TRACKING TWO-WAY HUMAN-MINK TRANSMISSION DURING AN OUTBREAK OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 ON A FARM IN BULGARIA

Ivelina Trifonova [1], Neli Korsun [1], Ivailo Alexiev [1], Ivan Ivanov [2], Lyubomira Grigorova [1], Ivan Stoikov [2], Dean Donchev [2], Simona Tchakarova[3], Lyudmila Lazarova[3], Iva Christova [1,2]

[1] Department of Virology, National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria
[2] Department of Microbiology, National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria
[3] Department of Exotic and Emerging Diseases, National Diagnostic and Research Veterinary Medical Institute, Sofia, Bulgaria

Highlights

• An outbreak of SARS-CoV-2 in a mink farm in Bulgaria.

• The FY.1.2 variant of SARS-CoV-2 was detected in a mink caretaker sample.

• Two variants, FY.1.2 and XBB.1.22, were observed in mink samples.

• Genetic analysis revealed the possibility of other infected mink farm workers.

• Human-mink two-way transmission is possible.

ABSTRACT

Introduction: During the COVID-19 pandemic, it was observed that SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) can be transmitted from humans to various animals. Our study aims to examine the genetic evidence for transmission of SARS-CoV-2 from humans to mink and potentially back to humans in the first reported outbreak on a mink farm in Bulgaria.

Materials and methods: Between October 2 and 29, 2023, 420 oropharyngeal samples from minks on a farm in Central Bulgaria were examined for SARS-CoV-2. Positive samples with Ct <32 were sequenced using Oxford Nanopore technologies.

Results: On 18 October 2023, 98 of 118 mink samples tested positive for SARS-CoV-2. In addition, on 25 October, a mink caretaker from the same farm was confirmed to be infected with SARS-CoV-2. Phylogenetic analysis of the isolated SARS-CoV-2 revealed that the mink caretaker and two minks had a variant similar to FY.1.2 (37.5%), while five other minks were infected with a different variant similar to XBB.1.22 (62.5%). This suggests the presence of another source of infection on the farm with the XBB.1.22 variant. Furthermore, we identified a substitution at position I478K in the receptor-binding motif (RBM) of the receptor-binding domain (RBD) of S-protein in 2 mink samples.

Conclusion: Based on the epidemiological and genetic analysis, our findings suggest a potential for human-to-mink and mink-to-human transmission of SARS-CoV-2. Tracing the route of transmission from an animal host to humans will help elucidate the route of origin and causality for the accumulation of mutations leading to the emergence of new human coronaviruses (HCoVs) and their variants with stronger or weaker pandemic potential.

Keywords: SARS-CoV-2, mink , whole genome sequencing, Oxford Nanopore technology, two-way transmissions

ADDRESS FOR CORRESPONDENCE:

Ivelina Trifonova, PhD
National Laboratory "Influenza and ARD,"
Department of Virology, NCIPD, Sofia, Bulgaria
26 Yanko Sazakov Blvd, 1504, Sofia, Bulgaria
phone: +359883585004
email: trifonova.ivelina@abv.bg

INTRODUCTION

Seven human coronaviruses are known so far, four of which are endemic: HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1. These four are a common cause of colds. The other three: severe acute respiratory syndrome (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 can lead to severe pneumonia.[1]. SARS-CoV can be easily transmitted from person to person, whereas MERS-CoV is rarely transmitted [2].SARS-CoV-2,

which appeared in the late 2019, has a high transmission rate but reduced pathogenicity compared to MERS-CoV and SARS-CoV [2]. The simultaneous infection of a host by different virus strains can lead to genetic recombination, creating new viral genomes. It is hypothesized that SARS-CoV-2 may have resulted from the recombination of pangolin and bat viruses in an unknown host. Virologists are concerned about recombinations as they could lead to the creation of a more dangerous viral strain [3,4]. During the COVID-19 pandemic, transmission of SARS-CoV-2 from domestic and wild animals, including cats, dogs, and minks has been observed [5]. The virus has been detected in wild animal zoos [6]. The uncontrolled spread of SARS-CoV-2 in different animals can lead to the accumulation of multiple mutations during repeated passaging. This inevitably leads to the emergence of new variants [7]. On the other hand, there is a risk of two-way human-mink-other mammal-human transmission of SARS-CoV-2 [8]. Passing through several different genetic species leads to the accumulation of different mutations, a prerequisite for the emergence of a "new coronavirus".

