



## VECTOR-BORNE INFECTIONS—PART 2: WILDLIFE & COMPANION ANIMALS

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# CCDR

## CANADA COMMUNICABLE DISEASE REPORT

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# Rabies in an imported dog, Ontario, 2021

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## Abstract

In July 2021, a dog was imported into Canada from Iran and subsequently developed clinical signs of rabies within 11 days of arrival. Following laboratory confirmation of the diagnosis of rabies, local, provincial and federal inter-agency collaboration was required to complete contact tracing to identify all persons and domestic animals that may have been exposed to the rabid dog during the potential virus shedding period. This case highlights the risks of importing animals from known canine rabies-endemic areas, identifies gaps in current dog importation policies that pose potential risk to human and animal health and prompts ongoing vigilance for this deadly disease among human and animal health partners, as well as members of the public who adopt imported dogs.

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**Keywords:** rabies, imported dog, Ontario, Canada

## Introduction

Requirements for importation of domestic animals into Canada are governed by the *Health of Animals Regulations* (1), with specific provisions for some categories of animals developed by the Canadian Food Inspection Agency (CFIA). For dogs, this may include proof of vaccination against rabies or a veterinary certificate confirming the animal has resided in a country considered free of terrestrial rabies for at least six months, though requirements differ and may be stringent, depending on age and purpose of the import (personal, assistive or commercial) (2). Rabies is the only disease for which Canada has specific importation requirements for dogs due to the significant public health and animal health consequences of this disease; however, the current requirements do not prevent the importation of dogs that may be incubating rabies infection in all cases.

Rabies is a viral disease that attacks the central nervous system of mammals, including humans, and is almost always fatal. Due to effective public health interventions—such as education and response to potential human exposures, effective risk assessment and management of potential domestic animal exposures, the availability of timely and reliable laboratory diagnostics, and the provision of timely rabies post-exposure prophylaxis—human cases of rabies in Canada remain rare (3) and Canada has been free of canine rabies since some time in the 1950s (3).

Nonetheless, vigilant monitoring and action by Canadian federal and provincial/territorial agencies remain crucial, particularly regarding imported dogs. The global burden of rabies is estimated to be approximately 60,000 human deaths each year, with 99% of cases associated with transmission from dogs (4).

This is concerning given increasing human and animal movement globally, as well as low rabies vaccination rates in domestic animals in many rabies-endemic areas. In the United States, there have also been increasing reports of fraudulent or questionable rabies vaccine certificates for dogs that were imported from canine rabies-endemic countries (5,6).

A recent case of rabies in a dog imported from a canine rabies-endemic country to Canada illustrates some of the risks to Canadians associated with canine importation and the coordinated actions required to protect human and animal health in such cases.

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## Case summary

An approximately 2-year-old mixed-breed dog (hereafter referred to as Dog 1) was imported from Iran via Europe through Toronto Pearson International Airport in Ontario, Canada on July 1, 2021. This dog was imported by a rescue organization that had prearranged its adoption by a family in Ontario.

On July 11, Dog 1 began exhibiting abnormal clinical signs, including an unspecified ocular issue, drooling and behaviour changes. The dog was assessed at a local veterinary clinic and sent home. On July 12, the dog's clinical signs had progressed, and based on the history of importation and compatibility of the dog's signs with rabies, the owner authorized euthanasia of the dog, and tissues were collected and submitted for rabies testing. The local public health unit (PHU) began its investigation into potential human exposures at this time, and details of the investigation are described below. The animal was confirmed to be positive for rabies based on fluorescent antibody testing performed by the CFIA rabies laboratory on July 15. Following

receipt of the positive result, the local PHU expanded its investigation, which required collaboration between eight local PHUs, the provincial Ministry of Health and Ministry of Agriculture, Food and Rural Affairs, Public Health Ontario, the Public Health Agency of Canada, CFIA and the Canada Border Services Agency.

Further testing by CFIA determined that the dog was infected with rabies antigenic variant IRAN-1 (7). Nucleotide sequencing and phylogenetic analysis corroborated the antigenic typing result and indicated the virus grouped with canine-variant viruses known to circulate in Iran and Iraq (Clade "D") (8). This confirmed that the dog was infected prior to departure from Iran, which is a high-risk area for canine rabies (**Figure 1**) (9). Rabies is an internationally notifiable animal disease and, given that this was a novel variant to Canada, an immediate notification was submitted to the World Organisation for Animal Health by the federal government in August 2021 (10).

Figure 1: Map of high-risk countries for dog rabies<sup>a</sup>



<sup>a</sup> Developed by Public Health Agency of Canada. Data source (9)



## Public health investigation

The public health investigation found that Dog 1 had travelled on international flights from Iran to Ontario via Frankfurt, Germany. When Dog 1 arrived in Ontario, it was met by a representative from the coordinating rescue organization and was transferred to a foster family for overnight lodging. On July 2, Dog 1 was transferred from the foster family to its adoptive family that subsequently introduced the dog to their extended family and friends. The dog also had contact with veterinary staff at two clinics prior to being euthanized on July 12. The dog's adoptive family provided a veterinary health certificate from Iran that included a record of a single rabies vaccination in October 2020 using a killed rabies vaccine product.

During the investigation, a second dog (Dog 2) was identified as having travelled from Iran to Ontario in the same shipment as Dog 1, but in a separate crate. Further investigation by the CFIA and the Canada Border Services Agency yielded no evidence that the two dogs had any direct contact. Therefore, Dog 2 was not considered at increased risk of rabies exposure from Dog 1. While Dog 2 also had a record of rabies vaccination prior to importation from Iran, the dog was re-vaccinated for rabies as a precaution to ensure it was effectively vaccinated using a Canadian-licensed product, as per the requirements of the *Ontario Health Protection and Promotion Act, Regulation 567* (11). No contact with any other animals (domestic or wildlife) was reported for Dog 1.

## Human contact tracing

An exposure period for contacts was established based on the defined period of communicability for rabies in domestic dogs, which is up to 10 days prior to the onset of clinical signs (12). Out of an abundance of caution, an exposure was defined as a person who had direct contact with Dog 1 involving a bite, scratch, or saliva exposure into a wound or mucous membrane from July 1 to July 12, 2021 (12 days).

A total of 24 individuals were identified as having contact with Dog 1 during this exposure period, of which 14 were considered exposed as described above and therefore received provincially funded post-exposure prophylaxis at an average cost of approximately CAD 2,000 per person (13,14). Due to the number and geographical distribution of these individuals, this required coordinated effort from multiple local PHUs and the provincial Ministry of Health. As all potential contacts were identified during this multi-jurisdictional investigation, there was no risk to the public and therefore no public risk communication was issued. High-risk contacts of Dog 1 included the foster and adoptive family members, veterinary staff, guests of the adoptive family and rescue organization personnel. No high-risk contacts were identified among airport staff. A notification was also sent to Iran via the International Health Regulations National Focal Point.

## Conclusion

This case highlights the need for ongoing vigilance for rabies among human and animal health partners, as well as members of the public who adopt imported dogs, particularly from high-risk countries. While the federal import requirement for rabies vaccination was met by the rescue organization involved, this case illustrates that this does not preclude the importation of animals incubating rabies infection and the severe consequences associated with the importation of rabid animals into Canada. Ineffective or improperly administered vaccines can also contribute to this risk, and fraudulent documentation of vaccination can be an additional compounding factor. As of July 14, 2021, the United States temporarily suspended importation of dogs from countries considered high-risk for canine rabies as a protective measure against such incidents (15).

Federal import requirements for dogs have been under review in Canada for several years; in May 2021 various changes were made to importation requirements for commercial dogs under eight months of age (16). Commercial dogs are those imported for breeding, resale and adoption end uses (16). This review should continue for all categories of dogs, with the aim of preventing animals infected with rabies from entering Canada. More stringent requirements for proof of vaccination with effective vaccine products (including a waiting period between vaccination and import), rabies titre testing and pre and/or post-importation quarantine requirements for dogs from designated high-risk countries could also be considered.

This incident also highlights the need for ongoing awareness among human and animal healthcare practitioners as well as public health agencies of the risks of rabies exposure from recently imported dogs (17,18). Public health professionals and veterinarians should strive to educate the public about the risks associated with importing animals from high-risk countries, promote consistent and timely vaccination of animals, and report any suspect imported animals to provincial and federal agencies promptly. Lastly, this incident underscores the financial and human resources costs associated with the number of local, provincial and federal agencies involved along with post-exposure prophylaxis required for high-risk contacts.

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## Competing interests

None.

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**CCDR** CANADA COMMUNICABLE DISEASE REPORT





# SARS-CoV-2 wildlife surveillance in Ontario and Québec

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## Abstract

**Background:** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the coronavirus disease 2019 pandemic, is capable of infecting a variety of wildlife species. Wildlife living in close contact with humans are at an increased risk of SARS-CoV-2 exposure and, if infected, have the potential to become a reservoir for the pathogen, making control and management more difficult. The objective of this study is to conduct SARS-CoV-2 surveillance in urban wildlife from Ontario and Québec, increasing our knowledge of the epidemiology of the virus and our chances of detecting spillover from humans into wildlife.

**Methods:** Using a One Health approach, we leveraged activities of existing research, surveillance and rehabilitation programs among multiple agencies to collect samples from 776 animals from 17 different wildlife species between June 2020 and May 2021. Samples from all animals were tested for the presence of SARS-CoV-2 viral ribonucleic acid, and a subset of samples from 219 animals across three species (raccoons, *Procyon lotor*; striped skunks, *Mephitis mephitis*; and mink, *Neovison vison*) were also tested for the presence of neutralizing antibodies.

**Results:** No evidence of SARS-CoV-2 viral ribonucleic acid or neutralizing antibodies was detected in any of the tested samples.

**Conclusion:** Although we were unable to identify positive SARS-CoV-2 cases in wildlife, continued research and surveillance activities are critical to better understand the rapidly changing landscape of susceptible animal species. Collaboration between academic, public and animal health sectors should include experts from relevant fields to build coordinated surveillance and response capacity.

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**Keywords:** SARS-CoV-2, wildlife, surveillance, Ontario, Québec

## Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the global coronavirus disease 2019 (COVID-19) pandemic and has been maintained through human-to-human transmission. However, humans are not the only species susceptible to infection. Over the course of the current pandemic, a range of domestic and wild animal species have been reported to either be naturally infected with SARS-CoV-2 or susceptible to the virus in experimental infections (1–4). As of April 30, 2022, 36 countries have reported positive SARS-CoV-2 cases in 23 different animal species to the World Organisation for Animal Health (5). Other species have been identified as potential hosts based on sequence analysis of the host cell receptor of SARS-CoV-2, angiotensin 1 converting enzyme 2 and predicted binding affinity (6,7).

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Many wild animal species, such as raccoons, skunks and bats, thrive in the ecological overlap with humans and are thus at an increased risk of being exposed to SARS-CoV-2 (8). Several peri-domestic species have been experimentally shown to become infected with and shed SARS-CoV-2 (9,10). SARS-CoV-2 infection has also been reported in wild or free-ranging animals that have been naturally exposed, including American mink (*Neovison vison*) in Spain (11) and, more recently, white-tailed deer (*Odocoileus virginianus*) in multiple locations across North America (12–16). In Ontario, this includes identification of a probable case of deer-to-human viral transmission (16). Infection in animals can result in mild to severe symptoms of respiratory disease up to and including death via interstitial pneumonia (e.g. mink) (17,18). Other species do not show clinical signs of infection (e.g. skunks) (9,10) or show only mild and transient symptoms in some individuals, such as elevated temperature (e.g. white-tailed deer) (19).

The concept of One Health recognizes that human and animal health are interdependent (20). The spillover of virus from humans or domestic animals into wildlife is concerning not only due to the possible deleterious effects on wildlife, but because these wild populations have the potential to act as reservoirs for SARS-CoV-2. Pathogens that have an animal reservoir are inherently more difficult to control and the spread of SARS-CoV-2 through animal populations could further contribute to the development of variants of concern (VoCs), potentially undermining the efficacy of countermeasures such as antivirals and vaccines (21,22). As such, there have been calls for increased surveillance at the human-wildlife interface (23). Urban areas around the world have been a particular area of concern and focus (24–26). The higher density of both human and some peri-urban wildlife species populations in urban centres can lead to more frequent human-animal contact and increased potential for disease transmission. Additionally, people who have close contact with wildlife, such as biologists, rehabilitators, and hunters and trappers, may be at higher risk of being exposed to the virus and of facilitating its spread among wildlife. The impact of SARS-CoV-2 infection on wildlife health is not fully understood. Early detection of any spillover is therefore critical to preventing and addressing these concerns.

Given the risk of reverse-zoonotic SARS-CoV-2 transmission and our lack of knowledge of the virus in local wildlife, there was an urgent need to elucidate the epidemiology of the virus at the human-wildlife interface to help wildlife management and public health officials better communicate risk and plan management strategies. We therefore conducted SARS-CoV-2 surveillance in wildlife across Ontario and Québec, with a major focus on the southern regions of both provinces. These areas have high human population densities and include major urban centres such as Toronto and Montréal. Between spring 2020 and spring 2021, incidences of COVID-19 peaked in Montréal and the surrounding regions in early January 2021, with rates exceeding 400 cases per 100,000 population in Montréal and Laval (27).

Incidences between spring 2020 and spring 2021 in the Greater Toronto Area peaked in April 2021, with case rates in the City of Toronto and Peel also exceeding 400 per 100,000 population (27).

## Methods

Many experts have recommended a One Health approach for animal SARS-CoV-2 testing, which balances concerns for both human and animal health and is based on knowledge of experts in both fields (28,29). As such, our work was conducted through consultation and cooperation among a wide variety of agencies: the Public Health Agency of Canada; the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry (NDMNRF); *le Ministère des Forêts, de la Faune et des Parcs du Québec*; the Canadian Wildlife Health Cooperative (CWHC); the Ontario Ministry of Agriculture, Food, and Rural Affairs; the Canadian Food Inspection Agency; the Western College of Veterinary Medicine; the Granby Zoo; the National Microbiology Laboratory (NML) of the Public Health Agency of Canada; and Sunnybrook Research Institute. All samples for testing were collected between June 2020 and May 2021 through pre-existing partnerships or over the course of other research, surveillance or rehabilitation work (Table 1).

