Zoonoses and Public Health

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Detection of a Reassortant Swine- and Human-Origin H3N2 Influenza A Virus in Farmed Mink in British Columbia, Canada

Kevin S. Kuchinski[1] | John Tyson[1,2] | Tracy Lee[2] | Susan Detmer[3] | Yohannes Berhane[4] | Theresa Burns[5] | Natalie A. Prystajecky[1,2] | Chelsea G. Himsworth[5]

[1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada | [2]Public Health Laboratory, British Columbia Centre for Disease Control, Vancouver, British Columbia, Canada | [3]Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada | [4]National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada | [5]Animal Health Centre, British Columbia Ministry of Agriculture and Food, Abbotsford, British Columbia, Canada

Correspondence: Kevin S. Kuchinski (kevin.kuchinski@bccdc.ca)

Received: 11 July 2024 | **Revised:** 18 November 2024 | **Accepted:** 14 December 2024

Funding: This work was funded by the Genome British Columbia as part of the One Health Genomics: COVID-19 Adaptation investigation in mink (Mink ALERT, Cov-200).

Keywords: genomics | human influenza | influenza A virus | mink | swine influenza | zoonoses

ABSTRACT

Introduction: In December 2021, influenza A viruses (IAV) were detected in a population of farmed mink in British Columbia, Canada. Circulation of IAVs in farmed mink populations has raised public health concerns due to similarities between mustelid and human respiratory physiology, potentially facilitating spillover of zoonotic influenzas from livestock.

Methods: Oropharyngeal specimens were collected from mink as part of a surveillance program for SARS-CoV-2. Diagnostic RT-qPCR testing was performed using a multiplex assay targeting SARS-CoV-2, IAV, influenza B virus and respiratory syncytial virus. Whole viral genome sequencing was conducted on IAV-positive specimens, followed by phylogenetic analysis with other animal and human IAV genome sequences from large global databases.

Results: IAVs were detected in 17 of 65 mink by RT-qPCR. Based on genomic sequencing and phylogenetic analysis, these IAVs were subtyped as H3N2s that originated from reassortment of swine H3N2 (clade 1990.4 h), human seasonal H1N1 (pdm09) and swine H1N2 (clade 1A.1.1.3). This reassortant has been subsequently observed in swine in several Midwest American states, as well as in swine and turkeys in Ontario, suggesting its spillover into farmed mink in British Columbia was incidental to its broader dissemination in North American swine populations.

Conclusions: These detections reaffirm the need for extensive genomic surveillance of IAVs in swine populations to monitor reassortments that might become public health concerns. They also highlight the need for closer surveillance of IAVs in mink to preserve animal health, protect agricultural interests, and monitor potential zoonotic threats.

1 | Introduction

Influenza A viruses (IAVs) infect diverse mammalian hosts due to conserved glycosylation of cell surface proteins that act as viral receptors. Specifically, sialic acids linked to galactose by α2,6-linkages (SA α2,6 GAL) within respiratory tract epithelium are exploited by IAVs for cell attachment and entry in humans and other mammals, notably swine (Zhao and Pu 2022). This has made swine a common source of zoonotic influenzas, which are often called 'variant' influenzas

Summary

- IAV infections in mink are likely under detected.
- Circulation of IAVs in mink raises public health concerns over zoonotic influenzas.
- IAV surveillance in mink can be useful for zoonotic risk awareness due to their exposure to other livestock and their physiological similarities to humans.

(Anderson et al. 2021). These are commonly acquired through occupational exposures to livestock, although recreational exposures, particularly among youth at petting zoos at agricultural fairs and exhibitions, have also been noted (Jhung et al. 2013; Greenbaum et al. 2015; Duwell et al. 2018). Mustelids are another mammalian host with SA α2,6 GAL on their respiratory epithelium, leading to the common use of ferrets as an animal model for studying IAV respiratory infections (Zhao et al. 2019; Ng et al. 2014).

Swine and certain mustelids, notably mink, also have sialic acid receptors with α2,6 and α2,3 galactose linkages (SA α2,3 GAL) on their intestinal epithelium, the latter of which is the preferred receptor for IAVs of avian origin (Zhao et al. 2019; Nelli et al. 2010). Consequently, swine have been recognised as 'mixing vessels' for avian- and mammalian-origin IAVs, implicating them in the emergence of past pandemic IAV strains (Ma, Kahn, and Richt 2008; Abdelwhab and Mettenleiter 2023; Ma et al. 2009). Increasingly, a similar mixing vessel role has been proposed for farmed mink, with suggestions of increased risk due to the high degree of similarity between human and mustelid respiratory physiology (Zhao et al. 2019). Indeed, mink can be infected, both naturally and experimentally, with IAVs originating from birds, swine and humans (Yagyu et al. 1982; Gagnon et al. 2009; Clayton et al. 2022; Agüero et al. 2023). These general concerns about the role of mink in zoonotic disease were reinforced during the COVID-19 pandemic by widespread spillover of SARS-CoV-2 into farmed mink populations, with worries of potential spillback of novel variants into humans (Pomorska-Mól et al. 2021; Koopmans 2021). This resulted in unprecedented surveillance and molecular testing of farmed mink populations with respiratory disease.