A study conducted in the Netherlands in 2020 reported a case of a mink infected with SARS-CoV-2, with additional infections found in dogs and cats on farms [9]. In Denmark, a Cluster 5 variant of SARS-CoV-2 was identified on a mink farm, showing the rapid transmission of the virus between minks and humans and the potential for emergence of new variants [10]. In the Netherlands and Denmark, cases of COVID-19 among farm workers occurred before the detection of infected minks, indicating that the animals were likely infected by humans [11]. Infections with SARS-CoV-2 in mink farms have been reported in several countries, including the Netherlands, Denmark, the United States, Greece, Sweden, France, Italy, Spain, Poland, Lithuania, and Canada (source: https://www.oie.int/en/, accessed 03 February 2021). The documented cases of SARS-CoV-2 outbreaks on mink farms worldwide and the evidence of transmission between humans and animals raise significant concerns. The emergence of a new variant could lead to another pandemic, making it essential to implement timely measures to control the virus spread. Ongoing monitoring of both animals and workers is crucial. Our team aims to clarify the transmission pathways of SARS-CoV-2 in the first reported outbreak on a Bulgarian mink farm, focusing on the potential transfer between humans and minks.

METHODS

The study was conducted from 2 to 29 October 2023, when a total of 472 oropharyngeal samples were collected from a mink farm located in Central Bulgaria and tested for the presence of SARS-CoV-2. Following the recommendations of the European Centre for Disease Prevention and Control (ECDC), a part of the mink population was tested weekly for SARS-CoV-2. This routine sampling consisted in collecting 118 mink samples each week in order to monitor the spread of SARS-CoV-2 among the mink population. The testing was performed by the Department of Exotic and Emerging Diseases at the National Veterinary Institute.   The samples were collected by a veterinarian and stored at 4°C.

2.1. Extraction and testing

Mink samples were extracted using the IndiSpin Pathogen Kit (QIAGEN GmbH for INDICAL BIOSCIENCE GmbH, Leipzig, Germany). The human sample was extracted using the ExiPrep Dx Viral DNA/RNA Kit from Bioneer, Daejeon, Republic of Korea.

In the Department of Exotic and Emerging Diseases, mink samples were tested for the presence of SARS-CoV-2 nucleic acids with a kit, INStest SARS-CoV-2 RT-qPCR (ACRO BIOTECH, Inc., California, United States), Rotor-Gene Q PCR (Qiagen, GmbH, Hilden, Germany).

To determine viral load before sequencing, 10 mink samples and the sample of the mink caretaker were retested with a commercial SARS-CoV-2 TaqPath COVID-19 CE-IVD PCR kit (Thermo Fisher Scientific, Singapore), identifying three regions in the SARS-CoV-2 genome: N-gene, S-gene and ORF ab. Amplification was performed using a QuantStudio™ 5 real-time PCR system, 96-well (ThermoFisher Scientific, Singapore). All eluates with a cycle threshold (Ct) value below 32 were sent to the National Referenve Laboratory "Influenza and ARD," at the National Center for Infectious and Parasitic Diseases for sequencing.

2.2. Sequencing and data analysis

A commercial Midnight RT-PCR Expansion Kit (Oxford Nanopore Technologies, Oxford, UK) with a

MinION Mk1C instrument (Oxford Nanopore Technologies) was used for SARS-CoV-2 sequencing. Normalization of libraries was performed with the Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, United States) and Invitrogen™ Quant-iT™ Broad Range dsDNA Assay Kit (Invitrogen, Thermo Fisher Scientific).

The genetic sequences of SARS-CoV-2 have been deposited in the GISAID database (EPI_ISL_18458688; EPI_ISL_19210102; EPI_ISL_18458689; EPI_ISL_19210103; EPI_ISL_18458692; EPI_ISL_18489918; EPI_ISL_19210130; EPI_ISL_18458693). SARS-CoV-2 variant assignment analysis was conducted using the Nextlade3.8.2c program (https://clades.nextstrain.org/). Geneious Prime software (GraphPad Software, LLC, Boston, MA, USA) was used for sequence alignment and phylogenetic tree construction. The overall tree design was created using Interactive Tree Of Life software (https://itol.embl.de). Amino acid analysis was performed with BioEdit (www.mbio.ncsu.edu/BioEdit/BioEdit.html) (RRID: SCR_007361).

