particles the impaction plate was removed from the Harvard impactor for a series of measurements at farm NB4. For inhalable dust sampling, GSP (Gesamtstaubprobenahme; JS Holdings, Stevenage, UK) sampling heads were used connected to a Gilian GilAir 5 pump (Sensidyne, St. Petersburg, USA) calibrated at a flow of 3.5 l/min (optimal flow rate for this sampling head type). The air flow was checked before and after sampling using a calibrated rotameter. Sampling duration was typically 4 days (hence 20.16 m³ air sampled), minimally 3 days (15.12 m³ air) and maximally 5 days (25.2 m³ air). Sampling heads were attached side-by-side onto a pole at 1.50m height (average breathing height of humans); see Figure S2 for pictures. Field blank controls were collected each measurement period, a Harvard impactor field blank and a GSP sampling head field blank. These field blanks underwent the same procedure except that no air was drawn through the sampling device.

Sampling inside farm and downwind/upwind

Six-hour stationary air sampling was performed and 8-hour personal air sampling by using Gilian GilAir 5 pumps with battery pack. Inhalable dust sampling was performed by means of GSP sampling heads at a flow of 3.5 l/min. PM_{10} Sampling was performed by means of PEM (Personal Environmental Monitor) sampling heads (MSP Corporation, Minnesota, USA) at a flow of 4.0 l/min (optimal flow rate for this sampling head type) which selectively samples PM_{10} by trapping larger particles on an impaction plate. The air flow was checked before and after sampling using a calibrated rotameter. For inhalable dust samples, 6-hour sampling resulted in 1.26 m³ air sampled and 8-hour sampling in 1.68 m³. For PM10 samples, 6-hour sampling resulted in 1.44 m³ air sampled and 8-hour sampling in 1.92 m³. Stationary air sampling was performed by attachment of the sampling heads side-by-side onto a pole at 1.50m height (average breathing height). Personal air samples were collected by attachment of the sampling heads within the breathing zone of the fieldworker and the pumps clipped on a belt; see Figure S2 for pictures of the measurement set-up. Inside the farm, stationary air sampling was performed at three spots distributed within the farm which remained the same per farm. The spots of the six-hour air sampling outside the farm were based on the wind direction of the measurement day. Upwind sampling was performed at 50 meter distance from the farm, and downwind sampling at 10-20 meter and 100 meter distance. Field blank controls were collected each measurement day, a GSP sampling head field blank and a PEM sampling head field blank. These field blanks underwent the same procedure except that no air was drawn through the sampling device. EDC samplers were placed in cardboard boxes on top of the minks’ housing units, on hanging PVC plates and on stands (see Figure S2 for pictures). EDC field blank control was collected each EDC measurement period. These
EDC field blanks underwent the same procedure including opening and closing of the folder except for being opened for 7 days inside the farm.

Technical details on sampling minks’ housing units (see figure S3 for pictures)

Swipe samples were collected by sterilized electrostatic cloths (polyester electrostatic cloth; Albert Heijn, Zaandam, the Netherlands) to collect material settled on hardboard rim. The swiped surface was 135mm in length and 65mm in width. Before every sampling, clean gloves were worn. Cloths were not-wetted. Swabbing of the drinker cup was performed by rolling the swab (Cultiplast (dry sterile swab rayon/plastic); LP Italiana, Milano, Italy) on the rim. Swabs were not-wetted. Bedding material was collected from the night/nest box by scooping. Pre-culling a pooled sample of both the top layer and bottom layer was collected. Post-culling, the top layer and bottom layer were sampled separately. Food residues were collected by scraping off the wire with remaining residues. Faecal material was collected by scooping either from within the cage when present or from the floor beneath the cage. These collected materials (bedding, faeces, food) were stored in containers (Cellstar tubes; Greiner bio-one; Kremsmünster, Austria).