IAVs infections in mink, however, are frequently asymptomatic and/or manifest in disease only when there is coinfection with another viral or bacterial pathogen; therefore, it is likely that cases and outbreaks are under detected (Liu et al. 2020; Bo-Shun et al. 2020). The source of IAV infections in farmed mink is also unclear. It has been hypothesised that contamination of the mink farm environment by wild birds could be source of infection, as could contact with infected farm workers (Clayton et al. 2022; Agüero et al. 2023; Jiang et al. 2017). Most commonly, infection is suspected to result from feeding raw, infected pork and poultry products (Gagnon et al. 2009; Jiang et al. 2017; Peng et al. 2015; Yoon et al. 2012). But overall, relatively little is known about the incidence, origin, or significance of IAV infections in farmed mink.

In December 2021, IAV infections were incidentally detected in a population of farmed mink (*Neovison vison*) in the province of British Columbia (BC), Canada. This mink herd had been under surveillance for SARS-CoV-2 following multiple coronavirus outbreaks on mink farms in BC (Paiero et al. 2022; Boyd et al. 2023), and diagnostic testing was conducted at a public health laboratory where the SARS-CoV-2 assay had been multiplexed with other human respiratory pathogens to improve throughput during the COVID-19 pandemic (Hempel et al. 2024). Following their detection, we performed genomic characterisation of the IAV associated with this outbreak and investigated potential sources.

2 | Materials and Methods

2.1 | SARS-CoV-2 Surveillance Program

Surveillance for SARS-CoV-2 in this mink herd began on 2 May 2021 following an outbreak. The outbreak continued until April 2022, at which point the farm was depopulated. During the surveillance period, SARS-CoV-2 prevalence and evolution was monitored by testing a random stratified sample of 65 mink every 2 weeks. Testing was conducted by the BC Centre for Disease Control Public Health Laboratory (PHL). After 2 December 2021, the SARS-CoV-2 assay was multiplexed with IAV, influenza B virus and respiratory syncytial virus for higher laboratory throughput during the COVID-19 pandemic.

2.2 | Specimen Collection

Oropharyngeal specimens were collected from manually restrained mink by inserting a sterile polyester swab into the mink's mouth through a gag (either a 3 cc syringe tube or a short length of sterilised 1 in. diameter copper pipe). The swab was swirled several times as far back in the oral cavity as possible, then placed into viral transport medium for storage and shipment.

2.3 | Nucleic Acid Extraction and Diagnostic Testing

Total nucleic acids were extracted from 200 μL of inoculated viral transport medium using the MagMax Express 96 Nucleic Acid Extractor (Applied Biosystems) and the MagMax Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher Scientific) according to the manufacturer's recommendations. Extracted nucleic acids were assayed by RT-qPCR for SARS-CoV-2, IAV, influenza B virus and respiratory syncytial virus as previously described (Hempel et al. 2024).

2.4 | Genome Sequencing and Bioinformatic Analysis

Influenza A virus cDNA was generated from specimen RNA using the amplification method described by Zhou et al. with modifications to the primers to improve amplicon complexity and specificity (Zhou et al. 2009). Sequencing libraries were constructed from cDNA using the Illumina DNA Prep Kit following a modified condensed protocol (Hickman et al. 2022). Libraries

were sequenced in-house on an Illumina MiSeq platform at the BC Centre for Disease Control Public Health Laboratory (PHL) using a V2 300 cycle micro kit (MS-103-1002).

Influenza A virus genome segment sequences were generated using FluViewer (v0.1.9) (https://github.com/KevinKuchinski/FluViewer). Default parameters were used, except for minimum read depth for base calling (-D), which was set to 10. Complete segment sequences (≥95% segment coverage and ≥95% nonambiguous base calls) were remotely queried against the NCBI Nucleotide Collection on 19 Feb 2024 using blastn (v2.14.1+) with subjects limited to the influenza A virus taxon (txid 11320) (Camacho et al. 2009). Alignments from blastn were grouped by subject sequence, then the median bitscore was calculated for each subject sequence to measure the similarity of each IAV reference sequence to the IAVs from the mink outbreak collectively. Median bitscores were used to identify the best-matching reference sequences for each genome segment. This provided a list of IAVs in GenBank with at least one segment closely related the IAVs from the mink outbreak. GenBank records for these closely related IAV strains were manually inspected, and a sample of them was selected for phylogenetic analysis based on the availability and completeness of all eight genome segments. Multiple sequence alignments were generated with MAFFT (v7.526) using the options --maxiterate 1000 and --localpair (Katoh and Standley 2013). Multiple sequences alignments were used to build maximum likelihood phylogenetic trees with IQTREE2 (v2.3.6) with automated model selection by ModelFinder and 1000 ultrafast bootstrap replicates (Minh et al. 2020; Kalyaanamoorthy et al. 2017; Hoang et al. 2018). Clades for H1 and H3 swine viruses were determined using the subspecies classification tools for Orthomyxoviridae provided by the Bacterial and Viral Bioinformatics Resource Centre (https://www.bv-brc.org).

3 | Results

On 3 December 2021, 17 of 65 mink specimens submitted under the SARS-CoV-2 surveillance program incidentally tested positive for IAV. No unusual clinical signs were reported in the herd, nor was there increased incidence of respiratory disease or mortality at this time. All subsequent surveillance samples from this herd were IAV-negative. The IAV positivity of mink surveillance samples before these detections is uncertain as they preceded the multiplexing of the SARS-CoV-2 and IAV assays. Unfortunately, they were not available for follow-up testing when preparing this report as freezer space shortages during the COVID-19 shortened specimen retention times.