We used an App called Paired Comparison Plot for pairwise comparisons versus Fisher's least significant difference (https://www.originlab.com/). Statistical significance was set at 0.05.
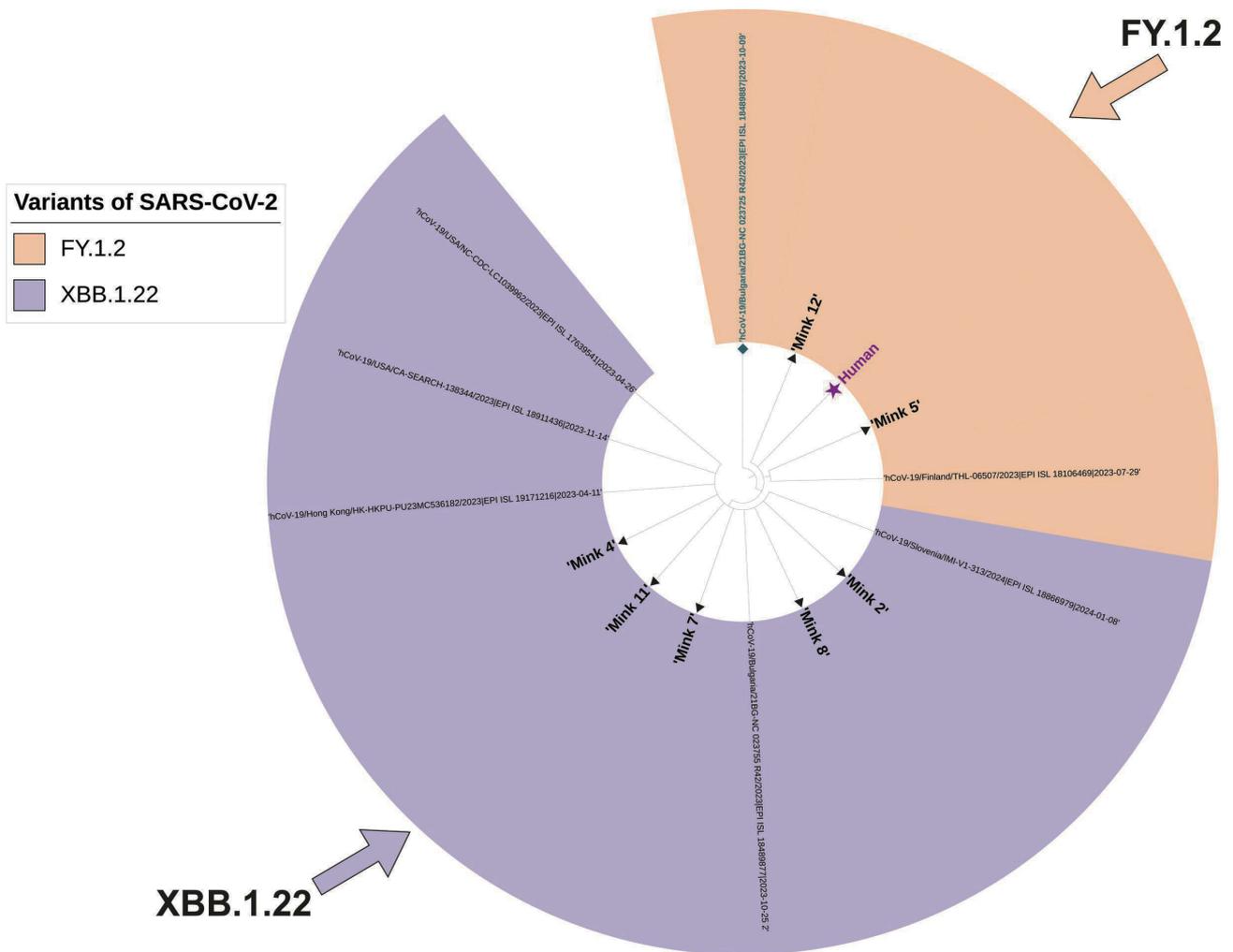

Figure 1. Phylogenetic tree based on the analysis of mink complete sequences published in GISAID. The tree was rooted against the Bulgarian sequence EPI_ISL_18489887 isolated during the same period marked with the symbol "◆". Other GISAID-derived sequences were used as reference strains. The isolated sequence from the person who cared for the minks is marked with the symbol "★" font and is called "Human" for short (EPI_ISL_18458688). The mink samples are labeled according to the names given by the veterinary laboratory marked with the symbol "▼". "Mink 2" (EPI_ISL_19210102), "Mink 4" (EPI_ISL_18458689), "Mink 5" (EPI_ISL_19210103), "Mink 7" (EPI_ISL_18458692), "Mink 8" (EPI_ISL_18489918), "Mink 11" (EPI_ISL_19210130), and "Mink 12" (EPI_ISL_18458693).
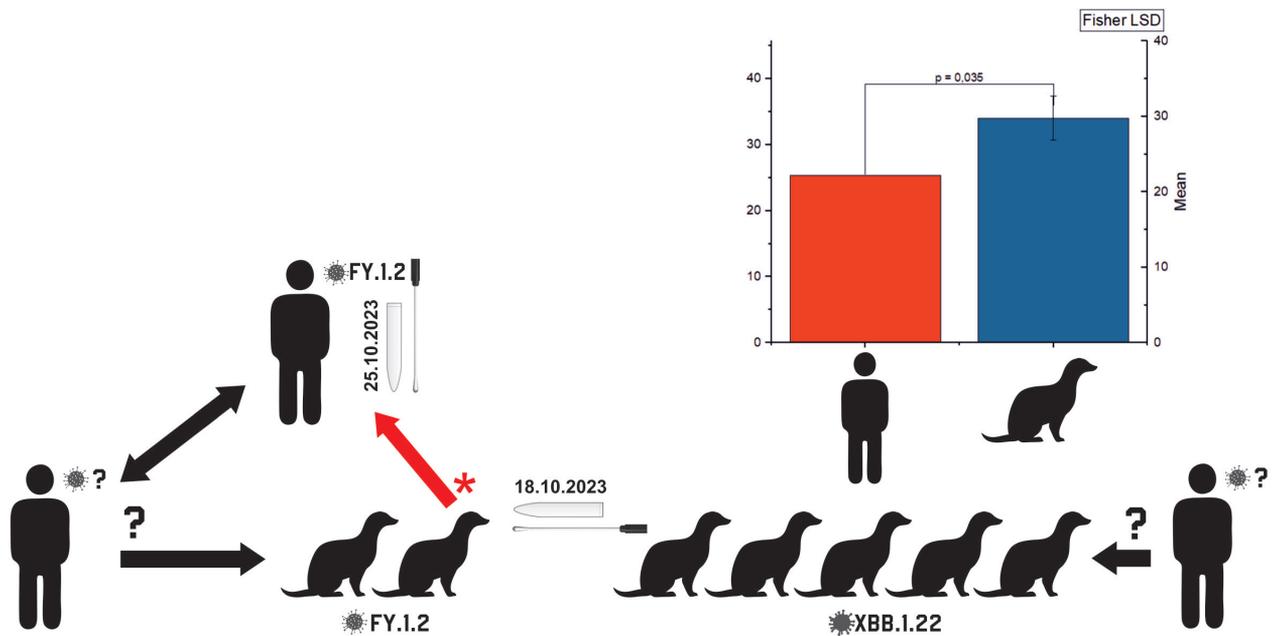
Figure 2. Transmission path of the SARS-CoV-2 infection in a mink farm on the territory of Bulgaria. The question mark indicates suspected transmission routes based on the comparison of SARS-CoV-2 Ct values from the mink caretaker and the 10 samples subjected to sequencing analysis. The arrow with a sign „*„ indicates the reverse transmission route of infection from mink to human.

