Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a pandemic in humans. Farmed mink (*Neovison vison*) are also susceptible. In Denmark, this virus has spread rapidly among farmed mink, resulting in some respiratory disease. Full-length virus genome sequencing revealed novel virus variants in mink. These variants subsequently appeared within the local human community.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the ongoing coronavirus disease (COVID-19) pandemic (1). Ferrets, cats, dogs, Syrian hamsters, and nonhuman primates can be infected with the virus and, in some cases, transmit it (2); however, other species, such as pigs and chickens, appear resistant (3,4). Thus, the virus has a restricted host range. Infection with SARS-CoV-2 has occurred in farmed mink in the Netherlands (5).

In Denmark, there are ≈1,200 mink farms (6). Because of contacts between persons with COVID-19 and mink farms, investigation of SARS-CoV-2 infection within mink in Denmark was undertaken. We documented 3 premises in the Northern Jutland region of Denmark with SARS-CoV-2-infected mink and analyzed virus transmission in mink and the local human community.

The Study

We collected blood and throat, nasal, and fecal swab samples from mink adults and kits (Table 1); we also sampled feed and air. We assayed viral RNA by quantitative reverse transcription PCR (qRT-PCR) (7). We performed SARS-CoV-2 Ab ELISA (Beijing Want-ai Biological Pharmacy Enterprise, http://www. ystwt.cn) as described (R. Lassaunière et al., unpub. data, https://doi.org/10.1101/2020.04.09.20056325).

SARS-CoV-2–positive RNA samples were sequenced and sequences aligned using Mafft (https://mafft. cbrc.jp/alignment/server/index.html). Phylogenetic analysis was performed in MEGA 10.1.7 (8) using the maximum-likelihood general time reversible plus invariant sites plus gamma (2 categories) method (9).

We selected mink farms for investigation because of COVID-19 in persons linked to them. During initial visits, we sampled 30 apparently healthy adult mink; we tested adults and kits in follow-up visits. We analyzed serum samples for SARS-CoV-2 antibodies and assayed swab samples for SARS-CoV-2 RNA (Table 1; Appendix, https://wwwnc.cdc.gov/EID/ article/27/2/20-3794-App1.pdf). At initial sampling, seroprevalence was high on farm 1 (>95%) and farm 3 (66%) but, in contrast, only 3% on farm 2. However, after the infection spread widely on farm 2, indicated by the increased prevalence of viral RNA (Table 1), a large increase in seroprevalence occurred, to >95%.

Air samples from farm 1 tested negative. However, on farms 2 and 3, multiple samples collected from exhaled air from mink or within 1 m of the cages scored positive, albeit with fairly high (>31) Ct values. None of the air samples collected outside the houses were positive. Feed samples collected at each farm tested negative. We also sequenced SARS-CoV-2 RNA from samples from each mink farm. The viruses found on farms 1–3 were very similar (Table 2). These sequences and those from humans (H1–H9) linked to
**Table 1.** Summary of laboratory analyses of mink samples from 3 mink farms tested for severe acute respiratory syndrome coronavirus 2 in Denmark, June–July 2020