To confirm these detections and further characterise these IAVs, whole genome sequencing was conducted. Twelve complete HA segment sequences and 13 complete NA segment sequences were obtained. When queried against the NCBI Nucleotide Collection (Table S1), these HA and NA sequences had their best alignments to swine-origin H3 and N2 sequences. We also obtained 65 complete internal segment sequences from these mink specimens (8 PB2, 8 PB1, 9 PA, 13 NP, 14 M and 13 NS). These internal segments had their best alignments to a mixture of swine-origin and human seasonal IAVs (Table S1), suggesting the H3N2 viruses detected in these mink originated through reassortment.

To investigate this apparent reassortment, we constructed phylogenetic trees for each segment using sequences from the mink specimens, closely related reference viruses from NCBI, and unpublished sequences from Canadian swine (Figures S1–S8). To assess possible epidemiological connections, these trees included four H3N2 viruses detected on local BC swine farms over the preceding 6 years (Figures S1–S8, pink leaves). The phylogenies suggested that the PA, HA, NA, M and NS segments of the mink viruses were derived from an H3N2 lineage that has been observed in Saskatchewan swine since 2017 (Figures S1–S8, purple leaves). This progenitor lineage belonged to clade 1990.4, also known as Cluster IV, the most prevalent swine H3N2 in North America in recent years (Olsen et al. 2006; Anderson et al. 2013; Walia, Anderson, and Vincent 2019). The trees also indicated that this H3N2 progenitor was itself a reassortant of earlier swine H3N2 (clade 1990.4) and H1N2 (clade 1A.1.1.3) viruses circulating in Western Canadian in the preceding years (Figures S1–S8, red and blue leaves respectively).

Phylogenetic analysis also indicated that the PB2, PB1 and NP segments of these mink viruses were derived from human seasonal H1N1pdm09 (Figures S1–S8, green leaves). Their best-matching human seasonal H1N1pdm09s circulated in 2018/19, suggesting reassortment of these segments had occurred 2 to 3 years prior to the mink farm outbreak. Temporal separation between reassortment and infection of these mink was further supported by the comparatively long branches separating mink-origin sequences from the human-origin sequences with which they formed clades.

Figure 1 summarises the proposed series of reassortments that gave rise to the H3N2 lineage detected in this mink farm outbreak. This IAV's constellation of genome segments has been subsequently observed in swine in Iowa and Minnesota in 2022 as well as turkeys and swine in Ontario in 2022 and 2023 (Figures S1–S8, orange leaves). This pairing of HA and NA segments was also observed in swine in Missouri in 2021 (Figures S4 and S6, orange leaves), but it is unknown if this was an additional instance of this full genome constellation or a subsequent reassortant because internal segment sequences were not submitted for the IAVs from Missouri. Taken together, these results suggest that the H3N2 lineage detected in the mink outbreak has become widespread in North American swine, and infection of these mink with this lineage was incidental to its broader dissemination in swine.

We interviewed the mink producers and their primary care veterinarians to assess several scenarios by which these minks could have been exposed to swine-origin IAVs. First, we ruled out direct contact between mink and infected swine because no swine were located on the mink farm premises. We also found no evidence of transmission via fomites; mink farm personnel could not recall having any contact with swine or visiting any premises with swine in the months preceding the outbreak. Exposure via contaminated pork was also considered, but the producers did not recall feeding the mink any pork products.

Swine-origin H3N2 infections have been reported in turkeys (Choi et al. 2004), so we also investigated potential direct and indirect exposures to turkeys. As with swine, there were no turkeys on the mink farm premises and no personnel or equipment