RESULTS

3.1. Epidemiological data

Between 2 and 29 October 2023, 472 oropharyngeal samples from minks were tested for the presence of SARS-CoV-2. On 18 October, 98 (83%) of 118 samples were found positive. However, the examination of 118 samples from other minks during the next week returned negative results. No other minks tested positive for SARS-CoV-2 in the weeks that followed. No deaths due to the infection were registered among minks infected with SARS-CoV-2.

On 25 October, the Regional Health Inspectorate received a report of a mink caretaker from the same farm with slightly elevated temperature of 37.5°C and respiratory symptoms, without a cough. A respiratory sample was sent to the National Laboratory "Influenza and ARI", where infection with SARS-CoV-2 was confirmed. The mink caretaker was a 51-year-old man with no known comorbidities. He was quarantined for home treatment. No contact persons infected with SARS-CoV-2 were identified.

3.2. Tracing the path of infection by phylogenetic analysis

Initial PCR screening showed that only 10% of SARS-CoV-2 positive samples were suitable for sequencing analysis. Therefore, whole genome sequencing (WGS) was performed on 10 mink samples and one mink caretaker sample on 26 October. The Omicron variant of SARS-CoV-2 was identified in the human sample and in seven mink samples. The FY.1.2 subvariant was detected in the mink caretaker's sample and two mink samples (37.5%), while a different subvariant, XBB.1.22 (62.5%), was observed in five mink samples (Figure 1). The genetic integrity of three out of ten mink samples was insufficient to identify subvariants of SARS-CoV-2. The remaining positive mink samples had Ct values above 32 and were not sequenced.

Based on the temporal sequence of events and the evolutionary path of the SARS-CoV-2 variants, we hypothesize that other mink caretakers or mink contacts were infected with the two different variants FY.1.2 and XBB.1.22. They have probably transmitted the infection to the minks from which these two variants of SARS-CoV-2 were islolated.