## Raccoons and skunks

Raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*) are peri-domestic species that are good candidates for reverse-zoonotic disease surveillance due to their high density in urban areas and their frequent close contact with people, pets and refuse. They are also subject to ongoing rabies surveillance operations in both Ontario and Québec, making them easy to sample. In Ontario, wildlife rabies surveillance and testing are conducted by the NDMNRF on roadkill, animals found dead for other reasons, and wildlife that were sick or acting strangely. Submissions are received mainly from southwestern Ontario, and most animals received by the program and subsequently sampled and tested for SARS-CoV-2 came from urban centres within this region or had a case history of close contact with people (Figure 1). In Québec, a similar wildlife rabies surveillance program is coordinated by *le Ministère des Forêts, de la Faune et des Parcs du Québec* and testing and other post-mortem examinations are performed by the Québec CWHC. As was the case in Ontario, animals sampled by the Québec CWHC for SARS-CoV-2 testing came mainly from urban areas (Figure 1). The Ontario CWHC laboratory also contributed a small number of raccoon and skunk samples from animals submitted to them for post-mortem examination. Carcasses were sampled using a combination of oral, nasal, and rectal swabs, respiratory tissue and intestinal tissue (Table 1). Swabs were stored in individual 2 mL tubes with ~1 mL of universal transport medium (UTM; Sunnybrook Research Institute) and 30–60 mg tissue samples were stored dry in tubes.



Table 1: Metadata for 776 animals from Ontario and Québec screened for severe acute respiratory syndrome coronavirus 2

Species	Sampling agency	Sample source	Sample location(s)	Dates of collection	Number of individuals sampled	Types of samples tested	Test performed <sup>a</sup>
Raccoon ( <i>Procyon lotor</i> )	CWHC	Rabies surveillance (Québec samples), post-mortem exam	Southern Ontario, Southern Québec	Aug 2020–Feb 2021	11	Respiratory tissue	PCR
			Southern Québec	Nov–Dec 2020	68	Respiratory tissue, rectal swab	
			Southern Ontario, Southern Québec	Oct 2020–June 2021	15	Respiratory and intestinal tissue	
			Southwestern Québec	Jan 2021	3	Nasal swab	
			Southern Québec	Jan–June 2021	54	Nasal and rectal swabs	
	NDMNRF and CWHC	Rabies surveillance, post-mortem exam	Hamilton, Ontario	Dec 2020	1	Oral and rectal swabs, respiratory and intestinal tissue	Antibody
	NDMNRF	Rabies surveillance	Southwestern Ontario	June 2020–Jan 2021	100	Oral and rectal swabs	
		Rabies seroprevalence study	Oakville, Ontario	Sept–Oct 2020	141	Oral and rectal swabs	
					Sera		
Total raccoons sampled					393	-	
Striped skunk ( <i>Mephitis mephitis</i> )	CWHC	Rabies surveillance (Québec samples), post-mortem exam	Southern Québec	Jan–June 2021	66	Nasal swab	PCR
			Southern Ontario, Southern Québec	July–Dec 2020	55	Respiratory tissue	
			Southern Ontario, Southwestern Québec, Saint-Félicien, Québec	Oct 2020–Apr 2021	9	Respiratory and intestinal tissue	
	NDMNRF	Rabies surveillance, rabies seroprevalence study	Southwestern Ontario	Sept 2020–May 2021	104	Oral and rectal swabs	Antibody
			Rabies seroprevalence study	Oakville, Ontario	Sept–Oct 2020	36	
Total skunks sampled					270	-	
American mink ( <i>Neovision vison</i> )	CWHC	Post-mortem exam	Thornhill, Ontario	July 2020	1	Respiratory tissue	PCR
	NDMNRF	Registered fur harvesters, roadkill, rabies surveillance	Southern Ontario	Fall 2020–Spring 2021	42 <sup>b</sup>	Oral and rectal swabs, lung and intestinal tissue	
						Cardiac blood or Nobuto strips	
Total mink sampled					43	-	
Big brown bat ( <i>Eptesicus fuscus</i> )	Granby Zoo	Rehabilitation program	Southwestern Québec	Nov 2020–Mar 2021	15	Oral swabs	PCR
					2	Guano	
					15	Oral swabs and guano	
Total big brown bats sampled					32	-	
Hoary bat ( <i>Lasiurus cinerus</i> )	CWHC	Post-mortem exam	Etobicoke, Ontario	Dec 2020	1	Respiratory and intestinal tissue	PCR
American marten ( <i>Martes americana</i> )	CWHC	Post-mortem exam	Sainte-Anne-de-Bellevue, Québec	Nov 2020	1	Respiratory and intestinal tissue	PCR





**Table 1: Metadata for 776 animals from Ontario and Québec screened for severe acute respiratory syndrome coronavirus 2 (continued)**

Species	Sampling agency	Sample source	Sample location(s)	Dates of collection	Number of individuals sampled	Types of samples tested	Test performed <sup>a</sup>
Fisher ( <i>Pekania pennanti</i> )	CWHC	Post-mortem exam	Western Québec	May 2021	2	Respiratory and intestinal tissue	PCR
American black bear ( <i>Ursus americanus</i> )	CWHC	Post-mortem exam	Northern Ontario	Sept 2020	2	Respiratory tissue	PCR
			Killaloe, Ontario	Oct 2020	1	Respiratory and intestinal tissue	
Total black bears sampled					3	-	
Atlantic white-sided dolphin ( <i>Lagenorhynchus actus</i> )	CWHC	Post-mortem exam	Carleton-sur-Mer, Québec	June 2021	1	Intestinal tissue	PCR
			Sept-Îles, Québec	March 2021	1	Respiratory and intestinal tissue	
Total Atlantic white-sided dolphins sampled					2	-	
Harbour porpoise ( <i>Phocoena phocoena</i> )	CWHC	Post-mortem exam	La Montée, Québec	Dec 2020	1	Respiratory and intestinal tissue	PCR
Harbour seal ( <i>Phoca vitulina</i> )	CWHC	Post-mortem exam	Matane, Québec	Dec 2020	1	Respiratory and intestinal tissue	PCR
Coyote ( <i>Canis latrans</i> )	CWHC	Post-mortem exam	Saint-Alexandre-d'Iberville, Québec	April 2021	1	Respiratory and intestinal tissue	PCR
Eastern wolf ( <i>Canus lupus lycaon</i> )	CWHC	Post-mortem exam	Algonquin Provincial Park, Ontario	Oct 2020	1	Respiratory tissue	PCR
			Southern and central Ontario		4	Respiratory and intestinal tissue	
Total eastern wolves sampled					5	-	
Grey fox ( <i>Urocyon cinereoargenteus</i> )	CWHC	Post-mortem exam	Châteauguay, Québec	Dec 2020	1	Respiratory and intestinal tissue	PCR
Red fox ( <i>Vulpes vulpes</i> )	CWHC	Post-mortem exam	Mercier, Québec	Jan 2021	1	Nasal and rectal swabs	PCR
			Southwestern Québec	Nov–Dec 2020	4	Respiratory tissue, rectal swabs	
			Southern, Ontario	July–Oct 2020	5	Respiratory tissue	
			Dunham, Québec	Dec 2020	1	Respiratory and intestinal tissue	
Total red foxes sampled					11	-	
Virginia opossum ( <i>Didelphis virginiana</i> )	CWHC	Post-mortem exam	Bolton-Est, Québec	June 2021	1	Nasal and rectal swabs	PCR
			Southern Ontario	July–Oct 2020	2	Respiratory tissue	
			Southwestern Ontario, Saint-Jean-sur-Richelieu, Québec	Oct 2020, March 2021	3	Respiratory and intestinal tissue	
Total Virginia opossums sampled					6	-	
White-tailed deer ( <i>Odocoileus virginianus</i> )	CWHC	Post-mortem exam	London, Ontario, Southwestern Québec	Oct–Dec 2020	3	Respiratory and intestinal tissue	PCR

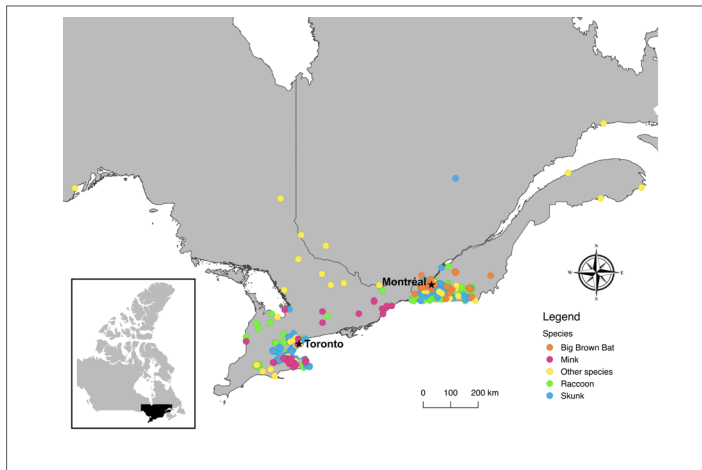
Abbreviations: CWHC, Canadian Wildlife Health Cooperative; NDMNRF, Northern Development, Mines, Natural Resources and Forestry; PCR, polymerase chain reaction; -, not applicable

<sup>a</sup> All PCR testing was performed at Sunnybrook Research Institute and all antibody testing was performed at the Public Health Agency of Canada's National Microbiology Laboratory

<sup>b</sup> Due to the condition of the carcasses, we were unable to collect lung tissue or cardiac blood from one individual, cardiac blood from a further two individuals and rectal swabs from two individuals. In cases where we could not collect cardiac blood, we instead submitted a Nobuto strip soaked in fluid from the chest cavity for antibody testing



**Figure 1: Original locations of animals submitted for severe acute respiratory syndrome coronavirus 2 testing June 2020–May 2021 (N=776)**



Additionally, samples were collected from live raccoons and skunks during an annual seroprevalence study conducted by the NDMNRF in Oakville, Ontario to assess the effectiveness of rabies vaccine baiting (NDMNRF Wildlife Animal Care Committee Protocol #358). Animals were captured in live traps and transported to a central processing station where they were anaesthetized. Oral and rectal swabs were collected for polymerase chain reaction (PCR) testing. Blood was drawn from the brachiocephalic vein and 0.2–1.0 mL of sera was collected for antibody testing. Following reversal and successful recovery, animals were returned to their point of capture and released.

## Mink

Instances of SARS-CoV-2 infection in mink have already been identified in multiple countries, including Canada, and infected farmed mink have proven capable of passing the virus to naive conspecifics, humans and companion animals (17,30–33). At the time of writing no mink farm outbreaks have been reported in Ontario or Québec, but mink farms in Ontario have previously been shown to act as points of infection for other viruses (e.g. Aleutian mink disease), which can spread to wild mink populations (34).

The majority of mink carcasses we sampled for SARS-CoV-2 testing were submitted to the NDMNRF by licensed fur harvesters through a collaboration with the Ontario Fur Managers Federation. The NDMNRF staff collected oral and rectal swabs, lung tissue and intestinal tissue from the carcasses, as well as cardiac blood samples via cardiac puncture for antibody testing. If blood could not be obtained from the heart, fluid was collected from the chest cavity on a Nobuto filter strip (Advantec MFS, Inc, Dublin, California, United States [US]). Nobuto strips were allowed to air dry, then placed in individual coin envelopes.

## Big brown bats

Bats are known carriers of coronaviruses (35–37). As such, concerns have been raised over the possible susceptibility of North American bats to SARS-CoV-2 (38). Species such as the big brown bat (*Eptesicus fuscus*) frequently roost in buildings, which brings them into close contact with people and increases the likelihood of SARS-CoV-2 exposure. Big brown bat oral swabs and guano samples for SARS-CoV-2 PCR testing were collected by staff at the Granby Zoo, which runs a rehabilitation program over the winter to care for bats that have been disturbed during their hibernation. Guano samples were stored dry in 2 mL tubes.

## Other species

Other samples for SARS-CoV-2 PCR testing were obtained opportunistically through the Ontario and Québec regional CWHC laboratories, which receive a wide variety of wildlife species for post-mortem examination (Table 1). Animals were selected for sampling based on potential for SARS-CoV-2 infection. This could be due to urban habitat, human contact or to predicted species susceptibility based on prior research. The number and type of samples collected varied by carcass and depended on carcass condition (Table 1).

## Ribonucleic acid extraction

Ribonucleic acid (RNA) extraction and PCR testing were performed at the Sunnybrook Research Institute in Toronto, Ontario. All swab, tissue and guano samples were stored at -80°C prior to testing. For oral, rectal or nasal swab samples, RNA extractions were performed using 140 µL of sample via the QIAmp viral RNA mini kit (Qiagen, Mississauga, Ontario) or the Nuclisens EasyMag using Generic Protocol 2.0.1 (bioMérieux Canada Inc., St-Laurent, Québec) according to manufacturer's instructions. The RNA from guano samples (80 mg) were extracted via the QIAmp viral RNA mini kit and eluted in 40 µL in containment level 3 at the University of Toronto. Tissue samples were thawed, weighed, minced with a scalpel, and homogenized in 600 µL of lysis buffer using the Next Advance Bullet Blender (Next Advance, Troy, New York, US) and a 5 mm stainless steel bead at 5 m/s for 3 minutes. The RNA from 30 mg tissue samples was extracted via the RNeasy Plus Mini kit (Qiagen, Mississauga, Ontario) or the Nuclisens EasyMag using Specific Protocol B 2.0.1; RNA was eluted in 50 µL. All extractions were performed with a positive and negative control. Extraction efficiency between kits was assessed through comparison of positive extraction controls.

## Severe acute respiratory syndrome coronavirus 2 polymerase chain reaction analysis

Real-time polymerase chain reaction (RT-PCR) was performed using the Luna Universal Probe One-Step RT-qPCR kit (NEB). Two gene targets were used for SARS-CoV-2 RNA detection: the 5' untranslated region (UTR) and the envelope (E) gene (39). This assay was adapted from the Shared Hospital Labs from The Research Institute of St. Joseph Hamilton for use in animals. The cycling conditions were as follows: one cycle of denaturation



at 60°C for 10 minutes then 95°C for 2 minutes followed by 44 amplification cycles of 95°C for 10 seconds and 60°C for 15 seconds. Quantstudio 3 software (Thermo Fisher Scientific Inc., Waltham, Massachusetts, US) was used to determine cycle thresholds (Ct). All samples were run in duplicate and samples with Cts less than 40 for both gene targets in at least one replicate were considered positive.