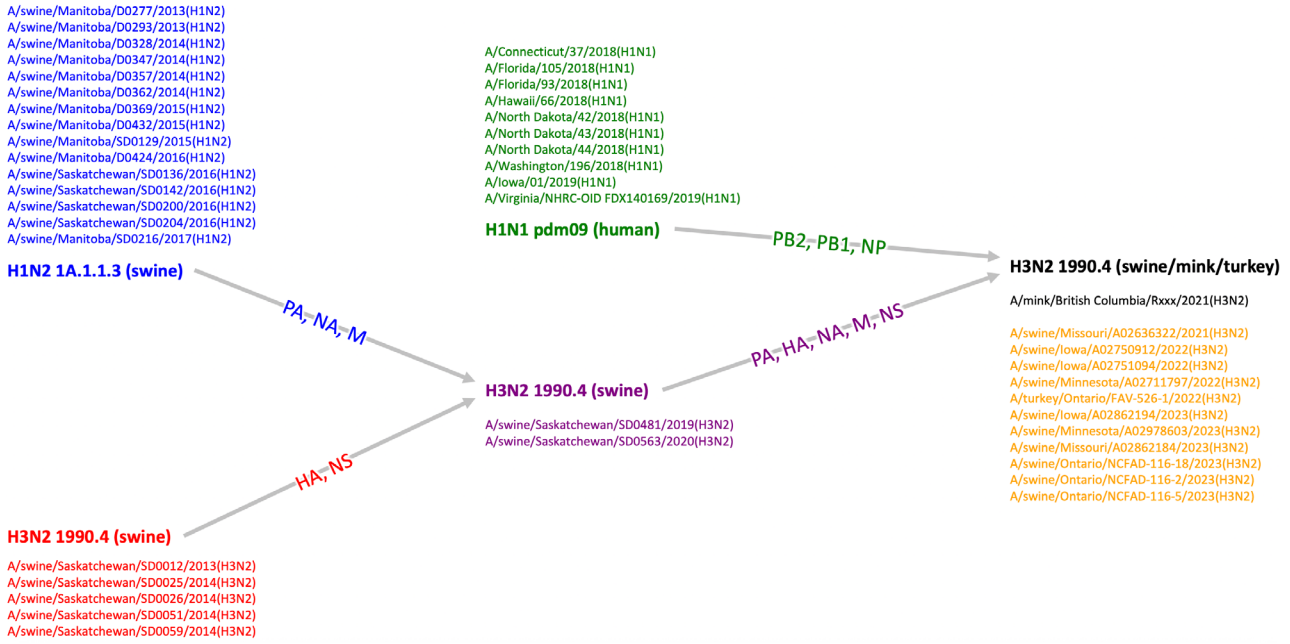

FIGURE 1 | Simplified schematic of re-assortments resulting in the H3N2 influenza A virus (IAV) detected in farmed mink. The origin of each genome segment in the mink H3N2 virus (black) was determined based on their best-matching reference sequences and the phylogenies presented in Figures S1–S8. The PB2, PB1 and NP segments were derived from human season H1N1 pdm09 *c*. 2018/19 (green). The PA, HA, NA, M and NS segments were derived from a swine H3N2 clade 1990.4 virus (purple), which itself was a reassortant of an earlier swine H3N2 clade 1990.4 (red) and a swine H1N2 clade 1A.1.1.3 (blue). This reassortant that was detected in mink has been subsequently detected in swine and turkeys in Ontario and American Midwest states (orange).

had been shared with turkey farms. Mink had been fed raw poultry necks and backs obtained from a local processing plant, but it was not known if the necks and backs were from turkeys or other poultry species.

We also considered if the mink had been exposed to humans or wild birds infected with swine-origin IAVs. There were no reported cases of humans infected with swine-origin IAVs in BC in 2021, and no farm personnel could recall any influenza-like illness around the time of the outbreak. Exposure to birds infected with swine-origin IAVs seemed equally unlikely; no swine-origin IAVs were detected through avian influenza surveillance programs during the 2021/22 season. This included passive surveillance of wild birds as well as novel environmental surveillance of avian habitats using targeted genomic sequencing of sediment from local wetlands (Kuchinski et al. 2024). Several wetlands in the same region as the mink farm were monitored, resulting in detections of diverse IAVs, but none were swine-origin. Taken together, this indicated that the mink were probably not exposed to humans or wild birds infected with swine-origin IAVs because swine-origin IAVs were not circulating in the human population or local wild bird community.

4 | Discussion

Our investigation could not conclude how these mink became exposed to swine-origin IAVs. Additional modes of transmission were considered, but they could not be assessed due to lack of available data. For instance, wild mustelids have been reported to visit mink farms and interact with captive animals resulting in the transmission of viruses (Nituch et al. 2015); it is possible that wild mustelids may have visited this farm unnoticed after becoming infected with IAVs on another premise where swine are raised.

We also considered environmental transmission of IAVs between local swine and poultry operations and this mink farm. Shortly before the outbreak, on 14 November 2021, a severe atmospheric river system inundated the region where the mink farm is located (Gillett et al. 2022), resulting in a regional state of emergency and historic flooding that submerged parts of the mink farm. It is conceivable that swine or poultry excreta were transported onto the premises in flood waters. Transmission of IAVs between birds in aquatic environments is well-established, and long-term persistence of IAVs in these aquatic environments has been demonstrated (Ramey et al. 2022, 1934). Water-borne transmission of IAVs into mammalian livestock has also been proposed in previous outbreaks (Karasin et al. 2000).

Another potential form of environmental transmission is atmospheric dispersion through wind. Airborne transmission has been invoked to explain spread between farms in both poultry and swine IAV outbreaks, and this has been corroborated to varying degrees with epidemiologic modelling, detection of viral RNA in air specimens, and IAV isolation from air specimens by viral culture (Corzo et al. 2013; Scoizec et al. 2018; Ypma et al. 2013; Ssematimba, Hagenaars, and de Jong 2012; Jonges et al. 2015). Mathematical modelling has proposed wind-borne transmission might be possible over distances of up to 25 km (Ssematimba, Hagenaars, and de Jong 2012). We did not find any reports in the literature of IAV detection in air specimens further than 2.1 km downwind from infected barns, however, and these were weak detections of viral RNA without successful

virus isolation (Corzo et al. 2013). Two swine farms were located within 10 km of the site of the mink outbreak; one 2.2 km away and the other 9.0 km away. Eleven turkey farms were located within 10 km of the mink farm.

Ultimately, the limited extent of genomic surveillance for IAVs in local swine and poultry populations constrained our ability to identify a local source for the outbreak. It also restricted our ability to assess the plausibility of different transmission routes. Although IAV is a reportable disease in swine in BC, the local industry is comparatively small so surveillance is conducted passively. Combined with the potential for asymptomatic or unremarkable infections, this means that under-reporting and under-detection is likely, especially in jurisdictions without large swine sectors where IAV surveillance in these animals may be less prioritised. Indeed, only four contemporaneous, local swine-origin H3N2 IAV genomes were available for analysis, opportunistically detected through an unrelated research study, and these viruses were not related to the mink farm outbreak. This suggests that IAV diversity within swine populations is under-characterised. This was further indicated by limited detections of IAVs with the same genome constellation as far afield as Iowa, Minnesota, Missouri and Ontario. This suggests that this IAV reassortant was able to disseminate across North America largely unnoticed. The uncomfortable corollary is that many other reassortant IAVs are likely emerging and disseminating unobserved within large, transnational, commercial swine populations.