Details on fieldworkers

All fieldworkers wore PPE including full face mask (silicon mask 6800 with filters 6035; 3M, St. Paul, U.S.A.) and coverall (Tyvek 500 Xpert Blue; Dupont, Wilmington, U.S.A.); fieldworkers remained SARS-CoV-2 negative.
**Supplemental Methods C. Details on laboratory analyses**

**Sample processing for storage at the end of fieldwork day**

For the sampling heads used for air measurements, this involved disassembly to collect the exposed filter and transfer it to an enclosed tube (Greiner 15ml, DNA/RNA-ase free). For the EDCs, this involved collection of the exposed electrostatic cloth out of the holder and place it in an enclosed tube (Greiner 50ml, DNA/RNA-ase free). The other samples were stored directly in their collection container at -80°C.

**Further processing for RNA extraction**

Per sample type, an adequate processing procedure was performed.

The exposed Teflon air filters, stored at -80°C in 15 ml Greiner tubes with the dust-surface facing the inner of the tube were thawed in the safety-cabinet of the BSL-2 lab. Using a 5 ml sterile pipet 4 ml of PBS-Dulbeco’s was added. To submerge the complete filter of 37 mm in diameter in the fluid, the filter was pushed carefully, without damaging the Teflon surface, to the bottom of the tube using the tip of this 5 ml pipet. Tubes were placed on an end-over-end roller and after 90 minutes of rolling at room temperature the tubes were placed vertical for a few minutes allowing the fluid to drip to the bottom of the tube. Using a 2 ml pipet the fluid and large dust particles that sank to the bottom, were homogenised and 1 ml of the suspension was transferred to a clean Eppendorf tube. After vortexing the Eppendorf, 200 µl of suspension was mixed with 200 µl lysis buffer of the ID Gene™ Mag Fast Extraction Kit (ID-VET) and RNA extraction was performed on the KingFisher (ThermoFisher). The remaining suspensions were stored at -80°C for potential virus isolation.

EDC cloths stored at -80°C in 50 ml Falcon tubes with the swiped surface facing the inner of the tube were thawed in the safety-cabinet of the BSL-2 lab. Using a sterile pipet 10ml PBS-PEFABLOC buffer was added (PBS-PEFABLOC buffer: 1% v/v of stock solution in PBS-Dulbecco’s. Stock solution: 1 PEFABLOC tablet [Sigma Aldrich, Zwijndrecht, the Netherlands] dissolved in 1ml PBS-Dulbecco’s and stored in aliquots at -20°C) and rolled for 1 hour on an end-over-end roller. Two hundred µl of the suspension with dust particles was collected from each tube and mixed with lysis buffer in a similar manner as described above for Teflon air filters.

Approximately 0.5 ml bedding material was transferred to a 15ml Falcon tube to which 3ml PBS-PEFABLOC buffer was added. After vortexing, tubes were incubated one hour at room temperature and centrifugated 10 minutes at 2500xg. Two hundred µl of intermediary fluid was collected and mixed with 200 µl of lysis buffer for RNA extraction.

For food residue samples as faecal material, approximately 0.5 ml was transferred to a 15ml Falcon tube. To each tube 2 ml PBS-PEFABLOC buffer was added and tubes were vortexed vigorously until the material was properly suspended. After incubation of one hour at room temperature, tubes were centrifuged for 10 minutes at 2500xg rpm and 200 µl of the supernatant was collected and mixed with 200 µl of lysis buffer for RNA extraction.

Sampled swabs from drinker cups) were transferred to a 15ml Falcon tube with 2 ml of medium used for maintaining Vero-E6 cells (Minimum Essential Medium with 5% v/v foetal bovine serum (Gibco, Thermo Fisher Scientific, Bleiswijk, The Netherlands), 1% v/v Antibiotic-Antimycotic mixture (anti-anti, Gibco), 1% L-Glutamine (Gibco), 1% nonessential amino acids (Gibco). Tubes were vortexed and incubated for one hour at room temperature. After centrifugation for 10 minutes at 2500 g, 200 µl of the supernatant was collected and mixed with 200 µl of lysis buffer for RNA extraction. See Supplemental Table S1 for a schematic overview of processed volume per sample type.