## Antibody testing

Antibody testing was performed on cardiac blood, chest cavity fluid and serum samples at the NML in Winnipeg, Manitoba. All samples were stored at -20°C prior to testing. Cardiac blood samples were collected onto Nobuto filter strips by saturating the length of the strip with 100 µl of blood. To obtain the 1:9 dilution required for testing, saturated Nobuto strips were cut into 4–5 pieces and placed into a 2 mL tube containing 360 µl phosphate buffered saline pH 7.4 containing 0.05% Tween 20 and eluted overnight at 4°C. Nobuto strips collected from chest cavity fluid were processed in the same way, whereas serum samples were diluted 1:9 with Sample Dilution Buffer. Samples were mixed by vortexing and tested using the GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript US, Inc. Piscataway, New Jersey, US) according to the manufacturer's protocol.

Briefly, 60 µl of a sample was added to 60 µl HRP-conjugated RBD solution and incubated at 37°C for 30 minutes. A 100 µl aliquot of the mixture was transferred to the ELISA microwell test plate and incubated at 37°C for 15 minutes. Microwells were washed four times with 260 µl wash buffer then 100 µl TMB substrate was added to each well. Following a 20-minutes incubation in the dark at room temperature, 50 µl of Stop Solution was added to each well. Absorbance was read immediately at 450 nm.

Each assay plate included positive and negative controls that met required quality control parameters. Percentage inhibition was calculated for each sample using the following equation:

$$\% \text{ inhibition} = (1 - \text{optical density sample} / \text{optical density negative control}) \times 100\%$$

Samples with greater than or equal to 30% inhibition were considered positive for SARS-CoV-2 neutralizing antibodies.

## Results

We tested 776 individual animals from 17 different wildlife species for SARS-CoV-2. These animals were collected primarily from urban areas in southern Ontario and Québec between June 2020 and May 2021 (Table 1). We found no evidence of SARS-CoV-2 viral RNA in any of the tested samples and no evidence of neutralizing antibodies in a subset of 219 individuals (141 raccoons, 36 striped skunks, 42 mink).

## Discussion

Our study did not detect any spillover of SARS-CoV-2 to wildlife in Ontario and Québec. Raccoons and skunks were the most commonly tested species. Results from experimental studies have suggested these species may be susceptible to SARS-CoV-2, but the lack of and low quantity of infectious virus shed by raccoons and skunks, respectively, suggest they are an unlikely reservoir for SARS-CoV-2 in the absence of viral adaptations (9,10). Similarly, a challenge study with big brown bats found that they are resistant to SARS-CoV-2 infection and do not shed infectious virus (40). Conversely, minks are susceptible to SARS-CoV-2 infection, but no evidence of SARS-CoV-2 was detected in any of the mink sampled. While this could be attributed to low effective sample size, to date SARS-CoV-2 has been infrequently detected in wild mink populations globally. It should be noted, however, that these experimental studies on raccoons, skunks and big brown bats (9,10,40) were conducted using parental SARS-CoV-2. The susceptibility of these species to VoCs is presently not known and may differ from susceptibility to the parental strain (41). Additionally, challenge studies assessing susceptibility tend to be conducted on small numbers of young, healthy individuals, so results may not be reflective of the full range of possible responses to infection in the wild.

As the pandemic progresses, new evidence is emerging on susceptible wildlife that may act as competent reservoirs for the virus. For example, white-tailed deer are now considered a highly relevant species for SARS-CoV-2 surveillance in light of their experimentally determined susceptibility as well as evidence of widespread exposure to the virus via antibody and PCR testing across North America (12–16,19). Continued surveillance efforts should be adaptive and include targeted testing of highly relevant species as they are identified. In Ontario and Québec, these would include mink, white-tailed deer and deer mice (*Peromyscus maniculatus*) (9,42). Continuing to include less susceptible species remains important given ongoing viral genomic plasticity and changing host range of VoCs.

## Limitations

There are several limitations for this study that need to be acknowledged. First, the majority of our SARS-CoV-2 testing was done by RT-PCR, which is only capable of detecting active infection. Antibody testing, which identifies resolved infection or exposure, is more likely to find evidence of SARS-CoV-2 in surveillance studies since results are less dependent on timing of sample collection. Antibody testing typically requires samples from live animals or fresh carcasses, which limited our ability to use it; however, the testing performed allowed for test validation in raccoons, skunks and mink, which may facilitate more antibody testing in future. Second, we relied on different kits for RNA extraction due to logistical challenges. Based on our extraction controls, the QIAamp RNA mini kit performed slightly better compared to the Nuclisens EasyMag (~2 Cts) for swab samples. Conversely, the Nuclisens EasyMag performed slightly better





(~2 Cts) compared to the RNeasy mini plus kit for tissue samples. Third, the type of samples we collected may also have limited our ability to detect SARS-CoV-2 infection. Viral replication can vary among tissue types and therefore some tissues are more optimal for viral RNA detection than others (1). In the present work, animals were sampled opportunistically as a part of pre-existing programs, and we were not able to consistently collect the same sample sets. Additionally, the sample types were from live animals and carcasses and not optimized; certain sample types were sometimes unavailable (e.g. tissue samples from live animals) or were not sufficient for collection.

## Conclusion

A One Health approach is critical to understanding and managing the risks of an emerging zoonotic pathogen such as SARS-CoV-2. We leveraged activities of existing research, surveillance, and rehabilitation programs and expertise from multiple fields to efficiently collect and test 1,690 individual wildlife samples. The absence of SARS-CoV-2-positive wildlife samples does not exclude spillover from humans to Canadian wildlife, given the limitations cited above. Continued research in this area is both important and pressing, particularly as novel VoCs emerge. Public and animal health sectors should continue to work collaboratively with academic and government partners to help prevent the spread of SARS-CoV-2 from people to wildlife, monitor for spillover, and address any issues should they arise. There is an urgent need for a coordinated wildlife surveillance program for SARS-CoV-2 in Canada. This approach will help protect the health of both Canadians and wildlife, now and in the future.

## Authors' statement

JEG and JDK contributed equally to the work.

JEG, JDK, JB, TB, PAB, CMD, LF, MG, CMJ, AM, PKM, LAN, SM — Conceptualization

JEG, LB, MG, CMJ, SL, AM, BS — Sample collection and coordination

JDK, AD, AH, LRL, AS, LY, SM — Sample testing

JEG, JDK — Resources

JEG, JDK, AD, LF — Writing, original draft

JEG, JDK, JB, LB, TB, PAB, CMD, AD, LF, MG, AH, CMJ, SL, LRL, AM, PKM, LAN, AS, BS, LY, SM — Writing, review and editing

JB, TB, PAB, CMD, PKM — Funding acquisition

## Competing interests

None.

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# SARS-CoV-2 wildlife surveillance surrounding mink farms in British Columbia, Canada

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## Abstract

**Background:** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can infect many wild and domestic animal species. Farmed American mink (*Neovison vison*) are particularly susceptible to infection. Outbreaks of SARS-CoV-2 were detected in farmed mink on three mink farms in British Columbia (BC), Canada between December 2020 and May 2021. In BC, mink farm density and proximity to wildlife habitats increase transmission risks from infected farmed mink. The objective of this study is to investigate the risk of SARS-CoV-2 spreading to and from wildlife in the area surrounding infected mink farms in BC, Canada, as well as to compare the effectiveness of physical and camera trapping surveillance methodologies.

**Methods:** A combination of physical and camera trapping was used on and around three BC mink farms with active SARS-CoV-2 infections between January 22, 2021, and July 10, 2021. Samples from trapped animals, including escaped farmed mink, were tested for SARS-CoV-2. Camera images from one mink farm were reviewed to determine species and proximity to the mink barn.

**Results:** Seventy-one animals of nine species were captured and sampled. Three captured mink tested positive for SARS-CoV-2 by polymerase chain reaction and serology; the remaining samples were negative for SARS-CoV-2. Genotyping of the three positive mink indicated these were domestic (vs. wild) mink. A total of 440 animals of 16 species were photographed at the one farm where cameras were deployed.

**Conclusion:** Detection of SARS-CoV-2 in escaped farmed mink is concerning and demonstrates the potential for transmission from farmed mink to wildlife, particularly given the observation of wildlife known to be susceptible to SARS-CoV-2 near infected mink farms. Combined use of physical and camera trapping contributed to the breadth of the results and is strongly recommended for future surveillance.

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**Keywords:** SARS-CoV-2, American mink, wildlife surveillance, physical trapping, camera trapping

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## Introduction

The ongoing coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for substantial morbidity and mortality of humans globally (1). SARS-CoV-2 is zoonotic in origin, but once the spillover to humans occurred the course

of the pandemic has been driven almost entirely by human-to-human transmission. Natural infections of SARS-CoV-2 have been detected in a wide range of animals, including castorids (Sino-Mongolian beaver) (2), cervids (white-tailed deer) (3), cricetids (hamsters) (4), felids: (domestic cats) (5,6), cougars, fishing cats,



lions, Canada lynx (7), snow leopards, tigers (8–10), domestic dogs and cats (5,6), gorillas, hippopotamus (11), mustelids (American mink) (12–16), Asian small-clawed otters and ferrets (17), procyonids (coatiundi), spotted hyenas (18) and viverrids (bearcats) (7) (Table 1).

**Table 1: Susceptibility of observed species to severe acute respiratory syndrome coronavirus 2<sup>a</sup>**

Order	Family	Species	Susceptibility to SARS-CoV-2
Carnivora	Canids	Coyote ( <i>Canis latrans</i> )	Unknown
	Felids	Cat ( <i>Felis catus</i> )	High
	Mustelids	Mink ( <i>Neovison vison</i> )	High
		Otter ( <i>Lontra canadensis</i> )	High
	Procyonids	Raccoon ( <i>Procyon lotor</i> )	Low
Lagomorpha	Leporidae	Rabbit ( <i>Sylvilagus</i> sp.)	Yes
Rodentia	Castoridae	Beaver ( <i>Castor canadensis</i> )	Yes
	Murids	Rat ( <i>Rattus</i> sp.)	Unknown
Anseriformes	Anatidae	Mallard duck ( <i>Anas platyrhynchos</i> )	Unknown
		Wood duck ( <i>Aix sponsa</i> )	Unknown
Galliformes	Phasianidae	Chicken ( <i>Gallus gallus domesticus</i> )	None
Passeriformes	Corvidae	Crow ( <i>Corvus brachyrhynchos</i> )	Unknown
	Sturnidae	Starling ( <i>Sturnus vulgaris</i> )	Unknown
Pelecaniformes	Ardeidae	Blue heron ( <i>Ardea herodias</i> )	Unknown
Strigiformes	Strigidae	Barred owl ( <i>Strix varia</i> )	Unknown

<sup>a</sup> Based on information from [Animals and COVID-19](#)

Transmission of SARS-CoV-2 in American mink (*Neovison vison*) is of particular concern. Mink are highly susceptible to the virus, and the virus has been found to undergo mutation at a higher rate in mink than in humans (19). Mink are farmed globally in high density environments, and there is evidence of transmission of SARS-CoV-2 from mink to humans and vice versa (20–23). These factors increase the transmission risk of SARS-CoV-2 in mink, potentially leading to viral mutations and the emergence of variants of concern for human health.

The SARS-CoV-2 in mink also poses a risk to wildlife. Indeed, free-ranging infected mink have been detected in the United

States (US) and Spain and, in both countries, these animals were believed to have escaped from nearby infected farms (24). Mink have also been shown to transmit SARS-CoV-2 to domestic dogs and cats in and around the farm environment (12,24) and other diseases, such as Aleutian disease, which have been shown to spillover from infected mink farms into wildlife populations (25). For this reason, the World Organisation for Animal Health (26) and US Department of Agriculture (27) have recommended surveillance for SARS-CoV-2 in wildlife potentially exposed to domestic animal reservoirs of the virus and Environment and Climate Change Canada have issued national guidelines recommending surveillance of wildlife around infected mink farms (24). This surveillance is focused on trapping and testing of target wildlife species in a 1–3 km range around infected farms and aligns with similar surveillance programs in the US (28).

In the province of British Columbia (BC), the mink farming industry is regulated by the BC Ministry of Agriculture, Food and Fisheries. In December 2020, there were nine active farms licensed in BC, all located in the Lower Mainland region. The SARS-CoV-2 was detected in farmed American mink on two mink farms in BC in December 2020 (Farm 1 and Farm 2) and on one farm in May 2021 (Farm 3). The original source of SARS-CoV-2 in mink on two of three affected mink farms was COVID-19 infections in mink farm workers. The source of infection of the third mink farm was not determined conclusively; however, genetic sequencing indicated that the strain was similar to human cases of COVID-19 in the local community at the time of detection (*N. Prystajewsky, personal communication, 2021*).

The detection of SARS-CoV-2 on the mink farms raised concerns about spread to wildlife in the surrounding area. It is of note that the aforementioned Environment and Climate Change Canada surveillance guidelines were published in November 2021, after surveillance around the infected farms was completed; however, the methods employed (including live trapping and SARS-CoV-2 testing of wildlife around farms, genetic testing of free-ranging mink and supplementing trapping data with information gleaned from camera footage) are largely aligned with the national recommendations.

Here we report on the results of wildlife surveillance for SARS-CoV-2 around the three infected mink farms in BC with a view to assessing the risk of the virus spreading to and from wildlife in the vicinity of mink farms. Furthermore, the broader purpose of this analysis is to compare physical and camera trapping surveillance strategies, and ultimately to inform future wildlife surveillance strategies to optimise risk assessments for both public health and wildlife health.