Similarly, this report suggests that IAV spillovers into mink may be underestimated. After all, these detections were incidental and relied on: (1) an historic SARS-CoV-2 global pandemic that led to unprecedented molecular testing of mink with respiratory signs, and (2) the fortuitous multiplexing of SARS-CoV-2 with IAV in the laboratory where the mink specimens were tested. Without these circumstances, this outbreak would likely have passed unrecognised, suggesting IAV spillovers into mink are more common than assumed. Indeed, serological evidence for frequent spillovers of diverse avian, swine, and human seasonal IAVs into mink has been previously reported (Yagyu et al. 1982; Gagnon et al. 2009; Clayton et al. 2022; Agüero et al. 2023; Jiang et al. 2017; Peng et al. 2015; Yoon et al. 2012; Okazaki et al. 1983; Sun et al. 2021). For example, a survey of sera collected from mink slaughterhouses in Eastern China between 2016 and 2019 revealed 47.3% and 11.4% seroconversion to H1N1 and H3N2 IAVs, which were presumed to be human seasonal lineages due to an association with incidences of these subtypes in local human populations (Sun et al. 2021). Furthermore, surveys of sera from mink slaughterhouses and live mink herds between 2013 and 2019, also in Eastern China, showed up to 47.5% seroconversion to avian-origin H9N2, the most common subtype infecting Chinese poultry at the time (Peng et al. 2015; Sun et al. 2021; Zhang et al. 2015). One of the major limitations of our work is that it was not conducted as a deliberate study on IAVs in mink like the aforementioned examples. Consequently, the epidemiological generalisability of these findings is limited and they do not contribute to our understanding of the incidence of IAV outbreaks on North American mink farms, the attack rates in these outbreaks, prevalence of IAV infections in North American mink populations, or the clinical spectrum of these infections.

Spillover of diverse IAVs into mink raise several biosecurity and infection prevention and control issues. For mink producers, primary care veterinarians, and agriculture officials, there are questions of animal welfare and economic impact. It has been observed that mink infections with IAVs do not typically produce remarkable disease (Gagnon et al. 2009; Liu et al. 2020; Bo-Shun et al. 2020), which was indeed the case in this outbreak. This does not preclude severe disease in mink after future IAV spillovers, however. Indeed, natural infections of mink with human season H1N1 and avian-origin H5N1 viruses have been shown to induce severe disease, especially in kits (Clayton et al. 2022; Jiang et al. 2017; Åkerstedt et al. 2012). It also possible that IAV impacts on mink are underestimated and largely unattributed due to limited molecular testing and confirmatory genomics for mink with respiratory disease.

These spillovers also raise issues for public health officials. Similarities between human and mustelid respiratory physiology suggest overlapping susceptibility to certain IAV lineages, as evidenced by the apparently frequent spillover of human seasonal IAVs into mink noted above. This means that IAVs detected in mink must be assessed as potential spillover threats to humans. Furthermore, the detection of avian- and swine-origin lineages circulating in mink raises the possibility that these animals may facilitate IAV genome segment reassortment, similar to the mixing vessel role played by swine (Zhao et al. 2019; Mok and Qin 2023). High seroconversion rates also suggest that mink have a larger exposure interface with poultry and swine populations than humans, which may allow them to serve as a selective conduit into human populations for reassortants arising in other livestock.

While this creates spillover risk, it also creates surveillance opportunities. The mink industry could be a valuable sentinel for reassortant zoonotic IAVs, especially those with high potential to successful replicate within and transmit between human hosts. Fortunately, no spillover between mink and humans was observed in this outbreak, and these IAVs were assessed to pose no immediate threat to public health. They are not related to other swine-origin H3N2 viruses like the H3N2v lineages that caused outbreaks in humans attending agricultural fairs (Jhung et al. 2013; Greenbaum et al. 2015; Duwell et al. 2018). Furthermore, any hypothetical threat from this particular IAV lineage via mink would appear trivial when compared to the threat from the much vaster transnational commercial swine populations in which this lineage is apparently widespread.

While this study was unable to identify the local source of this outbreak, it did provide valuable insights. Foremost, it highlighted the need for more extensive genomic surveillance of IAVs in classic livestock hosts like swine and poultry. It also highlighted the need to expand surveillance to other types of livestock that are not considered classic IAV hosts, like mink. Recent reports of highly pathogenic H5N1 avian influenza infections in American dairy cattle further demonstrate that IAVs may be more widespread in our livestock populations and food systems than previously imagined (Burrough et al. 2024; Caserta et al. 2024). Increased surveillance would assist outbreak investigations and further reveal the type of exposures that are responsible for IAV infections in mink and other livestock. Drawing these connections may also advance our

understanding of environmental transmission between farms, which would improve infection prevention and control practices for all livestock industries impacted by IAV spillovers. Finally, heightened genomic surveillance of IAVs in swine and mink would increase detections of well-adapted reassortments, improving pandemic preparedness.

Acknowledgements

We are deeply grateful to Terry Engebretson and Dr Dave McDermid for their invaluable contributions to this project. We also thank Dr Agatha Jassem, Frankie Tsang and the virology program at the BC CDC PHL for their assistance with molecular testing of mink specimens.

Ethics Statement

Patient consent, permission to reproduce material from other sources and clinical trial registration were not applicable for this work. The animal specimen collection protocol was approved by the Animal Care Review Board of the University of British Columbia on 6 April 2021 (protocol A21-106).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

IAV genome segment sequences have been uploaded to the National Center for Biotechnology Information (NCBI) GenBank database under the accession numbers PQ510505 to PQ510594. They have also been uploaded to the Global Initiative for Sharing All Influenza Data (GISAID) EpiFlu database under the accession numbers EPI_ISL_19132429 and EPI_ISL_19166148 to −63.