**PCR**

Subsequently, samples were tested for SARS-CoV-2 using the accredited E gene PCR as described by Corman et al. using the TaqMan Fast virus 1-Step Master Mix (Applied Biosystems), with minor modification in the reverse transcription conditions (52°C for 10 min). The calibration curve (-3 * log10(copies) + 39.249) was established based on serial dilutions of inactivated cell culture produced SARS-CoV-2 with known titer (SARS-CoV-
Samples of the same type were tested in one or maximally two runs. Negative controls (no template) and positive controls were included in each PCR run. To each sample, an internal control (intype IC-RNA, Qiagen) was added to check for inhibition of reverse transcription and amplification. Samples with Ct values below the threshold Ct, were defined positive. The threshold Ct was set at 36 based on optimization of sensitivity and specificity analysed by means of the ROC curve using the function spEqualSe from R package OptimalCutpoints.

**Attempted virus isolation from air filters**

After establishing the presence of SARS-CoV-2 RNA by E gene qPCR in air samples, samples with a Ct value below 32 were subjected to virus isolation using Vero-E6 cells. After thawing, 400 hundred µl of suspension was used to infect monolayers containing of $1 \times 10^6$ Vero-E6 cells (ATCC) grown in T25 cm$^2$ flask. Cells were cultured in MEM supplemented with 5% FCS, 1% antibiotic/antimycotic, 1%, glutamine and 1% non-essential amino acids at 37°C and 5% CO2. Monolayers were inspected regular with a microscope to identify the typical cytopathogenic effect induced by SARS-CoV-2. After five days of growth 200 µl of the medium was analysed by E-gene qPCR to detect replication of SARS-CoV-2.
### Supplemental Table S1. Schematic overview of quantitative Polymerase Chain Reaction analysed proportion per sample type

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Original sample</th>
<th>Volume of medium added (ml)</th>
<th>Volume aliquot of sample for qPCR (ul)</th>
<th>Volume lysisbuffer added to aliquot (ul)</th>
<th>Volume eluens (ul)</th>
<th>Volume in qPCR (ul)</th>
<th>Proportion volume qPCR/volume eluens</th>
<th>Proportion volume aliquot/sample volume medium</th>
<th>Proportion subsample of original sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teflon filter (filter used in active air sampling)</td>
<td>1 filter ~ cubic meter of air sampled dependent on flow rate and sampling duration</td>
<td>4.00</td>
<td>200.00</td>
<td>200</td>
<td>60</td>
<td>5</td>
<td>0.083</td>
<td>0.05</td>
<td>0.004167</td>
</tr>
<tr>
<td>EDC</td>
<td>1 EDC cloth ~ 0.2035 m*2</td>
<td>10.00</td>
<td>200.00</td>
<td>200</td>
<td>60</td>
<td>5</td>
<td>0.083</td>
<td>0.02</td>
<td>0.001667</td>
</tr>
<tr>
<td>Bedding material</td>
<td>1 patch ~ 0.5 ml</td>
<td>3.00</td>
<td>200.00</td>
<td>200</td>
<td>60</td>
<td>5</td>
<td>0.083</td>
<td>0.067</td>
<td>0.005556</td>
</tr>
<tr>
<td>Food residue</td>
<td>1 scoop ~ 0.5 ml</td>
<td>3.00</td>
<td>200.00</td>
<td>200</td>
<td>60</td>
<td>5</td>
<td>0.083</td>
<td>0.067</td>
<td>0.005556</td>
</tr>
<tr>
<td>Swab drinker cup</td>
<td>1 swab ~ swabbed rim drinker cup: 17 cm diameter</td>
<td>3.00</td>
<td>200.00</td>
<td>200</td>
<td>60</td>
<td>5</td>
<td>0.083</td>
<td>0.067</td>
<td>0.005556</td>
</tr>
<tr>
<td>Faecal material</td>
<td>1 scoop ~ 0.5 ml</td>
<td>3.00</td>
<td>200.00</td>
<td>200</td>
<td>60</td>
<td>5</td>
<td>0.083</td>
<td>0.067</td>
<td>0.005556</td>
</tr>
<tr>
<td>Swipe normal size</td>
<td>1 normal sized swipe ~ swiped hardboard: 60cm long and 2cm width</td>
<td>5.00</td>
<td>200.00</td>
<td>200</td>
<td>60</td>
<td>5</td>
<td>0.083</td>
<td>0.04</td>
<td>0.003333</td>
</tr>
<tr>
<td>Swipe large size</td>
<td>1 large sized swipe ~ swiped hardboard: 60cm long and 2cm width</td>
<td>10.00</td>
<td>200.00</td>
<td>200</td>
<td>60</td>
<td>5</td>
<td>0.083</td>
<td>0.02</td>
<td>0.001667</td>
</tr>
</tbody>
</table>
Supplemental Table S2. Results of multivariable modelling on SARS-CoV-2 RNA load in settling dust sampled by means of EDCs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Virus load (copies/m² per day)</th>
<th>Ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm NB1A (indicator)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Farm NB1B</td>
<td>0.43*</td>
<td>(0.21-0.90)</td>
</tr>
<tr>
<td>Farm NB2</td>
<td>15*</td>
<td>(6.8-32)</td>
</tr>
<tr>
<td>Timepoint 1 (indicator)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Timepoint 2</td>
<td>0.37*</td>
<td>(0.19-0.72)</td>
</tr>
<tr>
<td>Timepoint 3</td>
<td>0.075*</td>
<td>(0.038-0.15)</td>
</tr>
<tr>
<td>Positioned in close proximity to minks</td>
<td>2.3*</td>
<td>(1.1-4.9)</td>
</tr>
</tbody>
</table>