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>ELISA</th>
<th>qRT-PCR</th>
<th>Date of sample collection</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live adult mink</td>
<td>Serum</td>
<td>Throat swabs</td>
<td>Nasal swabs</td>
<td>Fecal swabs</td>
</tr>
<tr>
<td>Dead adult mink</td>
<td>NA</td>
<td>NA</td>
<td>5/30 (17)</td>
<td>2020 Jun 14</td>
</tr>
<tr>
<td>Live mink kits</td>
<td>NA</td>
<td>NA</td>
<td>4/100 (100)</td>
<td>2020 Jun 14</td>
</tr>
<tr>
<td>Live adult mink</td>
<td>NA</td>
<td>3/30 (10)</td>
<td>3/30 (10)</td>
<td>1/30 (3)</td>
</tr>
<tr>
<td>Retested adult mink</td>
<td>30/30 (100)</td>
<td>32/33 (13)</td>
<td>NA</td>
<td>0/23 (0)</td>
</tr>
<tr>
<td>Live adult mink</td>
<td>4/14 (100)</td>
<td>2/5 (40)</td>
<td>2/4 (50)</td>
<td>1/24 (50)</td>
</tr>
<tr>
<td>Dead adult mink</td>
<td>1/30 (3)</td>
<td>NA</td>
<td>NA</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>Dead adult mink</td>
<td>NA</td>
<td>1/10 (13)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Live mink kits</td>
<td>1/50 (2)</td>
<td>40/50 (80)</td>
<td>39/50 (78)</td>
<td>NA</td>
</tr>
<tr>
<td>Live adult mink</td>
<td>3/50 (6)</td>
<td>46/50 (92)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Dead adult mink</td>
<td>1/3 (33)</td>
<td>2/6 (66)</td>
<td>2/3 (66)</td>
<td>NA</td>
</tr>
<tr>
<td>Dead adult mink</td>
<td>NA</td>
<td>3/3 (100)</td>
<td>3/3 (100)</td>
<td>NA</td>
</tr>
<tr>
<td>Live adult mink (retest)</td>
<td>36/37 (97)</td>
<td>35/37 (95)</td>
<td>37/37 (100)</td>
<td>NA</td>
</tr>
<tr>
<td>Live adult mink</td>
<td>20/30 (67)</td>
<td>66/67 (100)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Dead adult mink</td>
<td>NA</td>
<td>5/5 (100)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Live mink kits</td>
<td>24/30 (80)</td>
<td>30/30 (100)</td>
<td>27/30 (90)</td>
<td>NA</td>
</tr>
<tr>
<td>Live adult mink</td>
<td>23/30 (77)</td>
<td>30/30 (100)</td>
<td>26/30 (87)</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not applicable; qRT-PCR, quantitative reverse transcription PCR; *Data from 30 mink were assayed in 6 pools of 5 swabs each.

**Table 2.** Location of nt differences identified in genome sequences of selected severe acute respiratory syndrome coronavirus 2 samples from mink and humans in Denmark, June–July 2020, compared with Wuhan and clade 20B reference sequences

<table>
<thead>
<tr>
<th>Virus sample</th>
<th>Genomic location and nt position</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCO455512 (Wuhan)</td>
<td>C</td>
</tr>
<tr>
<td>Humans in Jutland (to 2020 Jun 10)</td>
<td>T</td>
</tr>
<tr>
<td>Mink_AL25_Farm 1</td>
<td>T</td>
</tr>
<tr>
<td>Mink_AL30_Farm 1</td>
<td>T</td>
</tr>
<tr>
<td>Mink_AL40_Farm 1</td>
<td>T</td>
</tr>
<tr>
<td>Mink_AL40_Farm 3</td>
<td>T</td>
</tr>
<tr>
<td>H1/H7 + H9</td>
<td>T</td>
</tr>
<tr>
<td>In NB01 (NL) 6</td>
<td>T</td>
</tr>
<tr>
<td>In NB02 (NL) 6</td>
<td>C</td>
</tr>
<tr>
<td>In NB03 (NL) 6</td>
<td>T</td>
</tr>
<tr>
<td>In NB04 (NL) 6</td>
<td>T</td>
</tr>
<tr>
<td>Humans in Jutland (to 2020 Jul 1)</td>
<td>T</td>
</tr>
</tbody>
</table>

*Red text indicates nt differences from the Wuhan reference strain; pink shading indicates nt changes detected in mink and in human contacts (H1–H9) that differ from the clade 20B and index case; gray shading indicates a reference clade 20B sequence and the human index case sequence. NA, not applicable, as nt change in the noncoding region; ND, not determined; NL, the Netherlands; ORF, open reading frame.

† The proportions of each nt present at each of these positions in human sequences in Jutland are shown in Appendix Table 1 (https://wwwnc.cdc.gov/EID/article/27/2/20-3794-App1.pdf).

‡ Mink samples from the Netherlands also differ at other locations compared with the Wuhan sequence.

§ The mink sequences from the Netherlands also differ at other locations compared with the Wuhan sequence.

¶ Mink AD40_Farm 2 has a different nt sequence at this location.

†† Mink_AL30_Farm 3 has a different nt sequence at this location.

‡‡ Mink_AL40_Farm 3 has a different nt sequence at this location.