References

Abdelwhab, E. M., and T. C. Mettenleiter. 2023. "Zoonotic Animal Influenza Virus and Potential Mixing Vessel Hosts." *Viruses* 15, no. 4: 980. https://doi.org/10.3390/v15040980.

Agüero, M., I. Monne, A. Sánchez, et al. 2023. "Highly Pathogenic Avian Influenza A(H5N1) Virus Infection in Farmed Minks, Spain, October 2022." *Euro Surveillance* 28, no. 3: 2300001. https://doi.org/10.2807/1560-7917.ES.2023.28.3.2300001.

Åkerstedt, J., M. Valheim, A. Germundsson, et al. 2012. "Pneumonia Caused by Influenza A H1N1 2009 Virus in Farmed American Mink (*Neovison vison*)." *Veterinary Record* 170, no. 14: 362. https://doi.org/10.1136/vr.100512.

Anderson, T. K., J. Chang, Z. W. Arendsee, et al. 2021. "Swine Influenza A Viruses and the Tangled Relationship With Humans." *Cold Spring Harbor Perspectives in Medicine* 11, no. 3: a038737. https://doi.org/10.1101/cshperspect.a038737.

Anderson, T. K., M. I. Nelson, P. Kitikoon, S. L. Swenson, J. A. Korslund, and A. L. Vincent. 2013. "Population Dynamics of Cocirculating Swine Influenza A Viruses in the United States From 2009 to 2012." *Influenza and Other Respiratory Viruses* 7, no. S4: 42–51. https://doi.org/10.1111/irv.12193.

Bo-Shun, Z., L. J. Li, Z. Qian, et al. 2020. "Co-Infection of H9N2 Influenza Virus and *Pseudomonas aeruginosa* Contributes to the Development of Hemorrhagic Pneumonia in Mink." *Veterinary Microbiology* 240: 108542. https://doi.org/10.1016/j.vetmic.2019.108542.

Boyd, E., M. Coombe, N. Prystajecky, et al. 2023. "Hands off the Mink! Using Environmental Sampling for SARS-CoV-2 Surveillance in American Mink." *International Journal of Environmental Research and Public Health* 20, no. 2: 1248. https://doi.org/10.3390/ijerph20021248.

Burrough, E. R., D. R. Magstadt, B. Petersen, et al. 2024. "Highly Pathogenic Avian Influenza A(H5N1) Clade 2.3.4.4b Virus Infection in Domestic Dairy Cattle and Cats, United States, 2024." *Emerging Infectious Diseases* 30, no. 7: 1335–1343. https://doi.org/10.3201/eid3007.240508.

Camacho, C., G. Coulouris, V. Avagyan, et al. 2009. "BLAST+: Architecture and Applications." *BMC Bioinformatics* 10: 421. https://doi.org/10.1186/1471-2105-10-421.

Caserta, L. C., E. A. Frye, S. L. Butt, et al. 2024. "Spillover of Highly Pathogenic Avian Influenza H5N1 Virus to Dairy Cattle." *Nature* 634: 669–676. https://doi.org/10.1038/s41586-024-07849-4.

Choi, Y. K., J. H. Lee, G. Erickson, et al. 2004. "H3N2 Influenza Virus Transmission From Swine to Turkeys, United States." *Emerging Infectious Diseases* 10, no. 12: 2156–2160. https://doi.org/10.3201/eid1012.040581.

Clayton, M. J., E. J. Kelly, M. Mainenti, et al. 2022. "Pandemic Lineage 2009 H1N1 Influenza A Virus Infection in Farmed Mink in Utah." *Journal of Veterinary Diagnostic Investigation* 34, no. 1: 82–85. https://doi.org/10.1177/10406387211052966.

Corzo, C. A., M. Culhane, S. Dee, R. B. Morrison, and M. Torremorell. 2013. "Airborne Detection and Quantification of Swine Influenza A Virus in Air Samples Collected Inside, Outside and Downwind From Swine Barns." *PLoS ONE* 8, no. 8: e71444. https://doi.org/10.1371/journal.pone.0071444.

Duwell, M. M., D. Blythe, M. W. Radebaugh, et al. 2018. "Influenza A(H3N2) Variant Virus Outbreak at Three Fairs—Maryland, 2017." *Morbidity and Mortality Weekly Report* 67, no. 42: 1169–1173. https://doi.org/10.15585/mmwr.mm6742a1.

Gagnon, C. A., G. Spearman, A. Hamel, et al. 2009. "Characterization of a Canadian Mink H3N2 Influenza A Virus Isolate Genetically Related to Triple Reassortant Swine Influenza Virus." *Journal of Clinical Microbiology* 47, no. 3: 796–799. https://doi.org/10.1128/JCM.01228-08.

Gillett, N. P., A. J. Cannon, E. Malinina, et al. 2022. "Human Influence on the 2021 British Columbia Floods." *Weather and Climate Extremes* 36: 100441. https://doi.org/10.1016/j.wace.2022.100441.

Greenbaum, A., C. Quinn, J. Bailer, et al. 2015. "Investigation of an Outbreak of Variant Influenza A(H3N2) Virus Infection Associated With an Agricultural Fair—Ohio, August 2012." *Journal of Infectious Diseases* 212, no. 10: 1592–1599. https://doi.org/10.1093/infdis/jiv269.