In addition, the SARS-CoV-2 variant FY.1.2 detected in the mink caretaker was the same as the one found in two of the minks. The minks tested positive for SARS-CoV-2 on 18/10/2023, while the caretaker - on 25/10/2023. The Regional Health Inspectorate also reported that on October 25, 2023, the first signs of infection were registered in caretakers of minks.
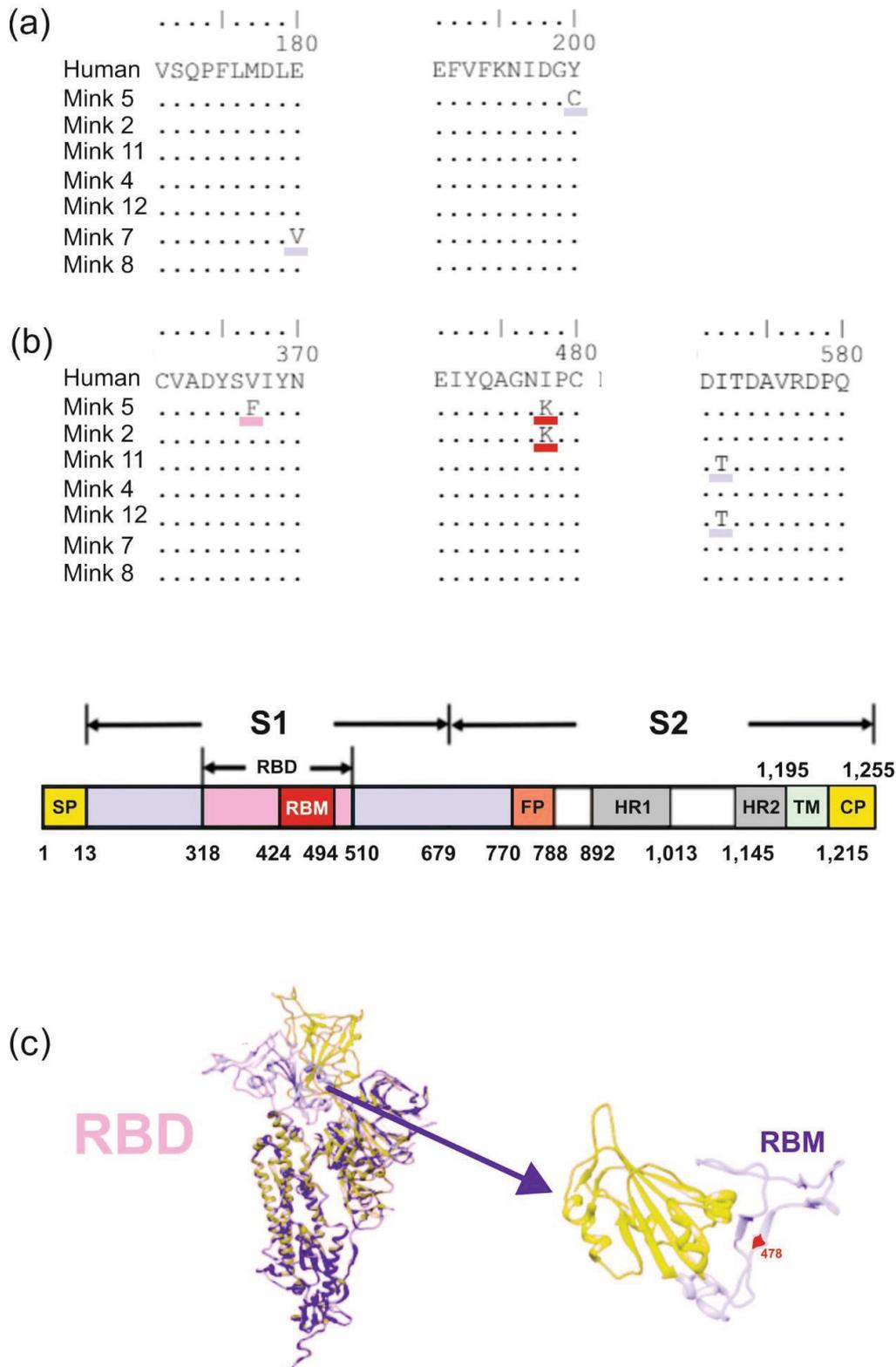
Figure 3. Amino acid analysis of the SARS-CoV-2 spike protein (S) isolated from a mink sample during an outbreak on a farm in Central Bulgaria: (a) cross-sections of the S analysis of the spike protein of SARS-CoV-2 isolated from samples of minks 5, 2, 4, 7, 8, 11, and 12 in the places of established replacement. An alignment was performed to the strain isolated from a person caring for these mink (EPI_ISL_18458688). (b) The structure of the S-protein; (c) The structure of RBD is depicted in yellow and purple, and the RBM is in purple, the site of the established mutation at position 478 is represented in red.

Based on this, we hypothesize that a transmission has occurred from a mink infected with the FY.1.2 variant to the positive mink caretaker (the path indicated by an arrow from mink to human in Figure 2). Regarding шге viral load, we observed that the mink caretaker's Ct value was lower than the mean Ct value observed in 10 mink samples that were subsequently sequenced (22.2 vs. 29.7 ± 2.9; p = 0.035). The remaining 88 minks had a Ct value in the range of 32 - 38.

3.3. Amino acid analysis

The S protein sequences isolated from seven mink samples were compared with those of the mink caretaker. The analysis identified five different substitutions in four of the mink samples. Specifically, one substitution was observed in mink 2 (I478K), three in mink 5 (Y200C; V367 F; I478K), two in mink 7 (E180V; I572T), and one in mink 11 (I572T) (Figure 3a). The substitutions at positions 367 and 478 were located in the receptor-binding domain (RBD) (Figure 3b). The sequences from minks 2 and 5 harbored a substitution at position 478 of the receptor-binding motif (RBM) (Figure 3c).