# Mink_AL40_Farm 3 has a different nt sequence at this location.
the infected farms grouped within the European 20B clade of the global SARS-CoV-2 tree (10,11) (Figure; Appendix Table 1). We deposited the SARS-CoV-2 genome sequences of virus from farm 1 (SARS-CoV-2/mink/DK/AD3_Farm1/2020) in GenBank (accession nos. MT919525–36). The sequences closely matched those of a human case, diagnosed in mid-May, with a direct epidemiologic link to farm 1. This index sequence (only 91% complete) matched the mink viruses at nt 15656 (rare globally) but had A at nt 22920 (Table 2). The nt 25936 in the index case could not be determined. The local phylogeny (Appendix Figure) showed that mink sequences from farm 1 fell into 3 subclusters (defined by the nucleotide changes at positions 5421 and 22920), but sequences from linked humans (H1–H9) and mink in farms 2 and 3 were within subcluster 2 (Appendix Figure).

We found 9 to 11 nt differences (mainly nonsynonymous) between the mink sequences in Denmark and the Wuhan-Hu-1 reference sequence (Table 2). One mutation at nt 23403 (resulting in substitution D614G in the spike protein) was present in all sequences from mink in Denmark and the Netherlands, except for NB02 from the Netherlands (Table 2) and was predominant in the human population in Jutland (Appendix Table 1) and globally (12). However, another mutation (nt C25936T [as cDNA] encoding H182 to Y within ORF3a) appeared in all mink sequences from Denmark (Table 2) and in human cases (H1–H9) linked to them. This change was not found in human SARS-CoV-2 sequences from Jutland before June 10, 2020 (Appendix Table 1), but reached ≈40% frequency during June 10–July 1, 2020 (Table 2; Appendix Table 2). This mutation has been found only rarely in other SARS-CoV-2 sequences (11) (Appendix Table 1) but was in mink farm NB03 from the Netherlands (SARS-CoV-2/mink/NED/NB03_index/2020; GenBank accession no. MT457400.1).

Another mutation in the spike gene (A22920T, encoding Y453 to F) was present in 4 of 8 sequences from farm 1, in all sequences from farms 2 and 3, and in 5 of 6 sequences from farm NB02 in the...
Netherlands (5). This change was not in the index case or the human population anywhere before June 10 but was subsequently detected in farm-linked humans (H1–H9) and in Jutland (Table 2; Appendix Table 2). Finally, the mutation in the open reading frame 1b gene (C15656T, encoding T730 to I) was present only in mink/human sequences from Denmark (Table 2) and a sequence from New Zealand (Appendix Table 1).

Conclusions
A high proportion of mink on farms can be infected with SARS-CoV-2 within a few days, which may provide major virus exposure to persons working with mink. The infections we describe here occurred with little clinical disease or increase in death (Appendix), making it difficult to detect the spread of infection; thus, mink farms could represent a serious, unrecognized animal reservoir for SARS-CoV-2. There is no evidence for spread of the virus outside of farm buildings, either in Denmark or in the Netherlands (5), except by infected persons. However, there appears to be some risk of virus transmission to persons working with infected mink as well as for their contacts and thus, indirectly, for the public.

On farm 1, the virus had probably been introduced some weeks before detection (Table 1). On farm 2, the low frequency (4%) of seropositivity and the high proportion of qRT-PCR positive animals at second sampling (Table 1) suggested that the virus had been recently introduced but was spreading. Indeed, a third sampling (8 days later) showed a much higher seroprevalence (>90%). Conceivably, the variant viruses that appeared in farm 1 and spread to farms 2 and 3 may be better adapted to mink and thus able to transmit rapidly. The infection at farm 3 was detected relatively late, with a high seroprevalence (66%) at first visit.

A likely scenario for the spread of infection in mink in Denmark is that the index human case-patient, who had nt T15656 introduced it into farm 1. Initially, we observed sequence heterogeneity at nt 22920 in mink on farm 1, but subsequently, we detected only the variant form (T22920) on farms 2 and 3 and in subsequent linked human cases (H1–H9) (Table 2). Remarkably, this heterogeneity also occurred on farm NB02 in the Netherlands. This change, possibly together with the mutation at nt 25936 (Table 2), may represent virus adaptation. It is not yet established whether these changes confer advantages in mink, but the variant viruses in farm 2 spread rapidly. It seems that the variant viruses on farm 1 spread to ≥1 human and were then transmitted, presumably by human–human contact, to other persons and to farms 2 and 3. The change at nt 22920 results in substitution Y453F in the S-protein (Table 2). This Y-residue, within the receptor-binding motif of the S-protein, is highly conserved among SARS-related coronaviruses and is close to residue L455 that is critical for interaction with the cellular ACE2 receptor (13).