Hempel, E. M., A. Bharmal, G. Li, et al. 2024. "Prospective, Clinical Comparison of Self-Collected Throat-Bilateral Nares Swabs and Saline Gargle Compared to Health Care Provider Collected Nasopharyngeal Swabs Among Symptomatic Outpatients With Potential SARS-CoV-2 Infection." *Journal of the Association of Medical Microbiology and Infectious Disease Canada* 8, no. 4: 283–298. https://doi.org/10.3138/jammi-2023-0002.

Hickman, R., J. Nguyen, T. D. Lee, et al. 2022. "Rapid, High-Throughput, Cost Effective Whole Genome Sequencing of SARS-CoV-2 Using a Condensed One Hour Library Preparation of the Illumina DNA Prep Kit." *MedRxiv* 2022.02.07.22269672. https://doi.org/10.1101/2022.02.07.22269672.

Hoang, D. T., O. Chernomor, A. von Haeseler, B. Q. Minh, and L. S. Vinh. 2018. "UFBoot2: Improving the Ultrafast Bootstrap Approximation." *Molecular Biology and Evolution* 35, no. 2: 518–522. https://doi.org/10.1093/molbev/msx281.

Jhung, M. A., S. Epperson, M. Biggerstaff, et al. 2013. "Outbreak of Variant Influenza A(H3N2) Virus in the United States." *Clinical*

Infectious Diseases 57, no. 12: 1703–1712. https://doi.org/10.1093/cid/cit649.

Jiang, W., S. Wang, C. Zhang, et al. 2017. "Characterization of H5N1 Highly Pathogenic Mink Influenza Viruses in Eastern China." *Veterinary Microbiology* 201: 225–230. https://doi.org/10.1016/j.vetmic.2017.01.028.

Jonges, M., J. van Leuken, I. Wouters, G. Koch, A. Meijer, and M. Koopmans. 2015. "Wind-Mediated Spread of Low-Pathogenic Avian Influenza Virus Into the Environment During Outbreaks at Commercial Poultry Farms." *PLoS ONE* 10, no. 5: e0125401. https://doi.org/10.1371/journal.pone.0125401.

Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. von Haeseler, and L. S. Jermiin. 2017. "ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates." *Nature Methods* 14, no. 6: 587–589. https://doi.org/10.1038/nmeth.4285.

Karasin, A. I., C. W. Olsen, I. H. Brown, S. Carman, M. Stalker, and G. Josephson. 2000. "H4N6 Influenza Virus Isolated From Pigs in Ontario." *Canadian Veterinary Journal* 41, no. 12: 938–939.

Katoh, K., and D. M. Standley. 2013. "MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability." *Molecular Biology and Evolution* 30, no. 4: 772–780. https://doi.org/10.1093/molbev/mst010.

Koopmans, M. 2021. "SARS-CoV-2 and the Human-Animal Interface: Outbreaks on Mink Farms." *Lancet Infectious Diseases* 21, no. 1: 18–19. https://doi.org/10.1016/S1473-3099(20)30912-9.

Kuchinski, K. S., M. Coombe, S. C. Mansour, et al. 2024. "Targeted Genomic Sequencing of Avian Influenza Viruses in Wetland Sediment From Wild Bird Habitats." *Applied and Environmental Microbiology* 90, no. 2: e0084223. https://doi.org/10.1128/aem.00842-23.

Liu, J., Z. Li, Y. Cui, H. Yang, H. Shan, and C. Zhang. 2020. "Emergence of an Eurasian Avian-Like Swine Influenza A (H1N1) Virus From Mink in China." *Veterinary Microbiology* 240: 108509. https://doi.org/10.1016/j.vetmic.2019.108509.

Ma, W., R. E. Kahn, and J. A. Richt. 2008. "The Pig as a Mixing Vessel for Influenza Viruses: Human and Veterinary Implications." *Journal of Molecular and Genetic Medicine* 3, no. 1: 158–166.

Ma, W., K. M. Lager, A. L. Vincent, B. H. Janke, M. R. Gramer, and J. A. Richt. 2009. "The Role of Swine in the Generation of Novel Influenza Viruses." *Zoonoses and Public Health* 56, no. 6–7: 326–337. https://doi.org/10.1111/j.1863-2378.2008.01217.x.

Minh, B. Q., H. A. Schmidt, O. Chernomor, et al. 2020. "IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era." *Molecular Biology and Evolution* 37, no. 5: 1530–1534. https://doi.org/10.1093/molbev/msaa015.

Mok, C. K. P., and K. Qin. 2023. "Mink Infection With Influenza A Viruses: An Ignored Intermediate Host?" *One Health Advances* 1, no. 1: 5. https://doi.org/10.1186/s44280-023-00004-0.

Nelli, R. K., S. V. Kuchipudi, G. A. White, B. B. Perez, S. P. Dunham, and K. C. Chang. 2010. "Comparative Distribution of Human and Avian Type Sialic Acid Influenza Receptors in the Pig." *BMC Veterinary Research* 6, no. 1: 4. https://doi.org/10.1186/1746-6148-6-4.

Ng, P. S. K., R. Böhm, L. E. Hartley-Tassell, et al. 2014. "Ferrets Exclusively Synthesize Neu5Ac and Express Naturally Humanized Influenza A Virus Receptors." *Nature Communications* 5: 5750. https://doi.org/10.1038/ncomms6750.

Nituch, L. A., J. Bowman, P. J. Wilson, and A. I. Schulte-Hostedde. 2015. "Aleutian Mink Disease Virus in Striped Skunks (*Mephitis mephitis*): Evidence for Cross-Species Spillover." *Journal of Wildlife Diseases* 51, no. 2: 389–400. https://doi.org/10.7589/2014-05-141.