DISCUSSION

During the early stages of COVID-19 pandemic, there were reports of SARS-CoV-2 outbreaks on mink farms [12,13,14]. Minks are vulnerable to infection and can carry the virus without symptoms [13]. Direct contact with infected minks poses a risk for SARS-CoV-2 transmission to humans [11]. This interspecies transmission can result in genetic mutations and the emergence of new SARS-CoV-2 variants [15]. Similar to our study, other researchers have aimed to trace the potential transmission routes of SARS-CoV-2 between humans and minks [6,16,17]. Despite existing research on this topic, it remains relevant and important. A study reported that human angiotensin-converting enzyme 2 (ACE2) receptors have a higher binding affinity to the SARS-CoV-2 RBD as compared to mink ACE2s. Therefore, only SARS-CoV-2 variants with a higher binding affinity would contribute to viral entry and replication in minks and thus acquire higher transmissibility [18]. SARS-CoV-2 is believed to have originated from animals in 2019. By the end of 2020, it had significantly adapted to humans, leading to the emergence of the alpha, beta, gamma, delta,

and Omicron variants. The Omicron variant, which appeared in November 2021, caused the largest wave of COVID-19, spawning multiple subvariants. The XBB line, comprising over 300 subvariants, was dominant in 2023 [19]. The XBB.1.22 subvariant was found in 71.4% of mink samples in our study. Other studies in the Netherlands and Denmark reported the transmission of infection from minks to humans [20]. Our study identified similar subvariants of SARS-CoV-2 in some mink samples and a mink caretaker (FY.1.2), suggesting an evolutionary mechanism of mutation accumulation. Human-to-mink transmission of the infection is assumed. Studies have shown that the period from the SARS-CoV-2 infection to the appearance of the first symptoms takes an average of 5.6 to 6.7 days [21,22]. People diagnosed with COVID-19 can transmit the virus from 1 - 2 days before, upto 8 - 10 days after the symptoms onset (https://www.ecdc.europa.eu/en/infectious-disease-topics/z-disease-list/covid-19/factsheet-covid-19). Omicron showed peak virus shedding 3–6 days after symptom onset, with viable virus cultured by day 10 [23]. Our observations suggest that another mink caretaker was the primary source of FY 1.2 infection on the farm, and third parties infected with XBB 1.22 likely transmitted the infection to minks. The higher SARS-CoV-2 viral load of the mink caretaker, along with the eight-day gap between the appearance of his symptoms and the detection of positive mink samples, suggests the possibility that the human was infected by these minks. In support of this claim, high viral load coincides with the peak development of infection [24]. The farm's minks showed attenuation of the infection, and no other humans in contact with the mink caretaker were identified as infected with SARS-CoV-2.

The S protein of SARS-CoV-2 is highly variable and plays an essential role for viral infectivity and virulence [25]. Amino acid analysis was performed to identify substitutions that may have occurred during potential transmission between humans and minks or vice versa [26]. This analysis reveals substitutions in the receptor-binding sites. RBM (438–506) directly interacts with the ACE2 receptor and plays a crucial role in determining its affinity [27]. A substitution at position 478 found in two mink samples could potentially affect the binding affinity of ACE2.

Therefore, we can conclude that the mutations were introduced during the possible transmission of infection from humans to mink, which changes the binding affinity between RBD and ACE2. Other studies have reported no such mutation occurring I478K. In 2020, mutations Y453F and F486L were reported in the sequence of SARS-CoV-2, for which the first source was most probably a mink from a farm in the Netherlands [24]. Those mutations were located in the RBM and were close to the one we found. In a subsequent study, the authors reported the prevalence of Y453F mutation in human samples in Denmark [28]. The transmition of such mutations poses a risk because they can affect the neutralizing ability of virus-specific antibodies [29].

The emergence of new mutations at receptor-binding sites is crucial for SARS-CoV-2 transmission between humans and animals. However, our study has limitations, as only 10% of positive mink samples could be sequenced, hindering our understanding of the infection pathways. Improved farm monitoring and more frequent sampling are necessary for future research. Additionally, cycle threshold (Ct) measurements may not accurately reflect primer amplification efficiency across different detection kits. Despite these challenges, the study highlights the importance of continuous monitoring and the potential of new SARS-CoV-2 variants of to pose pandemic threats.

CONCLUSIONS

The genetic analysis of the SARS-CoV-2 genome in both minks and humans suggested that there may have been other infected workers at the mink farm in addition to the caretaker who tested positive. This raises the possibility of SARS-CoV-2 being transmitted from humans to minks and then back to humans. This situation highlights the potential for mutations to occur within the spike protein, and affect the neutralizing ability of virus-specific antibodies thus increasing the public health risks.

CRediT authorship contribution statement

IT: conceptualisation, methodology, analysis, research design. **IT and NK**: writing the original manuscript**. ST and LL:** recruitment and testing of mink samples. **IT, IM, II, LG, IS DD**: Implementation of the experiments. **IT, IM, NK, IA, LG, II, IS, and DD:** Analysis of the results. **IC, II** project management and task execution. **NK, II, and IC**: review of the final manuscript.