Acknowledgments
We thank Mads Albertsen for guidance with Nanopore sequencing and Henrik B. Krarup for providing human samples containing SARS-CoV-2. We gratefully acknowledge the provision of genetic sequence data shared via GISAID (https://www.gisaid.org; see Appendix Table 3, https://wwwnc.cdc.gov/EID/article/27/2/20-3794-App1.pdf). We also thank Amalie E. Bedsted and Thea Kristensen for careful reading of the manuscript.

About the Author
Dr. Hammer, an associate professor at the University of Copenhagen, is a veterinary pathologist with special interest and expertise in pathological methods applied in diagnostics, research, and surveillance of diseases in fur animals and wildlife. Her research focus has been mainly on viral diseases of carnivorous species.

References

Address for correspondence: Anette Bøtner, Department of Veterinary and Animal Sciences, University of Copenhagen, Grønnegårdsvej 15, 1870 Frederiksberg C, Denmark; email: aneb@sund.ku.dk

May 2020

Respiratory Viruses

- Surveillance of Leprosy in Kiribati, 1935–2017
- Biphasic Outbreak of Invasive Group A Streptococcus Disease in Eldercare Facility, New Zealand
- Epidemiology of Tick-Borne Relapsing Fever in Endemic Area, Spain
- Food Safety and Invasive Cronobacter Infections during Early Infancy, 1961–2018
- Clinical Outcomes of Patients Treated for Candida auris Infections in a Multisite Health System, Illinois, USA
- Mosquito Control Activities during Local Transmission of Zika Virus, Miami-Dade County, Florida, USA, 2016
- Blastomycosis in Minnesota, USA, 1999–2018
- Effectiveness of Live Poultry Market Interventions on Human Infection with Avian Influenza A(H7N9) Virus, China
- Systematic Review and Meta-Analysis of Sex Differences in Social Contact Patterns and Implications for Tuberculosis Transmission and Control
- Effects of Air Pollution and Other Environmental Exposures on Estimates of Severe Influenza Illness, Washington, USA
- Epidemiologic and Clinical Progression of Lobomycosis among Kaiabi Indians, Brazil, 1965–2019
- Rhizopus microsporus Infections Associated with Surgical Procedures, Argentina, 2006–2014
- Zika Virus Circulation in Mali
- Possible Transmission Mechanisms of Mixed Mycobacterium tuberculosis Infection in High HIV Prevalence Country, Botswana
- Nonpharmaceutical Measures for Pandemic Influenza in Nonhealthcare Settings—International Travel-Related Measures
- Nonpharmaceutical Measures for Pandemic Influenza in Nonhealthcare Settings—Personal Protective and Environmental Measures
- Nonpharmaceutical Measures for Pandemic Influenza in Nonhealthcare Settings—Social Distancing Measures
- Candidatus Rickettsia xinyangensis as Cause of Spotted Fever Group Rickettsiosis, Xinyang, China, 2015
- Pretreatment Out-of-Pocket Expenses for Presumptive Multidrug-Resistant Tuberculosis Patients, India, 2016–2017
- Capybara and Brush Cutter Involvement in Q Fever Outbreak in Remote Area of Amazon Rain Forest, French Guiana, 2014
- Women’s Awareness and Healthcare Provider Discussions about Zika Virus during Pregnancy, United States, 2016–2017
- Genetic Characterization of Japanese Encephalitis Virus Genotype 5 Isolated from Patient, South Korea, 2015
- Update on Ebola Treatment Center Costs and Sustainability, United States, 2019
- A Neighbor-Based Approach to Identify Tuberculosis Exposure, the Kopanyo Study
- Species Distribution and Isolation Frequency of Nontuberculous Mycobacteria, Uruguay
- Crimean-Congo Hemorrhagic Fever Virus Endemicity in United Arab Emirates, 2019
- Zika Inquiries Made to the CDC-INFO System, December 2015–September 2017
- Serologic Detection of Middle East Respiratory Syndrome Coronavirus Functional Antibodies
- Novel Ehrlichia Strain Infecting Cattle Tick Amblyomma neumanni, Argentina, 2018

To revisit the May 2020 issue, go to: https://wwwnc.cdc.gov/eid/articles/issue/26/5/table-of-contents