## Methods

### Physical trapping

The outbreak on Farm 1 lasted from December 2, 2020, to February 24, 2021, and the outbreak on Farm 2 lasted from



December 23, 2020, to December 26, 2020, when the producer opted to euthanize the whole herd. Ring surveillance was used around Farm 1 and Farm 2. Seventy traps were placed in a three-kilometre perimeter surrounding the two farms from January 22, 2021, to March 19, 2021. Target species were selected based on what species were known to be present in the area and what was known about species susceptibility at the time. Primary target species included feral cats (*Felis catus*), escaped domestic mink (*N. vison*) and wild mustelids such as wild mink and otters (*Lontra canadensis*). Raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), Virginia opossum (*Didelphis virginiana*) and bobcat (*Lynx rufus*) were also expected in the areas and considered target species but likely presented lower likelihood of SARS-CoV-2 carriage. White-tailed deer (*Odocoileus virginianus*) were not targeted as the extent of their susceptibility was not known at the time of sampling. A mixture of live and kill traps (Tomahawk Durapoly small, 120 Conibear, 330 Conibear, Havahart 1079, Havahart 1081) were used based on trapper experience and target species. Where live traps were used, the animal was then humanely euthanized. Note that the target species were used to inform the trapping methodology; however, all animals trapped, regardless of species, were included in the surveillance sample, including opportunistically collected roadkill animals. Live and kill traps were selected to meet certification and requirements of the *Agreement on International Humane Trap Standards*. All physical trapping was carried out by experienced wildlife trappers who were familiar with the geographical area and the patterns of local wildlife.

The outbreak on Farm 3 lasted from April 2, 2021, to February 11, 2022. Risk-based surveillance was implemented on Farm 3 by focusing on mustelids (the species group in the area considered most susceptible to SARS-CoV-2) within and immediately adjacent to the farm. This approach was adopted because trapping occurred during the breeding season; therefore, it was critical to target specific higher-risk species and exclude pregnant and lactating female. Twenty-four live traps were placed from June 23, 2021, to July 10, 2021, in three areas: on farm property (n=6); around the perimeter of the farm property (n=6); and in adjacent suitable mustelid habitat (n=12) that consisted of farmland and river habitat. Animals were assessed in the live traps and those that were neither pregnant nor lactating were humanely euthanized.

Samples collected from euthanized animals included nasal swabs for SARS-CoV-2 polymerase chain reaction (PCR), which were placed in viral transport medium prior to testing at the Animal Health Centre, Abbotsford. Whole blood for serological analysis was collected by saturating the length of Nobuto filter strips (Fisher Scientific, Waltham, Massachusetts, US) with cardiac blood. These were air dried and stored in individual envelopes at 4°C until shipped to the National Microbiology Laboratory, Winnipeg, Manitoba for testing. Skin samples were collected from three SARS-CoV-2-positive mink for microsatellite genotyping to investigate their ancestry (i.e.

domestic vs. wild) and analyzed at the Wildlife Genetics Lab of the Wildlife Research and Monitoring Section, Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry, Peterborough, Ontario.

## Severe acute respiratory syndrome coronavirus 2 polymerase chain reaction testing

Approximately 1.5 ml nasal swab in Virus Transport Media (VTM) was clarified by centrifugation at 2,000 g for two minutes. Viral ribonucleic acid (RNA) was isolated using the Applied Biosystems Incorporated MagMax-96 Express magnetic particle processor (ThermoFisher Scientific, Waltham, Massachusetts, US) with the MagMax™-96 Viral RNA Isolation Kit (ThermoFisher, catalog number: AM1836) as per kit instructions. The MagMax program (AM1836\_DW\_v50) was available on the ThermoFisher website (thermofisher.com). Primers and probe that target the E gene to create a 113-base pair (bp) amplicon were used to detect SARS-CoV-2. Forward primer 5'-ACAGGTACGTTAATAGTTAATAGCGT-3'; probe 5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1-3', reverse primer 5'-ATATTGCAGCAGTACGCACACA-3'. Reaction concentrations of the SARS-CoV-2 primers and probe were 800 nM and 200 nM, respectively. An enterovirus exogenous PCR control (Asuragen, catalog number: 42050) was spiked in the RNA isolation step and the 61 bp amplicon was detected with the following primers and probe: forward primer 5'-ATGCGGCTAATCCCAACCT-3'; probe 5'-VIC-CAGGTGGTCACAAAC-MGBNFQ-3'; and reverse primer 5'-CGTTACGACAGGCCAATCACT-3' (VIC and MGBNFQ are proprietary dyes to Applied Biosystems). The reaction concentration for the enterovirus primers and probe were 200 nM each. The AgPath-ID™ One-Step RT-PCR Reagents was used as per kit instructions (ThermoFisher, catalog number: 4387391): 5 µl of extracted RNA template was added to the master mix. Real-time PCR (RT-PCR) was performed on the Applied Biosystems 7500 Fast Real-Time PCR System thermocycler using with the following amplification profile: one cycle of 50°C, 30 minutes; one cycle of 95°C, one minute; 40 cycles of 95°C, 15 seconds and 60°C, one minute. Change in fluorescence was recorded at the elongation step of each cycle.

## Severe acute respiratory syndrome coronavirus 2 serology

Serological testing of whole blood was conducted using the GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (catalog number: L00847, GenScript US, Inc. Piscataway, New Jersey, US) according to manufacturer's protocol. Samples with more than 30% inhibition were considered positive for SARS-CoV-2 neutralizing antibodies. To minimize possible risk of exposure to the zoonotic pathogen, *Francisella tularensis*, by laboratory staff, serum samples were not collected from beavers as per laboratory guidelines at the BC Animal Health Centre.

## Genotyping of mink

Microsatellite profiling of free-ranging mink samples followed the procedure detailed in Beauclerc *et al.* (29,30) with minor modifications. Briefly, whole genomic DNA was extracted from approximately 10 mg of muscle with the E.Z.N.A.®Tissue DNA Kit (Omega Bio-Tek) and quantified with PicoGreen dye (Invitrogen). Samples were amplified at 15 microsatellite loci in 2 multiplexes, each consisting of 12 µL reactions with 1 ng DNA, primer labels and concentrations as shown in **Table A1**. Genotyping was performed on an ABI 3730 with GeneScan 500HD ROX (Applied Biosystems). Fragments were scored automatically in GeneMarker v.2.6.4 (SoftGenetics) and verified by eye; ambiguous alleles were reamplified.

### Camera trapping

In addition to physical trapping, more in-depth camera trapping was implemented on Farm 3 from February 7, 2021, to July 25, 2021, based on experience from Farm 1 and Farm 2. Camera trapping was utilized to gather more information on the presence of animals and their use of the habitats surrounding mink farms and to avoid physical disruption during the breeding season of relevant species. This involved the placement of 11 wildlife cameras inside the fenced area surrounding the mink barn (n=1), outside but adjacent to the fenced barn (n=4) and near the river adjacent to the perimeter of the farm property (n=6). Images of animals captured on camera were then analyzed visually. The species present in each image was identified based on morphology.

## Results

### Physical trapping

A total of 71 animals of nine different species were trapped, including 63 from Farm 1 and Farm 2 and 8 from Farm 3 (**Table 2**). All trapped animals appeared healthy upon visual examination. Two trapped cats were observed as acting aggressively. Several of the trapped species are known to be susceptible to infection with SARS-CoV-2, specifically domestic cats, mink, otters, rabbits and raccoons (31). The susceptibility for many other species is currently unknown (Table 2) (31).

Mink were assigned to their population of origin using Bayesian assignment tests in STRUCTURE v.2.2 for assumed number of clusters (K) of two, as described in Bowman *et al.* (30,32). Previously analysed samples, consisting of domestic and free-ranging samples from Ontario, Nova Scotia and Prince Edward Island (n=902), provided the reference dataset within which the new samples were analysed (29,30). Membership in a cluster used the average ancestry coefficient (q): individuals with q>0.8 were assigned to a single cluster, while those with q<0.8 were considered hybrids (33).

**Table 2: Species captured during physical trapping around severe acute respiratory syndrome coronavirus 2-infected mink farms in British Columbia (n=71)**

Order	Family	Species	Number of captures	Percent of total
Carnivora	Felids	Cat ( <i>Felis catus</i> )	5	7
	Mustelids	Mink ( <i>Neovison vison</i> )	12	17
		Otter ( <i>Lontra canadensis</i> )	1	1
	Procyonids	Raccoon ( <i>Procyon lotor</i> )	4	6
Didelphimorphia	Didelphids	Opossum ( <i>Didelphis virginiana</i> )	6	8
Rodentia	Castorids	Beaver ( <i>Castor canadensis</i> )	9	13
	Cricetids	Muskrat ( <i>Ondatra zibethicus</i> )	6	8
	Murids	Rat ( <i>Rattus</i> sp.)	10	14
	Sciurids	Grey squirrel ( <i>Sciurus carolinensis</i> )	18	25

### Severe acute respiratory syndrome coronavirus 2 polymerase chain reaction, serology and genotyping of mink

All sampled animals were negative for SARS-CoV-2 using PCR and serology, with the exception of three mink trapped on the property of Farm 3 outside the barrier fence that were both PCR-positive and had antibodies against SARS-CoV-2. These three mink were genotyped and genotyping highly assigned these mink to the domestic cluster (q=0.94–0.99), indicating that they were domestic (vs. wild) mink that had likely escaped from their cages. Note that none of the other trapped mink was genotyped.

### Camera trapping

There were 440 camera images showing 1 or more animals of 1 of 16 species (**Table 3**). Of note, cats and crows were observed inside the barrier fence with access to the mink barn. Additionally, some species were observed near the mink barn but outside the barrier fence, specifically coyotes, cats, mink, rabbits, crows, starlings and owls (Table 3).





Table 3: Species observed during camera trapping around Farm 3, (n=440)

Order	Family	Species	Number of observations	Percent of observations	Percent within proximity of mink barn	
					%	n
Carnivora	Canids	Coyote ( <i>Canis latrans</i> )	144	33	61	n=88/144
	Felids	Cat ( <i>Felis catus</i> )	59	13	49	n=29/59
	Mustelids	Mink ( <i>Neovison vison</i> )	5	1	40	n=2/5
		Otter ( <i>Lontra canadensis</i> )	3	<1	0	n=0/3
	Procyonids	Raccoon ( <i>Procyon lotor</i> )	7	2	0	n=0/7
Lagomorpha	Leporidae	Rabbit ( <i>Sylvilagus</i> sp.)	14	3	100	n=14/14
Rodentia	Castoridae	Beaver ( <i>Castor canadensis</i> )	11	3	0	n=0/11
	Murids	Rat ( <i>Rattus</i> sp.)	2	<1	0	n=0/2
Anseriformes	Anatidae	Mallard duck ( <i>Anas platyrhynchos</i> )	21	5	0	n=0/21
		Wood duck ( <i>Aix sponsa</i> )	26	6	0	n=0/26
Galliformes	Phasianidae	Chicken ( <i>Gallus gallus domesticus</i> )	1	<1	0	n=0/1
Passeriformes	Corvidae	Crow ( <i>Corvus brachyrhynchos</i> )	110	25	22	n=24/110
	Sturnidae	Starling ( <i>Sturnus vulgaris</i> )	7	2	43	n=3/7
Pelecaniformes	Ardeidae	Blue heron ( <i>Ardea herodias</i> )	21	5	0	n=0/21
Strigiformes	Strigidae	Barred owl ( <i>Strix varia</i> )	2	<1	100	n=2/2
Bird—unknown classification			7	2%	14	n=1/7

Note: A severe acute respiratory syndrome coronavirus 2-infected mink farm in British Columbia

It is particularly of interest that three mink were observed outside of the barrier fence surrounding the mink barn. While it is not certain that these were escaped farmed mink, it is very likely, given that mink trapped in similar locations were genotyped as domestic mink.

## Discussion

Wildlife surveillance involving physical and camera trapping surrounding mink farms in BC infected with SARS-CoV-2 identified 71 animals of nine different species from physical trapping and 440 observations of 16 different species from camera trapping. Three mink trapped on one farm property were PCR-positive and seropositive for SARS-CoV-2. Additionally, mink were observed on camera that were likely escaped farmed mink.

The observation of wildlife in proximity to infected mink farms, particularly those species known to be susceptible to SARS-CoV-2, demonstrates the risk of transmission from farmed mink to wildlife. Of particular concern was the capture of three escaped farmed mink that tested positive for SARS-CoV-2, as well as the observation of mink on camera footage (although it could not be confirmed whether these animals represent additional escapees). These were consistent with findings from the US and Spain in which SARS-CoV-2 surveillance was conducted around infected mink farms (28,34). In those studies, exposure and infection was only detected in free-ranging mink that were thought to have escaped from infected farms (28,34). For the infected mink caught on the farm property in this study, it

is problematic that they were able to escape from the caging and barrier fence; however, being found within the farm property is less of a concern than if they had been found outside the farm as they are less likely to have had extensive contact with wildlife.

Feral cats and crows were observed (via cameras) inside the fence in the immediate area of the mink barn. Continued surveillance of these species is prudent, particularly for cats as they are known to be susceptible to SARS-CoV-2, appear to have greater access to mink barns compared with other species and can often be in close contact with humans. Furthermore, a previous study reported that a feral cat on a mink farm in the Netherlands tested positive for SARS-CoV-2 (12). In combination, these factors could allow cats to facilitate interspecies transmission of SARS-CoV-2 (35). Continued surveillance of birds should also be considered. While birds are not known to carry or transmit the virus to conspecifics, other wildlife or humans, they may act as fomites through contact with and carriage of contaminated material or surfaces (36). Additionally, surveillance of wild ungulates should be considered due to their high susceptibility to SARS-CoV-2 infection and transmission (31). Although outside the barrier fence, other wildlife known to be susceptible to SARS-CoV-2 (e.g. raccoon, rabbit, otter and beaver) (31) were trapped or observed in close proximity to the mink farms. Overall, although no spillover from farmed mink to any wildlife species was detected, the potential for farmed mink to come into close contact with wildlife species or feral and domestic animals and transmit SARS-CoV-2 to wildlife, via aerosol transmission, exists.



This implementation of different surveillance methods demonstrated that both physical and camera trapping provided important information, and the conclusions drawn were strengthened by the combined data. Physical trapping using ring surveillance was beneficial when little was known about SARS-CoV-2 and the potential for spillover. This is because a greater number of animals from more diverse species were caught. Once more information was known, a more focused approach at one facility using risk-based surveillance reduced the removal of healthy, uninfected wildlife and successfully identified three infected mink. Camera trapping showed that there were multiple species present around the farm that were not identified by physical trapping. Both physical trapping and camera trapping have a number of strengths and limitations (37). Physical trapping allowed for the collection of biological samples, as well as for the evaluation of the physical condition of the animals; however, physical trapping was labour-intensive and necessitated the euthanasia of trapped animals. Camera trapping was easier to implement and allowed for the collection of a greater quantity of data; however, camera trapping did not allow for the collection of biological samples or for determination of whether the same animal was captured multiple times.