Okazaki, K., R. Yanagawa, H. Kida, and H. Noda. 1983. "Human Influenza Virus Infection in Mink: Serological Evidence of Infection in Summer and Autumn." *Veterinary Microbiology* 8, no. 3: 251–257. https://doi.org/10.1016/0378-1135(83)90077-9.

Olsen, C. W., A. I. Karasin, S. Carman, et al. 2006. "Triple Reassortant H3N2 Influenza A Viruses, Canada, 2005." *Emerging Infectious Diseases Journal* 12, no. 7: 1132–1135. https://doi.org/10.3201/eid1207.060268.

Paiero, A., E. Newhouse, E. Chan, et al. 2022. "SARS-CoV-2 in Mink Farms in British Columbia, Canada: A Report of Two Outbreaks in 2020–2021." *Canada Communicable Disease Report* 48, no. 6: 274–281. https://doi.org/10.14745/ccdr.v48i06a05.

Peng, L., C. Chen, H. Kai-yi, et al. 2015. "Molecular Characterization of H9N2 Influenza Virus Isolated From Mink and Its Pathogenesis in Mink." *Veterinary Microbiology* 176, no. 1–2: 88–96. https://doi.org/10.1016/j.vetmic.2015.01.009.

Pomorska-Mól, M., J. Włodarek, M. Gogulski, and M. Rybska. 2021. "Review: SARS-CoV-2 Infection in Farmed Minks—An Overview of Current Knowledge on Occurrence, Disease and Epidemiology." *Animal* 15, no. 7: 100272. https://doi.org/10.1016/j.animal.2021.100272.

Ramey, A. M., A. B. Reeves, J. Z. Drexler, et al. 1934. "Influenza A Viruses Remain Infectious for More Than Seven Months in Northern Wetlands of North America." *Proceedings of the Royal Society B: Biological Sciences* 2020, no. 287: 20201680. https://doi.org/10.1098/rspb.2020.1680.

Ramey, A. M., A. B. Reeves, B. J. Lagassé, et al. 2022. "Evidence for Interannual Persistence of Infectious Influenza A Viruses in Alaska Wetlands." *Science of the Total Environment* 803: 150078. https://doi.org/10.1016/j.scitotenv.2021.150078.

Scoizec, A., E. Niqueux, R. Thomas, P. Daniel, A. Schmitz, and S. Le Bouquin. 2018. "Airborne Detection of H5N8 Highly Pathogenic Avian Influenza Virus Genome in Poultry Farms, France." *Frontiers in Veterinary Science* 5: 15. https://doi.org/10.3389/fvets.2018.00015.

Ssematimba, A., T. J. Hagenaars, and M. C. M. de Jong. 2012. "Modelling the Wind-Borne Spread of Highly Pathogenic Avian Influenza Virus Between Farms." *PLoS ONE* 7, no. 2: e31114. https://doi.org/10.1371/journal.pone.0031114.

Sun, H., F. Li, Q. Liu, et al. 2021. "Mink Is a Highly Susceptible Host Species to Circulating Human and Avian Influenza Viruses." *Emerging Microbes & Infections* 10, no. 1: 472–480. https://doi.org/10.1080/22221751.2021.1899058.

Walia, R. R., T. K. Anderson, and A. L. Vincent. 2019. "Regional Patterns of Genetic Diversity in Swine Influenza A Viruses in the United States From 2010 to 2016." *Influenza and Other Respiratory Viruses* 13, no. 3: 262–273. https://doi.org/10.1111/irv.12559.

Yagyu, K., R. Yanagawa, Y. Matsuura, H. Fukushi, H. Kida, and H. Noda. 1982. "Serological Survey of Influenza A Virus Infection in Mink." *Nihon Juigaku Zasshi* 44, no. 4: 691–693. https://doi.org/10.1292/jvms1939.44.691.

Yoon, K. J., K. Schwartz, D. Sun, J. Zhang, and H. Hildebrandt. 2012. "Naturally Occurring Influenza A Virus Subtype H1N2 Infection in a Midwest United States Mink (*Mustela vison*) Ranch." *Journal of Veterinary Diagnostic Investigation* 24, no. 2: 388–391. https://doi.org/10.1177/1040638711428349.

Ypma, R. J. F., M. Jonges, A. Bataille, et al. 2013. "Genetic Data Provide Evidence for Wind-Mediated Transmission of Highly Pathogenic Avian Influenza." *Journal of Infectious Diseases* 207, no. 5: 730–735. https://doi.org/10.1093/infdis/jis757.

Zhang, C., Y. Xuan, H. Shan, et al. 2015. "Avian Influenza Virus H9N2 Infections in Farmed Minks." *Virology Journal* 12: 180. https://doi.org/10.1186/s12985-015-0411-4.

Zhao, C., and J. Pu. 2022. "Influence of Host Sialic Acid Receptors Structure on the Host Specificity of Influenza Viruses." *Viruses* 14, no. 10: 2141. https://doi.org/10.3390/v14102141.

Zhao, P., L. Sun, J. Xiong, et al. 2019. "Semiaquatic Mammals Might Be Intermediate Hosts to Spread Avian Influenza Viruses From Avian to Human." *Scientific Reports* 9: 11641. https://doi.org/10.1038/s41598-019-48255-5.

Zhou, B., M. E. Donnelly, D. T. Scholes, et al. 2009. "Single-Reaction Genomic Amplification Accelerates Sequencing and Vaccine Production for Classical and Swine Origin Human Influenza A Viruses." *Journal of Virology* 83, no. 19: 10309–10313. https://doi.org/10.1128/JVI.01109-09.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.