Declaration of competing interest

The corresponding author affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained. The authors have no conflicts of interest to declare. No funding was received for this work.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of 00006384, November 2022 (SPREAD AND CLINICAL IMPACT OF MONO- AND CO-INFECTIONS WITH ENDEMIC CORONAVIRUS 229E, OC43, NL63 AND HKU1 DURING THE COVID-19 PANDEMIC).

Database linking

https://gisaid.org/(EPI_ISL_18458688;EPI_ISL_19210102;EPI_ISL_18458689;EPI_ISL_19210103;EPI_ISL_18458692;EPI_ISL_18489918;EPI_ISL_19210130; EPI_ISL_18458693).

REFERENCES

1. Kesheh MM, Hosseini P, Soltani S, Zandi M. An overview on the seven pathogenic human coronaviruses. Rev Med Virol. 2022 Mar;32(2):e2282. https://doi.org/10.1002/rmv.2282
2. Zhu Z, Lian X, Su X, Wu W, Marraro GA, Zeng Y. From SARS and MERS to COVID-19: a brief summary and comparison of severe acute respiratory infections caused by three highly pathogenic human coronaviruses. Respir Res. 2020 Aug 27;21(1):224. https://doi.org/10.1186/s12931-020-01479-w
3. Zhou H, Yang J, Zhou C, Chen B, Fang H, Chen S et al. A Review of SARS-CoV2: Compared With SARS-CoV and MERS-CoV.

Front Med (Lausanne). 2021 Dec 7;8:628370. https://doi.org/10.3389/fmed.2021.628370

4. Voskarides K. SARS-CoV-2: tracing the origin, tracking the evolution. BMC Med Genomics. 2022 Mar 18;15(1):62. https://doi.org/10.1186/s12920-022-01208-w

5. Banerjee A, Doxey AC, Mossman K, Irving AT. Unraveling the Zoonotic Origin and Transmission of SARS-CoV-2. Trends Ecol Evol. 2021 Mar;36(3):180-184. https://doi.org/10.1016/j.tree.2020.12.002

6. Domańska-Blicharz K, Orłowska A, Smreczak M, Munnink BO, Trębas P, Socha W et al. SARS-CoV-2 Monitoring on Mink Farms in Poland. J Vet Res. 2022 Dec 3;66(4):449-458. https://doi.org/10.2478/jvetres-2022-0066

7. Pramod RK, Nair AV, Tambare PK, Chauhan K, Kumar TV, Rajan RA et al. Reverse zoonosis of coronavirus disease-19: Present status and the control by one health approach. Vet World. 2021 Oct;14(10):2817-2826. https://doi.org/10.14202/vetworld.2021

8. Greenhorn JE, Kotwa JD, Bowman J, Bruce L, Buchanan T, Buck PA et al. SARS-CoV-2 wildlife surveillance in Ontario and Québec. Can Commun Dis Rep. 2022 Jun 9;48(6):243-251. https://doi.org/10.14745/ccdr.v48i06a02

9. Mallapaty S. COVID is spreading in deer. What does that mean for the pandemic? Nature. 2022 Apr;604(7907):612-615. https://doi.org/10.1038/d41586-022-01112-4

10. Oreshkova N, Molenaar RJ, Vreman S, Harders F, Oude Munnink BB, Hakze-van der Honing RW et al. SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. Euro Surveill. 2020 Jun;25(23):2001005. https://doi.org/10.2807/1560-7917.ES.2020.25.23.2001005

11. Erratum in: Euro Surveill. 2021 Mar;26(12). https://doi.org/10.2807/1560-7917.ES.2021.26.12.210325c

12. Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, Molenaar RJ, Munger E, Molenkamp R et al. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. Science. 2021 Jan 8;371(6525):172-177. https://doi.org/10.1126/science.abe5901 Epub 2020 Nov 10.

13. Hammer AS, Quaade ML, Rasmussen TB, Fonager J, Rasmussen M, Mundbjerg K et al. SARS-CoV-2 Transmission between Mink (Neovison vison) and Humans, Denmark. Emerg Infect Dis. 2021 Feb;27(2):547-551. https://doi.org/10.3201/eid2702.203794

14. Chaintoutis SC, Thomou Z, Mouchtaropoulou E, Tsiolas G, Chassalevris T, Stylianaki I et al. Outbreaks of SARS-CoV-2 in naturally infected mink farms: Impact, transmission dynamics, genetic patterns, and environmental contamination. PLoS Pathog. 2021 Sep 7;17(9):e1009883. https://doi.org/10.1371/journal.ppat.1009883

15. Fenollar F, Mediannikov O, Maurin M, Devaux C, Colson P, Levasseur A et al. SARS-CoV-2, and the Human-Animal Interface. Front Microbiol. 2021 Apr 1;12:663815. https://doi.org/10.3389/fmicb.2021.663815