From this specific implementation of wildlife surveillance, a number of considerations have been identified that should inform future surveillance strategies. Factors that should be considered include the species of interest, the season and its impact on the species' behaviour and lifecycle, the landscape of interest, the practicality of placing and monitoring physical traps or cameras, and the need to collect biological samples to answer the research questions.

## Conclusion

When implementing future surveillance, it is recommended to begin with camera trapping to assess the species present and the frequency of observations. These initial observations can be followed by targeted physical trapping as needed to collect biological samples from specific species of interest. Use of or consultation with experienced wildlife trappers with knowledge of the local area is a critical component and was a significant factor in the success of this project.

## Authors' statement

CH, CS, EF, CT — Study design and execution  
KB — Genotyping methodology and testing  
AD, NT, RL — Serological testing  
TS, EF, CT, CH — Writing, original draft  
All authors — Writing, reviewing and editing

## Competing interests

None.

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## Annex

**Table A1: Microsatellite loci and polymerase chain reaction conditions used to genotype American mink (*Neovison vison*)**

Locus	Final concentration (μM)	Source
<b>Multiplex 1</b>		
Mvi1006 FAM	0.6	(34)
Mvi1016 FAM	0.05	(34)
Mvi075 HEX	0.15	(35)
Mvi1272 HEX	0.25	(36)
Mvi072 HEX	0.1	(35)
Mvi114 NED	0.4	(37)
Mvi002 NED	0.03	(35)
<b>Multiplex 2</b>		
Mvi1321 FAM	0.05	(36)
Mvi1354 FAM	0.5	(36)
Mvi099 FAM	0.2	(35)
Mvi111 HEX	0.15	(37)
Mvi1342 HEX	0.15	(36)
Mvi1302 HEX	0.6	(36)
Mvi2243 NED	0.15	(36)
Mvi4001 NED	0.5	(38)



# One Health response to SARS-CoV-2-associated risk from mink farming in British Columbia, Canada, October 2020 to October 2021

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## Abstract

**Background:** Mink farms are susceptible to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreaks and carry an associated risk of novel SARS-CoV-2 variant emergence and non-human reservoir creation. In Denmark, control measures were insufficient to prevent onward transmission of a mink-associated variant, contributing to the nation-wide culling of farmed mink. To date, British Columbia (BC) is the only Canadian province to report mink farm SARS-CoV-2 outbreaks. The objective of this study is to describe BC's One Health response to SARS-CoV-2-associated risk from mink farming, its outcomes, and insights from implementation.

**Methods:** The detection of two mink farm outbreaks in December 2020 catalyzed BC's risk mitigation response for both infected and uninfected farms, including the following: farm inspections and quarantines; Public Health Orders mandating mink mortality surveillance, enhanced personal protective equipment, biosafety measures and worker coronavirus disease 2019 vaccination, at-a-minimum weekly worker viral testing, and wildlife surveillance.

**Results:** A One Health approach enabled a timely, evidence-informed and coordinated response as the situation evolved, including the use of various legislative powers, consistent messaging and combined human and mink phylogenetic analysis. Ongoing mink and worker surveillance detected asymptomatic/subclinical infections and facilitated rapid isolation/quarantine to minimize onward transmission. Voluntary testing and mandatory vaccination for workers were acceptable to industry; enhanced personal protective equipment requirements were challenging. Regular farm inspections helped to assess and improve compliance.

**Conclusion:** British Columbia's One Health response reduced the risk of additional outbreaks, viral evolution and reservoir development; however, a third outbreak was detected in May 2021 despite implemented measures, and long-term sustainability of interventions proved challenging for both industry and governmental agencies involved.

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**Keywords:** SARS-CoV-2, COVID-19, mink farm, One Health, spillover, reservoir



## Introduction

In 2020, Denmark reported community spread of a mink-associated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant reducing antibody-mediated neutralization (1,2). Implemented measures, including surveillance, enhanced biosecurity and use of personal protective equipment (PPE), did not prevent SARS-CoV-2 transmission to other mink farms and humans (3), contributing to the Danish government's decision to cull all farmed mink to prevent further mutation and spread (3,4).

Mink farm SARS-CoV-2 outbreaks had occurred in 12 countries (5) by the end of 2021, indicating high mink susceptibility (6–10). Mink-to-human transmission during mink farm outbreaks is, to date, the only confirmed animal-to-human SARS-CoV-2 transmission (8,11,12). The SARS-CoV-2 infection in a new non-human host is of public health (PH) concern because of viral adaptation to that host (13–16) and the potential creation of a reservoir. These factors can promote the emergence and re-introduction of variants of interest into humans (13) or other animals, further increasing opportunities for viral mutation (14–18).

Canadian (19), European (15) and international (16) organizations all recommend a One Health approach for managing SARS-CoV-2 risk on mink farms to enable a timely and coordinated response between the agricultural, animal health and human health sectors. The One Health approach also facilitates data sharing for surveillance and outbreak detection and response; however, there is a paucity of literature on the practical implementation, evolution and outcomes of such One Health approaches for mink farming (14–16). While the majority of Canadian mink farms are located in Eastern Canada, as of January 2022, British Columbia (BC) remains the only Canadian province with reported mink farm SARS-CoV-2 outbreaks (5). The objective of this work is to describe the implementation of BC's One Health response to SARS-CoV-2-associated risk on mink farms from October 2020 to October 2021, detailing the interventions and outcomes, and discuss the insights gained.

## Setting

In 2020, all nine active mink farms in BC were located in the Fraser Health Authority (FH), near large urban centres. The mink fur industry was regulated and licensed by the BC Ministry of Agriculture, Food and Fisheries (MAFF), recently renamed to Ministry of Agriculture and Food. The industry operated in a cycle of breeding mink in spring, whelping offspring in summer and pelting in fall and winter, with pelts sold early the following year. British Columbia farms produced approximately 240,000 pelts in 2020 (20). Some farms operated independently, while two pairs of farms partially integrated operations, resulting in seven independent farm units.

Following the report of large SARS-CoV-2 outbreaks in mink farms in Europe (5,15), a provincial One Health Committee (OHC) was established in October 2020 to assess, mitigate and respond to risks from SARS-CoV-2 in farmed mink. Committee members included the BC Centre for Disease Control (BCCDC), FH, MAFF veterinarians and relevant organizations from other sectors, such as WorkSafeBC (Table 1). The OHC held weekly to semi-weekly meetings to share information and expertise, improve coordination and enable joint decision-making related to surveillance strategies, biosafety/control measures and other aspects of the One Health response (Table 2).

**Table 1: Membership of British Columbia's One Health Committee to address severe acute respiratory syndrome coronavirus 2 risk on British Columbia mink farms**

Organization	Role/mandate
British Columbia Centre for Disease Control	Providing provincial public health leadership in British Columbia and acting as Chair
Fraser Health Authority	Regional health authority with jurisdiction for local outbreak management under the <i>BC Public Health Act</i>
WorkSafeBC	Overseeing the protection of workers, including mink farmworkers
Ministry of Agriculture, Food and Fisheries	Regulatory responsibility of fur farming, animal health programs, and control of reportable animal diseases
Ministry of Forests, Lands, Natural Resource Operations and Rural Development	Responsible for wildlife monitoring and issuing export permits for mink pelts
Ministry of Environment	Regulatory responsibility for environmental discharge (as needed)
Canadian Food Inspection Agency	Providing technical expertise (as needed)

Under the BC Animal Health Act (28), SARS-CoV-2 infection in animals was made reportable; mink farms and herd veterinarians were to report mink signs or symptoms compatible with SARS-CoV-2, including excess mortality, as well as any confirmed infections. By November 2020, MAFF had informed all mink farms of SARS-CoV-2-associated risks and assessed biosafety measures. Mink farm operators reported implementing physical distancing, signage related to not working while sick and non-medical mask use. A set of draft federal guidelines (19), shared with the BC Mink Producers Association by the OHC, recommended implementation of further biosecurity measures. In November 2020, PH attempted to discuss enhanced measures with a tepid response by industry.

**Table 2: Sequential interventions to manage and mitigate risk from severe acute respiratory syndrome coronavirus 2 on mink farms in British Columbia, October 2020 to October 2021**

Trigger	Purpose and considerations/actions	Outcomes and challenges
<b>1. OHC, formed in October 2020</b>		
Large outbreaks reported in mink farms in European countries	<p>Purpose: To assess, mitigate, and respond to risks from SARS-CoV-2 on BC mink farms using a One Health approach.</p> <p>Action: The OHC held weekly to semi-weekly meetings to: share information and contextualized technical and on-the-ground expertise of all members; coordinate human, animal, and environmental surveillance strategies; jointly identify biosafety gaps to be addressed and request for funds or other response tools; collaborate on decision-making based on shared situational assessments and evidence review; coordinate communication with mink farm operators; and liaise with other jurisdictions and organizations such as the Public Health Agency of Canada, United States Centers for Disease Control and Prevention and the World Health Organization.</p>	<p>The OHC enabled timely and effective response to mink farm outbreaks, realistic and implementable regulations, policies and guidelines, and optimization and sharing of technical, financial and human resources. The OHC also helped to provide unified and coordinated messaging to mink farm operators.</p> <p>Challenges in differing perceptions of risk or related decisions were typically surmountable and consensus was possible to reach in most areas. In some circumstances where specific legal jurisdiction clearly identified the most responsible organization, decisions were left to that organization.</p>
<b>2. Mink farm inspections, starting December 4, 2020</b>		
<b>2.1 Initial inspections at Farm 1</b>		
Outbreak investigation at Farm 1	<p>Purpose: To assess adherence to enhanced biosafety measures and identify gaps for improvement.</p> <p>Action: Coordinated inspections were carried out by OHC partners (i.e. PH, MAFF, and/or WorkSafeBC).</p>	<p>Inspections at Farm 1 found limited biosafety measure implementation. Out of concern for workers potentially contracting a mink-adapted COVID-19 variant, only activities necessary for animal welfare were immediately allowed to continue at Farm 1, halting the pelting process.</p> <p>Based on Farm 1 findings, a letter was issued to all mink producers urging the implementation of enhanced biosafety measures as outlined by the draft federal guidelines.</p>
<b>2.2 Repeated farm inspections on all mink farms</b>		
Finding of limited safety measures at Farm 1	<p>Purpose: To monitor implementation and feasibility of biosafety measures required by PH, MAFF or WorkSafeBC.</p> <p>Action: Inspections were repeated at all active mink farms on an ongoing basis.</p>	<p>Initial inspections on all mink farms found weaker biosafety measure implementation than those recommended by the mink farm biosafety advisory group. Implementation of recommended enhanced biosafety measures improved over time with the issuance of a FH PH Class Order mandating enhanced measures, along with subsequent inspections and feedback to mink farm operators.</p>
<b>3. Formal communications with mink farm operators, including Provincial Health Officer and Chief Veterinarian letter to operators on December 6, 2020, and follow-up meetings between PH, MAFF and industry in January and February 2021</b>		
Weak biosafety measures observed on Farm 1 during outbreak investigation, and in other farms' inspections triggered by the Farm 1 outbreak	<p>Purpose: To communicate PH concerns to mink farm operators and achieve improved biosafety measures on mink farms.</p> <p>Action: The letter reminded operators of the mandatory requirement for a written COVID-19 Safety Plan and for those plans to be posted. It strongly recommended all mink farms to immediately review and strengthen those Safety Plans to implement the measures recommended for mink farms that were outlined in a biosecurity advisory from the Canadian Food Inspection Agency and the Public Health Agency of Canada. Those measures included the use of fitted respirators (N95 or equivalent) especially for pelting (or, if unavailable, medical masks), gloves, and eye protection, as well as viral testing of workers before pelting and on a weekly basis until pelting conclusion.</p> <p>Follow-up meetings (one with a "town hall" format) were instituted for mink farm operators to share information about the industry operations, for public health and MAFF to share more about the science, and to support discussion about control measures.</p>	<p>Some operators' COVID-19 Safety Plans were found lacking, and some reported that they thought recommendations were challenging, confusing, and unnecessary. To improve compliance, FH issued a Class Order to all mink farms mandating enhanced measures be implemented before pelts, animals, or products could be moved on or off farms.</p> <p>OHC subcommittees were also created to issue BC-specific biosafety recommendations balancing risk reduction with practicality considerations and challenges.</p> <p>Meetings led to greater understanding of mink farm operations and increased overall buy-in for public health measures, although perceptions still varied across the industry.</p>





**Table 2: Sequential interventions to manage and mitigate risk from severe acute respiratory syndrome coronavirus 2 on mink farms in British Columbia, October 2020 to October 2021** (*continued*)