Note. Censored regression was applied. Associations expressed in ratio’s = estimate of associations to the power 10 to represent ratio in viral load. Marked * then P-value < 0.05
**Supplemental Table S3.** Comparisons of SARS-CoV-2 RNA detection in 14 housing units at NB4 sampled pre- and post-culling

<table>
<thead>
<tr>
<th>Sample type</th>
<th>% positive samples pre-culling</th>
<th>% positive samples post-culling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swipe</td>
<td>100%</td>
<td>14%</td>
</tr>
<tr>
<td>Bedding material</td>
<td>Mix of layers top and bottom: 100%</td>
<td>Bottom layer: 85% Top layer: 57%</td>
</tr>
<tr>
<td>Faeces material</td>
<td>71%</td>
<td>21%</td>
</tr>
<tr>
<td>Swab drinker cup</td>
<td>50%</td>
<td>0%</td>
</tr>
<tr>
<td>Food residue</td>
<td>14%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Supplemental Figures

Supplemental Figure S1. Map showing the sampling locations at farm NB4. The blue blocks are mink stables.
Supplemental Figure S2. Pictures showing sampling of air and settling dust

Picture showing personal air sampling (1), stationary air sampling (2) and sampling of settling dust in close proximity of minks (3).

Close-up of EDC placed in cardboard box
Pictures showing sampling of settling dust at further distances from minks; left by placement of EDC on hanging PVC plate (red circle), right by placement of EDC on stand in empty row
Supplemental Figure S3. Pictures showing sampling of mink’s housing units

Picture of mink’s housing unit

<table>
<thead>
<tr>
<th>Number</th>
<th>Sample type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Swipe</td>
</tr>
<tr>
<td>2</td>
<td>Bedding material</td>
</tr>
<tr>
<td>3</td>
<td>Food residue</td>
</tr>
<tr>
<td>4</td>
<td>Swab of drinker cup</td>
</tr>
<tr>
<td>5</td>
<td>Faeces material</td>
</tr>
</tbody>
</table>

Picture showing typical way of feeding minks by placing suspension of raw meat on top of the cage
1. Collection of swipe

2. Collection of bedding material
3. Collection of food residue

4. Collection of swab of drinker cup
5. Collection of faeces material
Supplemental figure S4. Overview of viral load in swipes and bedding material samples per farm in housing units belonging to recently deceased minks versus minks still alive

Note. Percentiles: 25th, 50th and 75th of virus load detected in swipes of 2.06E+06, 6.03E+06, 1.60E+07; respectively.

Percentiles: 25th, 50th and 75th of virus load detected in bedding material of 6.36E+04, 8.16E+05, 7.41E+06; respectively.
**Supplemental Figure S5.** Overview of viral loads in bedding material collected pre-culling versus post-culling (divided in bottom layer sampling and top layer sampling)

Note. Pre = bedding material samples collected pre-culling
Post-B = bedding material samples collected post-culling of bottom layer
Post-T = bedding material samples collected post-culling of top layer