16. Wasniewski M, Boué F, Richomme C, Simon-Lorière E, Van der Werf S, Donati F, Enouf V et al. Investigations on SARS-CoV-2 and other coronaviruses in mink farms in France at the end of the first year of COVID-19 pandemic. bioRxiv [Preprint]. 2023 Feb 2:2023.02.02.526749. https://doi.org/10.1101/2023.02.02.526749

17. Cossaboom CM, Wendling NM, Lewis NM, Rettler H, Harvey RR, Amman BR et al. One Health Investigation of SARS-CoV-2 in People and Animals on Multiple Mink Farms in Utah. Viruses. 2022 Dec 29;15(1):96. https://doi.org/10.3390/v15010096

18. Sharun K, Tiwari R, Natesan S, Dhama K. SARS-CoV-2 infection in farmed minks, associated zoonotic concerns, and importance of the One Health approach during the ongoing COVID-19 pandemic. Vet Q. 2021 Jan 1;41(1):50-60. https://doi.org/10.1080/01652176.2020.1867776

19. Lu L, Sikkema RS, Velkers FC, Nieuwenhuijse DF, Fischer EAJ, Meijer PA et al. Adaptation, spread and transmission of SARS-CoV-2 in farmed minks and associated humans in the Netherlands. Nat Commun. 2021 Nov 23;12(1):6802. https://doi.org/10.1038/s41467-021-27096-9

20. Su C, He J, Han P, Bai B, Li D, Cao J et al. Molecular Basis of Mink ACE2 Binding to SARS-CoV-2 and Its Mink-Derived Variants. J Virol. 2022 Sep 14;96(17):e0081422. https://doi.org/10.1128/jvi.00814-22

21. Feng Y, Su Q, Li L, He X, Niu P, Guo X et al. Genomic Surveillance for SARS-CoV-2 Variants: Dominance of XBB Replacement - China, January-June 2023. China CDC Wkly. 2024 Apr 12;6(15):324-331. https://doi.org/10.46234/ccdcw2024.061

22. Zaki N, Mohamed EA. The estimations of the COVID-19 incubation period: A scoping reviews of the literature. J Infect Public Health. 2021 May;14(5):638-646. https://doi.org/10.1016/j.jiph.2021.01.019

23. Quesada JA, López-Pineda A, Gil-Guillén VF, Arriero-Marín JM, Gutiérrez F, Carratala-Munuera C. Incubation period of COVID-19: A systematic review and meta-analysis. Rev Clin Esp (Barc). 2021 Feb;221(2):109-117. https://doi.org/10.1016/j.rceng.2020.08.002

24. Kandel C, Lee Y, Taylor M, Llanes A, McCready J, Crowl G et al. Viral dynamics of the SARS-CoV-2 Omicron Variant among household contacts with 2 or 3 COVID-19 vaccine doses. J Infect. 2022 Dec;85(6):666-670. https://doi.org/10.1016/j.jinf.2022.10.027

25. Burkholz S, Pokhrel S, Kraemer BR, Mochly-Rosen D, Carback RT 3rd, Hodge T et al. Paired SARS-CoV-2 spike protein mutations observed during ongoing SARS-CoV-2 viral transfer from humans to minks and back to humans. Infect Genet Evol. 2021 Sep;93:104897. https://doi.org/10.1016/j.meegid.2021.104897

26. Greaney AJ, Starr TN, Gilchuk P, Zost SJ, Binshtein E, Loes AN et al. Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. Cell Host Microbe. 2021 Jan 13;29(1):44-57.e9. https://doi.org/10.1016/j.chom.2020.11.007

27. Yang Y, Guo L, Yuan J, Xu Z, Gu Y, Zhang J et al. Viral and antibody dynamics of acute infection with SARS-CoV-2 omicron variant (B.1.1.529): a prospective cohort study from Shenzhen, China. Lancet Microbe. 2023 Aug;4(8):e632-e641. https://doi.org/10.1016/S2666-5247(23)00139-8. Epub 2023 Jul 14. Erratum in: Lancet Microbe. 2023 Aug;4(8):e576. https://doi.org/10.1016/S2666-5247(23)00223-9

28. Almehdi AM, Khoder G, Alchakee AS, Alsayyid AT, Sarg NH, Soliman SSM. SARS-CoV-2 spike protein: pathogenesis, vaccines, and potential therapies. Infection. 2021 Oct;49(5):855-876. https://doi.org/10.1007/s15010-021-01677-8 Epub 2021 Aug 2.

29. Du Y, Wang H, Chen L, Fang Q, Zhang B, Jiang L, Wu Z, Yang Y, Zhou Y, Chen B, Lyu J, Wang Z. Non-RBM Mutations Impaired SARS-CoV-2 Spike Protein Regulated to the ACE2 Receptor Based on Molecular Dynamic Simulation. Front Mol Biosci. 2021 Jul 27;8:614443. https://doi.org/10.3389/fmolb.2021.614443