Trigger	Purpose and considerations/actions	Outcomes and challenges
<b>4. Mink euthanasia for the purpose of pelt production on Farm 1, December 16–24, 2020</b>		
Concern that maintaining a stock of thousands of mink infected with SARS-CoV-2 at Farm 1 would promote further viral replication and mutation, on the one hand, versus concern of viral transmission to workers	<p><b>Purpose:</b> To decrease further viral replication with associated risk of mutation among infected mink on Farm 1.</p> <p><b>Considerations:</b> There were thousands of animals left to skin at Farm 1 when the process was halted. On the one hand, keeping the herd at its large size could enable further viral replication and promote the emergence of more mutations, and from the producer's perspective, mink needed to be skinned as soon as possible before aging decreased pelt value, among other considerations. On the other hand, the skinning process is considered high risk because of compression of the mink's lungs expelling respiratory secretions, potentially generating aerosols, and workers being in very close proximity to each other and to the mink.</p> <p>Culling of the entire herd, disposal, and disinfection was considered, to decrease ongoing risk of transmission from regular operation. However, this was ultimately decided against as it would have exposed a significant number of additional workers, was logistically complicated and had significant negative implications for the producers.</p>	<p>A decision was reached to allow euthanasia and skinning under strict biosafety measures, which could vary considering whether performed by previously infected workers or not.</p> <p>Farm 1 producer decided to proceed with euthanasia/skinning. Skins were not ultimately processed into pelts as processing facilities were unable to accept skins from an infected herd, causing financial strain.</p>
<b>5. Surveillance of farmed mink mortalities, starting in December 2020</b>		
Concerns of potentially undetected or delayed detection of mink outbreaks	<p><b>Purpose:</b> To quickly detect SARS-CoV-2 infection in mink herds.</p> <p><b>Considerations and action:</b> The OHC had concerns that clinical surveillance with weekly written monitoring of illness and mortality, as suggested by the Canadian COVID-19 One Health working group (21) was unlikely to be adequate, and active surveillance had been recommended by both the World Health Organization and the World Organisation for Animal Health (16). Farm 1 had submitted mortalities upon request by MAFF following detection of the farmworker outbreak, while Farm 2 submitted mortalities for testing based on herd signs or excess mortality.</p> <p>Participation in mandatory mink mortality surveillance regardless of excess mortality or compatible signs was ordered December 14, 2020, with the start of collection in the following month. The goal of mandatory mortality surveillance was to monitor for SARS-CoV-2 infection among mink herds in a timely manner, regardless of signs or symptoms, enabling swift quarantine and detection of mutations and minimizing transmission to workers. Based on the Canadian Food Inspection Agency guidelines, it was estimated that weekly collection of 15 mink mortalities per farm would provide a 95% surveillance sensitivity to detect an outbreak; therefore, farms were required to provide up to 15 per week. Logistical considerations, both from farms and from testing processing capacity, suggested five mortalities per week would be more feasible, estimated to provide 65% sensitivity.</p> <p>FH environmental health officers collected frozen and sealed mink carcasses from both non-infected and infected premises each week and brought them to MAFF's Animal Health Centre for SARS-CoV-2 testing. Any non-negative samples were sent to the National Centre for Foreign Animal Disease for confirmatory polymerase chain reaction testing and to the BCCDC Public Health Laboratory for whole-genome sequencing, if positive.</p>	<p>On December 23, 2020, mink mortalities collected the prior week from a second farm (Farm 2) returned SARS-CoV-2-positive, and an additional outbreak was declared; mink displayed slight clinical signs and increased mortality (fewer than 3%). Farm 2 owners euthanized their small herd (fewer than 1,000 mink) without request by PH or MAFF.</p> <p>Farms had challenges in providing even the five mortalities per week due to the low mortality rate for many months of the year and small herd sizes in BC.</p> <p>On May 14, 2021, Farm 3 mink mortalities collected in early May were confirmed SARS-CoV-2-positive. The outbreak investigation uncovered mink exposure to an infectious worker who tested positive approximately 6 weeks earlier (within 14 days of the first dose of the vaccine), harbouring the same strain as positive mink.</p> <p>Ongoing mortality collection enabled detection of Farm 3 mink cases months after the outbreak start, enabling timely assessment of viral propagation and evolution in the herd; however, mink mortality freezing, collection, thawing and testing was resource-intensive.</p>
<b>6. Farm quarantine by BC Chief Veterinarian</b>		
Suspicion or confirmation of a SARS-CoV-2 infection in a mink herd (Farm 1, Farm 2 and Farm 3)	<p><b>Purpose:</b> To limit potential spread of the virus from infected mink farms.</p> <p><b>Action:</b> The Chief Veterinarian placed a quarantine order on infected premises that restricted all movements of animals, products and goods off of the farm. New enhanced protocols of disinfection of vehicles, products, and goods were put in place before authorization was given for non-essential activities.</p>	<p>On Farm 1, the herd was deemed free of disease as of February 24, 2021, after 2 sets of 65 samples taken 2 weeks apart were found to be all negative. As the Farm 2 herd was culled, it did not need to be declared free of disease. The Farm 3 herd was still considered infected by the end of this study period.</p> <p>Farm sites remained in quarantine until the determination that the farm environment was decontaminated.</p>

**Table 2: Sequential interventions to manage and mitigate risk from severe acute respiratory syndrome coronavirus 2 on mink farms in British Columbia, October 2020 to October 2021** (*continued*)

Trigger	Purpose and considerations/actions	Outcomes and challenges
<b>7. Mandatory worker COVID-19 testing, December 2020–January 2021</b>		
Concerns of undetected asymptomatic worker infection or avoidance of testing by symptomatic workers	<p>Purpose: To detect past or current COVID-19 infection in mink farmworkers.</p> <p>Action: In Mid-December 2020, mink farmworkers (n=102) were mandated to complete COVID-19 virology and serology tests before being allowed back on farms. Farm 2 workers had repeated viral and serological testing in January 2021 after pelting completion, to detect missed infections.</p>	No viral or serological tests taken in December 2020 and January 2021 returned SARS-CoV-2-positive.
<b>8. Voluntary worker COVID-19 surveillance, starting in January 2021</b>		
Concerns of undetected asymptomatic worker infection or avoidance of testing by symptomatic workers	<p>Purpose: To improve detection of COVID-19 infection in mink farmworkers.</p> <p>Action: Public Health implemented a free-of-charge, weekly surveillance program for mink farmworkers in January 2021, utilizing self-collected saline gargle samples (22,23). BCCDC PH nurses trained workers on gargle sample self-collection and associated processes, minimizing ongoing PH staffing requirements and increasing testing acceptability, while maintaining sensitivity compared to nasopharyngeal swabs (22,23) and still enabling whole genome sequencing by the BCCDC Public Health Laboratory. A same-day medical courier collected samples from farms for delivery to the BCCDC Public Health Laboratory, with 0–2 days from sample collection to results. Indeterminate results led to repeated tests.</p>	<p>All active farms (6 farm units including Farm 1) participated by the end of February 2021. Between February 21 and May 31, 2021, an audit showed active workers' weekly participation at 86%–100% per farm.</p> <p>The worker surveillance program detected 11 COVID-19 cases. One additional positive worker was detected through community testing following household exposure. Detection of positive workers triggered increased testing (2–3 times per week). Further, if an infectious worker had been near mink, 3 weeks of live mink sampling also occurred. Some farms voluntarily maintained twice or thrice-weekly testing.</p>
<b>9. Wildlife surveillance, starting in January 2021</b>		
Concern regarding SARS-CoV-2 transmission to surrounding wildlife	<p>Purpose: To detect potential SARS-CoV-2 transmission to wildlife from escaped mink or feral cats (17,18,24).</p> <p>Action: Wildlife surveillance around Farm 1 and Farm 2, utilizing wildlife trapping, testing, and video footage, occurred from January to March 2021. Wildlife surveillance was also undertaken around Farm 3 in summer 2021.</p>	Virology and serology tests were negative on all 65 animals sampled around Farm 1 and Farm 2. Repeated wildlife surveillance surrounding Farm 3 in summer 2021 located no infected wildlife but did detect 3 escaped mink that tested positive (25).
<b>10. Mandatory worker COVID-19 vaccination, April 2021</b>		
Availability of COVID-19 vaccine supply and prioritization of vaccines for high-risk workplaces, including mink farms	<p>Purpose: To reduce risk of SARS-CoV-2 transmission to mink herds from mink farmworkers.</p> <p>Action: On April 15, 2021, a new FH PH Order permitted only vaccinated workers to work in proximity to mink.</p>	<p>Most workers opted to be vaccinated (~90% first dose at the time of the Order including unmandated workers). Pfizer-BioNTech (BNT162b2) vaccination was offered to workers beginning March 17 and subsequently to their household members with excellent uptake; second doses were offered in May–June, with more than 90% worker uptake.</p> <p>Out of the 12 COVID-19-positive workers, 33% were unvaccinated, 25% partially vaccinated (onset or positive test more than 14 days post-first dose), and 42% fully vaccinated (more than 14 days post-second dose) at the time of infection.</p>
<b>11. Joint rapid qualitative risk assessment, June 2021</b>		
Need for an up-to-date, BC-specific assessment on the risk of mink farm-related SARS-CoV-2 variant of interest emergence and community transmission to inform further response	<p>Purpose: A formal risk assessment was undertaken to support decision-making regarding concerns related to SARS-CoV-2 and the mink farm industry in BC.</p> <p>Action: In June 2021, a multi-jurisdictional risk assessment was conducted as per best practices (26). National and provincial experts assessed potential scenarios' probabilities, impacts, and uncertainties, using a modified Delphi approach (<i>personal communication, V. Clair, 2021</i>).</p>	The likelihood of a variant of interest emerging in mink and circulating in the community over the next 5 years was evaluated as unlikely (moderate-high uncertainty) with minor to moderate impacts (moderate-high uncertainty). As a result, BCCDC recommended a moratorium on mink farming expansion. Following detection of SARS-CoV-2-positive escaped mink on Farm 3, the Provincial Health Officer issued a moratorium on expansion of the mink industry in late July 2021 (27).

Abbreviations: BC, British Columbia; BCCDC, British Columbia Centres for Disease Control; COVID-19, coronavirus disease 2019; FH, Fraser Health Authority; MAFF, Ministry of Fisheries and Oceans; OHC, One Health Committee; PH, Public Health; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2



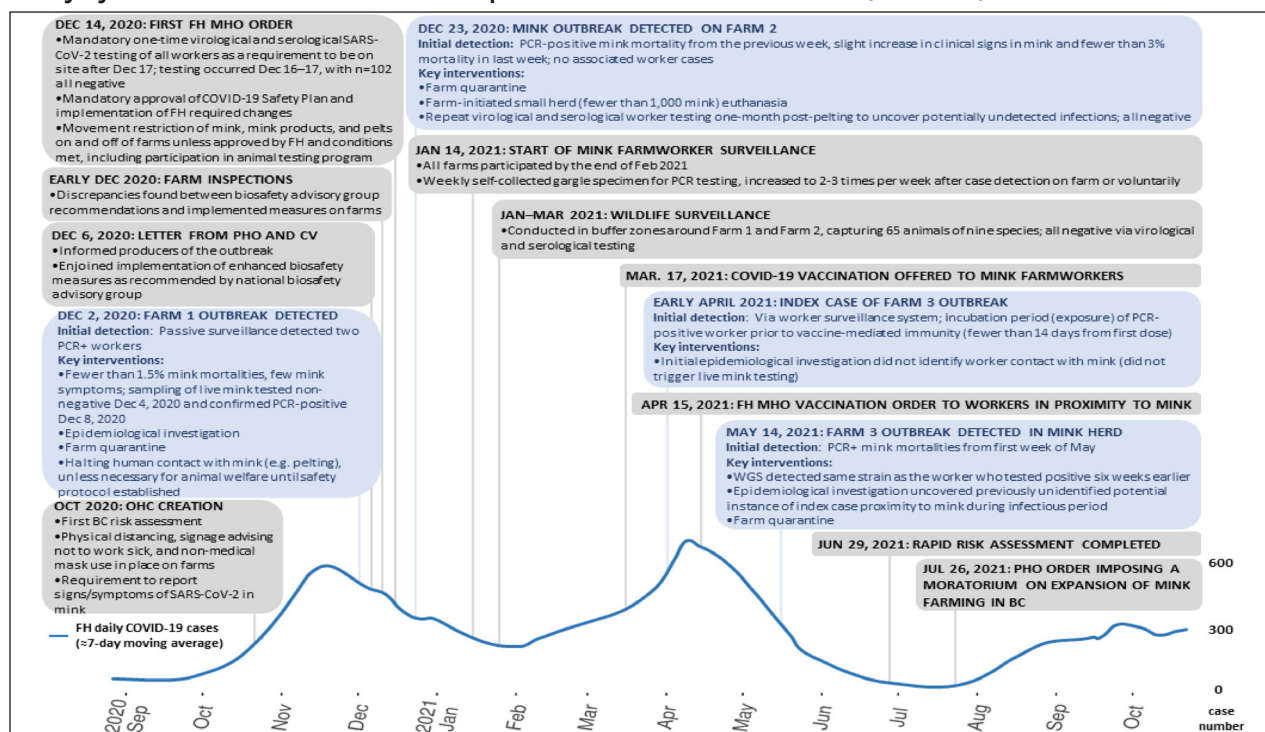
On December 2, 2020, a SARS-CoV-2 outbreak was detected on mink Farm 1 (29), triggering urgent OHC meetings to optimize outbreak management and a coordinated provincial response. The Farm 1 outbreak ultimately involved 11 coronavirus disease 2019 (COVID-19) cases among 12 workers (29). Mink on Farm 1 displayed few clinical signs and fewer than 1.5% mortality.

## Interventions, challenges and outcomes

The One Health approach included on-going evidence review and risk assessment informing the response. The main actions implemented were farm inspections, the use of public health orders to mandate worker testing and vaccination, mink mortality viral surveillance and biosafety control measures, a voluntary asymptomatic worker viral surveillance system, and wildlife surveillance. Table 2 and **Figure 1** provide a timeline and full details on the triggering events, actions, challenges and outcomes of BC's One Health response. Other measures on infected farms included animal and human epidemiological investigations, live animal testing, biocontainment and disinfection measures and quarantine of sites and workers (29).

Initial farm inspections revealed weak biosafety measure implementation, resulting first in communication encouraging strengthening of those measures followed by a Public Health Order mandating specific measures (**Table 3**). Before implementation of improved biosafety measures and availability of human vaccine, an outbreak in mink on a second farm (Farm 2) was detected due to the herd displaying slight clinical signs and increased mortality (fewer than 3%). The Farm 2 owners euthanized their small herd (fewer than 1,000 mink) without request by PH or MAFF. After the Farm 1 outbreak, all mink farmworkers in FH (n=102) were mandated to complete COVID-19 viral and serological testing, with no infections detected. After the Farm 2 outbreak, Farm 2 workers underwent a second round of viral and serological testing, again with no worker infections detected.

**Figure 1: Timeline of significant events and interventions in public health response related to severe acute respiratory syndrome coronavirus 2 in mink production in British Columbia, Canada, 2020 to 2021**



Abbreviations: BC, British Columbia; COVID-19, coronavirus disease 2019; CV, Chief Veterinarian; FH, Fraser Health Authority; MHO, Medical Health Officer; OHC, One Health Committee; PCR, polymerase chain reaction; PHO, Provincial Health Officer; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

**Table 3: Public Health Orders in British Columbia relating to mink farms, 2020 to 2021**

Public Health Orders	Descriptions
<b>December 14, 2020: Fraser Health Authority Medical Health Officer Order</b>	
COVID-19 Safety Plans and enhanced biosecurity	Mandating provision of the COVID-19 Safety Plans to FH, for review and approval by FH. As part of the safety plan requirements, enhanced use of personal protective equipment was required, including the usage of N95 or equivalent protection, eye protection, protective clothing, safety footwear that can withstand disinfection, for all activities occurring in close proximity to mink or mink feed.
Worker registry and human testing	Providing a list and contact information of all employees, contractors, volunteers, owners and operators or other individuals who have worked at the mink farm in the last three months, to facilitate epidemiological investigation if needed and ascertain workers compliance with testing or other measures as needed.  Asymptomatic testing of all mink farmworkers (not already tested) to occur by a specific date, after which untested workers would not be allowed on premises, for initial case ascertainment.  Serological testing of workers, to clarify if workers might have had an infection in the past.
Animal surveillance and testing	Mandating participation in an animal surveillance system, to be specified by FH, which included weekly submission of mink mortality for testing, with the hope of detecting infections early in asymptomatic herds to implement further monitoring and risk mitigation measures to prevent mink related strain transmission and spread to humans.
Movement restriction of mink, mink products, and pelt	Restriction on moving mink or mink-related products between farms, to limit opportunities for the spread of the virus, which occurred in other zoonoses outbreak in BC and COVID-19 outbreaks in other countries.  Restriction on moving pelts until compliance with the terms of the order, as assessed by FH.
<b>April 15, 2021: Fraser Health Authority Medical Health Officer Order</b>	
Vaccination	Mandatory vaccination of workers who work in close proximity to mink.  Mandatory record keeping of workers' vaccination status.
<b>July 26, 2021: Provincial Health Officer Order</b>	
Moratorium on mink farming expansion	Farms must report the number of breeding mink stock, non-breeding mink and total mink on the farm.  Must not allow the number of breeding mink or non-breeding mink stock to exceed their reciprocal number as the date of this order.  Must not acquire new live mink.

Abbreviations: BC, British Columbia; COVID-19, coronavirus disease 2019; FH, Fraser Health Authority

Mandatory vaccination and the worker COVID-19 surveillance program were acceptable to the industry; however, mandatory enhanced PPE usage and other biosafety measures were challenging. Skepticism regarding effectiveness or necessity,

costs and discomfort of PPE constituted some of the impediments. To address challenges with specificity and feasibility of national biosafety recommendations, a local OHC subcommittee was formed to swiftly generate BC-specific recommendations. An effective short-term mode of compliance was the restriction of pelt, animal and product movement unless biosafety requirements were met. Ongoing farm inspections were also helpful in assessing and improving compliance.

After implementation of strengthened biosafety measures, mink mortality surveillance and voluntary worker surveillance, only small clusters of human cases (one or two persons/cluster) occurred between January 14 and May 31, 2021, unlike the outbreak among workers on Farm 1 in December 2020 (29) prior to enhanced measures (Table 4 and Figure 2 detail findings from worker surveillance).

**Table 4: Coronavirus disease 2019-positive mink farmworkers in British Columbia, January 14 to October 31, 2021**

Date range	N	%	Context
January 14 to March 17, 2021	2 <sup>a</sup>	16.6	Before vaccination being offered to farmworkers
March 18 to May 31, 2021	5	41.6	After offering vaccination (mandatory vaccination not in place until April 15, 2021):  n=1 had chosen not to be vaccinated  n=1 was positive within 14 days of vaccination with first dose (not considered partially immunized)  n=3 were positive more than 14 days after receipt of a first dose of vaccine (considered partially immunized)
June 1 to October 31, 2021	5	41.6	Following receipt of 2 doses of vaccine:  n=5 were considered fully vaccinated and part of the outbreak on Farm 3
Total	12	100.0	Human cases arose on 3 of 6 remaining farm units <sup>b</sup> from the start of the surveillance system until the end of the study period

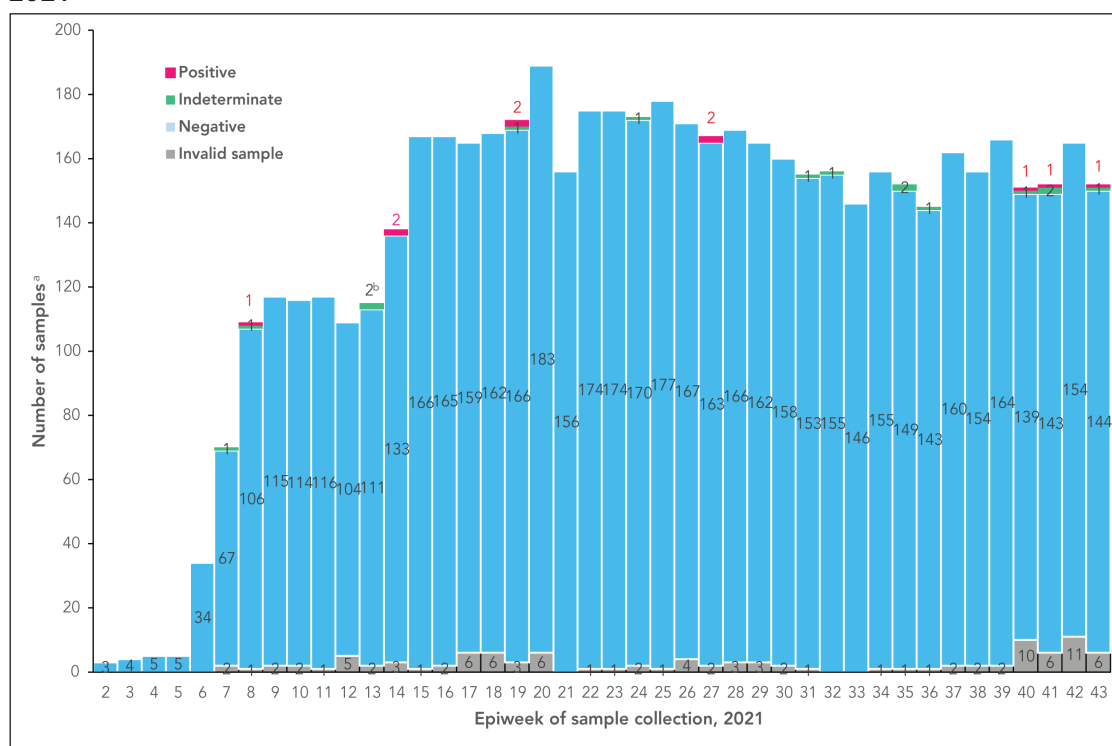
<sup>a</sup> One worker was detected through community testing following household exposure rather than through the worker surveillance system

<sup>b</sup> Of the original nine active mink farms in British Columbia, two pairs of farms were submitting human surveillance samples jointly as they had integrated operations and were in close proximity to each other with workers working on both sites. It was not possible to separate these farms through the human surveillance system. From these original seven independent farm units, one farm (Farm 2) had ceased operation, culling all of its mink after an outbreak was detected in the mink herd, resulting in six remaining farm units by January 2021





**Figure 2: Mink farmworker coronavirus disease 2019 surveillance virologic testing results, January 14 to October 31, 2021**



<sup>a</sup> The number of weekly samples exceeds the number of mink farmworkers as sampling was increased to twice or three times weekly after detection of a case on some farms; the number of workers per farm varies significantly depending on the mink farm production cycle phase. As of October 31, 2021, 11 cases of coronavirus disease 2019 infection in mink farmworkers were detected through the human surveillance program (10 positive via saline gargle and 1 indeterminate on gargle subsequently positive on follow-up nasopharyngeal [NP] swab) out of 5,673 tests among 123 unique workers since January 14, 2021

<sup>b</sup> One individual who returned an indeterminate result during epiweek 13 was positive on follow-up NP swab

One worker case triggered a mink herd outbreak on a third farm (Farm 3). Farm 3 was the only outbreak detected through the mink mortality testing, with phylogenetic analysis identifying the same strain as a previously positive worker who had not originally been thought to have had contact with mink. Mortality surveillance enabled timely monitoring of SARS-CoV-2 propagation and evolution in Farm 3 over several months, despite a lack of symptoms of infection (29). Following two-dose vaccination of workers in contact with mink, with more than 90% uptake among all workers, there were no further mink herd outbreaks, although five worker cases were detected through the surveillance system during this time.

Wildlife surveillance around Farm 1 and Farm 2 occurred in January to March 2021, to detect potential SARS-CoV-2 transmission to wildlife from escaped mink or feral cats. Virology and serology tests were negative on all 65 animals sampled (25). Repeated wildlife surveillance surrounding Farm 3 in summer 2021 also failed to detect infected wildlife but located three escaped mink that tested positive.

In June 2021, a formal risk assessment was conducted based on best practices (**Table 5**). After consideration of the risk portrayed in the report, BC's Provincial Health Officer placed a moratorium on mink farming expansion in the province (27).

**Table 5: Joint rapid qualitative risk assessment on severe acute respiratory syndrome coronavirus 2-associated risk for mink farms in British Columbia, June 29, 2021**

Methods
<p>The scope of the assessment was limited to the mink farms in the Fraser Health Authority and British Columbia. The outcome of interest was the circulation of a mink-induced SARS-CoV-2 variant of interest (VOI) in the community that could potentially increase transmission, cause more severe disease in humans, escape vaccines or significantly decrease the effectiveness of therapeutic and diagnostic technologies, compared with what is seen with currently circulating variants. The pathways assessed were human-mink-human and human-mink-wildlife-human. Two timeframes were assessed: 1) short term: completion of the current production cycle, up to before the start of the next breeding season; and 2) long term: the next five years. A multi-jurisdictional expert group jointly completed all steps of the process. Using a modified Delphi approach, the expert group assessed probability, impact, and uncertainty estimates. Probabilities along the scenario pathways were combined as per accepted qualitative risk assessment methodologies. Several assumptions were made by the expert group that influence the results. There is often a high degree of uncertainty related to the assumptions. They are important to highlight because changes to the assumptions may affect the final estimates, and significant changes may indicate the need for reassessment.</p>
Probability level, impact and uncertainties
<p>The combined assessment of probability and the assessment of consequence the emergence and circulation of a VOI in the community of mink/wildlife origin were as follows:</p> <p>1. What is the likelihood and impact of emergence and circulation of a SARS-CoV-2 VOI in the community due to virus evolution in mink or "wildlife after exposure to mink" during completion of this cycle, compared to what is seen with currently circulating variants and evolving public health measures?</p> <p>Probability: very unlikely (VOI in wildlife pathway) to very unlikely/unlikely (VOI in mink pathway)</p> <p>Uncertainty: moderate (VOI in mink pathway) to high (VOI in wildlife pathway)</p> <p>Impact: minor to moderate at the local/regional level, and slightly less at the provincial level</p> <p>Uncertainty: moderate to high</p> <p>2. What is the likelihood and impact of emergence and circulation of a SARS-CoV-2 VOI in the community due to virus evolution in mink or "wildlife after exposure to mink" WITHIN THE NEXT FIVE YEARS, compared to what is seen with currently circulating variants and evolving public health measures?</p> <p>Probability: very unlikely (VOI in wildlife pathway) to unlikely (VOI in mink pathway)</p> <p>Uncertainty: moderate (VOI in mink pathway) to high (VOI in wildlife pathway)</p> <p>Impact: minor to moderate at the local/regional level, and slightly less at the provincial level</p> <p>Uncertainty: moderate to high</p> <p>The combined probability estimates for both timeframes of the human-mink-human pathway were driven primarily by the probability of the evolution of the virus into a VOI in a mink herd, with higher uncertainty associated with the probability in the five-year assessment due to the higher uncertainty in the expected number of mink herd outbreaks per year as time goes on and higher uncertainty regarding the evolution of a VOI. The risk assessment for the next five years assumed limited control measures. In the pathway involving wildlife, most steps were estimated as less probable than for the direct pathway from mink-to-humans, with a similar level of uncertainty. The mode of the overall probability for the human-mink-wildlife-human pathway during both periods was very low, regardless of the spread scenario in wildlife (limited spread or reservoir). These estimates were driven primarily by 1): the probability of evolution of the virus into a VOI in wildlife that was assessed as very unlikely to occur and 2) the probability that a person would contract the virus from wildlife was assessed as very unlikely. Experts expressed it is more likely that a VOI will arise in humans rather than in mink. If there was emergence and circulation of a VOI in the community that was of mink/wildlife origin, the magnitude of the impact on the health of the population above the current/ongoing pandemic impacts for this cycle were estimated as likely to be minor to moderate at the local/regional level, and slightly less at the provincial level. The uncertainty associated with this was moderate to high. The magnitude of the impact at the five-year timeframe was assessed as likely to be similar, with a higher level of uncertainty.</p>

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VOI, variant of interest

## Discussion

The OHC realized all the benefits of a timely, coordinated, evidence-based and jointly accountable One Health response (30). The main interventions, which were similar to responses in other jurisdictions (3,6), included sequential situational assessments followed by voluntary and mandated measures such as human, mink and wildlife surveillance, farm inspections, enhanced biosafety measures and a moratorium on mink farming expansion. While we did not find evidence of spread between farms or to the community following implementation of interventions akin to Denmark (3,4), phylogenetic analyses indicated mink-to-human transmission at the subsequent Farm 3 outbreak despite enhanced biosafety measures and two-dose worker vaccination (29).

## Strengths and limitations

Existing PH and animal health regulations were paramount in improving compliance with new interventions and/or measures. The sequential approach enabled the response to continually adapt as the situation evolved, considering new scientific evidence and past successes, challenges, and outcomes. Regarding OHC's joint decision-making, consensus on most approaches was reached in a timely manner because of the ongoing dialogue and sharing of information. Some decisions clearly lay within a single organization's purview and consensus was not required; however, the One Health approach enabled effective coordination and integration of multiple perspectives into decision-making.



The first two mink farm outbreaks occurred in December 2020 during the second COVID-19 wave in BC, before vaccination, when minimal biosafety measures were in place, and with increased staffing and mink-worker and/or worker-worker interactions during the pelting season. Structural disincentives for testing among farmworkers (31–33) may have delayed worker testing at Farm 1, thus delaying outbreak detection (29). Combined, ongoing human and mink mortality surveillance were able to successfully overcome case detection difficulties such as asymptomatic/mildly symptomatic mink and human infection (34,35) or testing avoidance (31–33). Rapid human and mink case detection from surveillance also enabled timely whole genome sequencing and combined phylogenetic analyses.

Only one outbreak occurred following implementation of interventions, despite human cases detected on three out of six farm units in January–October 2021, suggesting that our multi-layered approach including PPE, biosafety measures, surveillance and mandatory worker vaccination combined to reduce outbreak risk. A systematic review indicated that physical distancing of more than one metre substantially decreased human-to-human transmission (adjusted odds ratio [AOR] 0.18, 95% CI 0.09–0.38), as did consistent use of face masks (AOR 0.15, 95% CI 0.07–0.34), with stronger associations with respirators (36). Lapses in PPE usage and other biosafety measures would be unsurprising, as these occur even in healthcare settings where workers receive extensive training and monitoring (37–39). The human surveillance program also decreased the probability of a worker COVID-19 outbreak occurring, further decreasing the risk of transmission to the herd. In humans, modelling studies suggest weekly testing reduces secondary infection by 23%–60%, increasing to 90% with twice-weekly testing (40,41). As no outbreaks were seeded after workers had spent 14 days following dose one vaccination, immunization with a highly effective vaccine likely further decreased outbreak risk over the next five months, past the peak of the fourth wave in BC. Despite support from the literature with plausible timelines and mechanisms suggesting the control measures were effective to a point, it is difficult to establish causality between measures and the number of cases or outbreaks detected after their implementation.

Without mandatory mink mortality surveillance, the outbreak on Farm 3 might not have been detected until much later, if at all, in part because SARS-CoV-2 infection in mink frequently results in asymptomatic or mildly symptomatic infections (42). The BC farms had difficulty providing even five mink mortalities weekly, lowering the estimated infection detection sensitivity to less than 65% (21). With at least weekly worker surveillance and infectious worker contact with mink triggering live mink testing as part of our One Health approach, it is unlikely an outbreak was missed.

Spillover into wildlife from infected mink herds and associated feral cats could promote the emergence of SARS-CoV-2 genetic

mutation of concern or of a reservoir (17,18,24). Repeated wildlife surveillance surrounding all three infected farms failed to detect infected wildlife, despite locating three escaped mink that tested positive. A limitation of this monitoring was that the sensitivity of the wildlife surveillance was uncertain (43,44).

One of the main limitations of BC's comprehensive One Health response was its resource-intensive nature. Evidence review, risk assessment, mink mortality surveillance and inspections all were resource-intensive at a time when most of the OHC's organizations were already overstretched by the pandemic response. Although the use of self-collected saline gargle specimens for human surveillance decreased PH resource requirements while maintaining sensitivity and improving acceptability (22,23), the materials needed, specimen transportation, and ongoing lab processing and analysis were not without cost.

## Implications

In BC, sustaining many of the implemented interventions long term, despite some evidence of their effectiveness, was challenging for the industry and the various agencies involved. Worker vaccination likely reduces the risk of subsequent outbreaks and is less resource-intensive, but is contingent on vaccine effectiveness against prevailing strains, which continues to evolve. Furthermore, immunization does not resolve infection detection difficulties in workers and mink. Without ongoing worker and mink herd surveillance, it is possible that mink farm outbreaks and the associated risk of mink-related viral adaptation and transmission back to the community are occurring undetected in other jurisdictions including other provinces.

## Conclusion

A One Health response tailored to mitigating SARS-CoV-2 risk on mink farms in BC, led by an issue-specific OHC, was triggered following two mink farm outbreaks in December 2020. The One Health approach enabled ongoing communication between relevant agencies and a timely and coordinated response. A third mink farm outbreak occurred in mid-2021 despite implemented enhanced PPE and biosafety measures, worker and mink surveillance programs and regular farm inspections. A comprehensive One Health approach, involving animal health, public health, worker safety and industry regulation organizations, should be implemented to respond to complex and evolving threats such as risks from emerging zoonotic pathogens in farmed animals.

## Authors' statement

VC — Conceptualization, investigation, writing—original draft, data visualization, review and editing

YLEC — Conceptualization, investigation, writing—original draft, data curation, data visualization, review and editing

AP — Investigation, data curation, review and editing



EF — Investigation, review and editing  
RG — Investigation, review and editing  
EN — Conceptualization, investigation, writing—original draft,  
data curation, review and editing

## Competing interests

None.

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# SARS-CoV-2 in mink farms in British Columbia, Canada: A report of two outbreaks in 2020–2021

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## Abstract

**Background:** Since April 2020, mink have been recognized as a potential reservoir for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a potential source of new variants. The objective of this report is to describe the epidemiological investigation and public health response to two coronavirus disease 2019 (COVID-19) outbreaks that involved both humans and farmed mink.

**Methods:** An outbreak was declared on December 4, 2020, following detection of two COVID-19-positive farmworkers and elevated mink mortality on a mink farm (Farm 1) in British Columbia. The second cluster was detected on Farm 3 following detection of 1) a COVID-19 case among farm staff on April 2, 2021, 2) an indeterminate result from farm staff on May 11, 2021, and 3) subsequent SARS-CoV-2-positive mink in May 2021. Quarantine of infected farms, isolation of workers and their close contacts, and introduction of enhanced infection control practises were implemented to break chains of transmission.

**Results:** Among mink farmworkers, 11 cases were identified at Farm 1 and 6 cases were identified at Farm 3. On both Farm 1 and Farm 3, characteristic COVID-19 symptoms were present in farm employees before signs were observed in the minks. The viral sequences from mink and human samples demonstrated close genetic relation. Phylogenetic analyses identified mink intermediates linking human cases, suggesting anthro-po-zoonotic transmission.

**Conclusion:** These were the first COVID-19 outbreaks that included infected mink herds in Canada and identified potential anthropogenic and zoonotic transmission of SARS-CoV-2. We provide insight into the positive impact of regulatory control measures and surveillance to reduce the spillover of SARS-CoV-2 mink variants into the general population.

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**Keywords:** mink, spillover, zoonotic disease, SARS-CoV-2, COVID-19, One Health

## Introduction

Mink—a semiaquatic, carnivorous mammal of the genus *Neogale*—have been identified as a potential reservoir of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19) (1). Since April 2020, twelve countries have reported SARS-CoV-2 infection in mink (2,3). Genetic analyses of outbreaks in Denmark and the Netherlands suggest potential anthro-po-zoonotic origins and spillover of mink-divergent SARS-CoV-2 into the greater community, highlighting a biosecurity risk resulting from genetic diversification following adaptation of the virus in a new host (4–7).

The Fraser Valley contains all mink farms in British Columbia (BC), Canada, and produced 23% of Canadian mink pelts in 2020 (8). BC's farms are located close to major population centres. The historical herd size is significantly smaller than Danish and Dutch farms, with less reliance on centralized infrastructure for feeding and pelting. Since the beginning of the pandemic, three BC mink farms have experienced SARS-CoV-2 transmission within mink herds, with two of these outbreaks (Farm 1, Farm 3) involving documented human cases (9).



We report on the epidemiological investigation of the two outbreaks that involved genetically linked human and mink infections with SARS-CoV-2 occurring between December 2020 and November 2021 in BC. We reflect on the impact of control measures, vaccines and active surveillance, highlighting how these interventions may have created the unique epidemiological profile observed on Farm 3 and provide comparisons to Farm 1 and other outbreaks described in the literature.

## Methods

### Overview

The outbreak on Farm 1 was detected on December 2, 2020, during the pelt-harvesting season after two farmworkers tested COVID-19-positive at a community testing site. The farm owner identified an increased overall mortality rate of approximately 1.5% in the herd of 15,000 mink in the preceding week. A herd veterinarian was deployed to sample mink mortalities on the farm. On December 4, 2020, Public Health (PH) declared an outbreak in mink and farmworkers and the Ministry of Agriculture, Food and Fisheries (MAFF) quarantined the farm after four of five mink samples tested non-negative. On February 24, 2021, the outbreak was declared over.

On April 2, 2021, a farmworker on Farm 3 tested positive prior to achieving vaccine-mediated immunity through the mink farmworker surveillance system established in February 2021 (9). On May 11, 2021, an indeterminate result from another worker and SARS-CoV-2-positive mink mortalities were identified. On May 12, 2021, MAFF placed the farm under quarantine and an outbreak investigation began and was ongoing as of November 1, 2021. For both farms, the criteria for an outbreak to be declared over was for the farm to have neither positive nor indeterminate human or mink samples detected for two consecutive 14-day incubation periods.

### Laboratory investigation

The British Columbia Centre for Disease Control (BCCDC) Public Health Laboratory conducted real-time polymerase chain reaction-based (RT-PCR) SARS-CoV-2 testing using RdRP and E gene targets; specimens were confirmed SARS-CoV-2 positive at a cycle threshold (Ct) value  $\leq 35$ . The MAFF performed preliminary animal testing (reported as negative or non-negative based on RT-PCR of the E gene target) that was confirmed with similar assays validated in animals at the National Centre for Foreign Animal Disease laboratory in Winnipeg, Canada.

All human and animal samples with a PCR-confirmed positive test underwent next-generation whole genome sequencing (WGS) using laboratory methods described in detail elsewhere (10). Briefly, samples were sequenced on an Illumina MiSeq or NextSeq instrument using a tiled 1,200 bp amplicon scheme and analyzed using a modified ARCTIC Nextflow pipeline (10). Sequences passing quality control (85% genome completeness,

10X depth of coverage and no quality flags) were included in the phylogenetic analysis. Phylogenetic trees were constructed using Nextstrain (11) and samples were manually assigned to a genetic clade based on an inclusion criterion of three mutations or fewer. According to our clade calling scheme, 0 mutations were "identical", 1–2 mutations were "nearly identical", 3 mutations were "similar" and more than 3 mutations were "different". This scheme aligns with the previously reported mutation rate in humans of approximately one mutation per two-week period (12). Samples were assigned a sub-clade designation (e.g. Clade 1.1) to denote clusters of genetically identical sequences. Lineage assignment was performed using the Phylogenetic Assignment of Named Global Outbreak Lineages tool (PANGOLIN) Version V.3.1.17 (13).

### Case finding and investigation

For these investigations, the case definitions were as follows:

- Confirmed case: an individual who worked on the farm **and** had a positive RT-PCR (Ct: $\leq 35$ )
- Epidemiologically linked case: an individual who worked on the farm **and** had an indeterminate RT-PCR (Ct:36–50) **and** reported respiratory symptoms consistent with SARS-CoV-2 in the two weeks prior to testing **or** was a household contact of a confirmed case

Public Health conducted investigations of confirmed cases within 24 hours of notification. Interviews explored illness onset date, symptoms, exposure history, risk factors, close contacts and connections to other mink farms. Owners of infected farms confirmed each case's duties and identified farm contacts. Cases were instructed to isolate for 10 days. Close contacts of SARS-CoV-2-positive mink and humans were advised to self-isolate for 14 days since their last exposure and seek testing if symptoms developed.

### Animal and wildlife investigation

Herd veterinarians conducted weekly animal sampling on site. Animal sampling included nasopharyngeal swabbing of mink mortalities and live mink. Wildlife captured by hunters and trappers in the less than 2 km perimeter of each premise provided insight into the spillover from escaped mink into the surrounding wildlife, as described in Strang *et al.* (14). No wildlife samples tested positive for SARS-CoV-2.

### Epidemiological and statistical analyses

Case and contact management details were available through PARIS, Fraser Health's PH information system. Descriptive analyses were generated using Microsoft Excel (2020) software. The crude attack rate for secondary cases is the number of confirmed cases over the number of susceptible persons (i.e. close contacts who were not employed on the farm). Shared contacts were counted once.





## Interventions

### Farm 1

The provincial One Health Committee, detailed in **Table 1**, launched a tandem response to the Farm 1 outbreak. Public Health conducted an environmental health and occupational risk assessment on site, tested all workers and reviewed biosecurity practises. Simultaneously, MAFF placed Farm 1 under quarantine with restrictions on the transportation of animals, products, goods and people in and out of the site. Farm activities were restricted to those necessary for animal welfare; activities outside of this scope needed MAFF approval. An animal outbreak investigation identified no transmission between Farm 1 and other BC mink farms.

The One Health Committee provided instructions for animal care, including minimizing interaction length and frequency, limiting care to asymptomatic individuals, and enhanced hand hygiene. Enhanced biosecurity measures for mink care included the use of full personal protective equipment (i.e. N95 masks, disposable Tyvex suit, long rubber gloves, rubber boots), the establishment of a quarantine zone for donning and doffing personal protective equipment, and sanitation procedures for soiled boots and gloves. Surgical masks were sufficient when not in close proximity to mink.

Three recovered farmworkers were permitted to euthanize the herd for the purposes of pelt production December 16–24 2020, as pelting would remove infected mink and reduce risk of further transmission. Breeding stock was retained. All euthanized mink were stored in the farm freezer for later processing. Farmworkers involved in pelting were released from isolation 14 days after the last mink was euthanized. Following pelting, no SARS-CoV-2 was detected in the herd.

### Farm 3

While the response to the Farm 3 outbreak had many similarities to Farm 1, the most significant difference in the two responses was the presence of industry-wide, preventative measures and surveillance infrastructure that was established from mid-December 2020 to April 2021. These measures included the creation of a weekly voluntary human surveillance system to detect asymptomatic or pre-symptomatic cases among farmworkers, as well as public health mandates to ensure weekly testing of mink mortalities, enhanced biosecurity measures (as described for Farm 1) and farmworker vaccination (9).

In addition to the above measures, PH required all Farm 3 workers exposed to the mink herd to follow a work-home quarantine May 11–June 9, 2021. The voluntary farmworker screening was increased to 2–3 times a week. Concurrent environmental health inspections found an acceptable compliance with the newly established provincial-level biosecurity requirements.

**Table 1: Role of mink outbreak management working group**

Name of outbreak management working group	Roles
Fraser Health Authority	<p>Medical Health Officer—Clinically directed case and contact management, responsible for human outbreak control, enacted Public Health Orders</p> <p>Environmental Health Officer—Completed environmental health inspections, provided review of COVID-19 safety plans, retrieved mink mortalities from farms</p> <p>Communicable Disease Nurse Coordinator—Oversaw Cluster Investigators, provided clinical support to team, oversaw logistics of outbreak management</p> <p>Cluster Investigator—Reviewed cases that are employed at farms, input laboratory results, followed up with farms regarding issues related to vaccinations/testing, completed clinical assessment of farm cases</p> <p>Analyst—Monitored and processed laboratory data, summarized and analyzed epidemiology</p>
BC Centre for Disease Control	<p>Physician epidemiologist—Provided leadership in supporting One Health group, pulled together scientific literature, connected national stakeholders and international organizations</p> <p>Public health veterinarian—Provided expertise on the intersection of animal and human health, connected to federal advisory working groups</p> <p>Epidemiologist—Designed and implemented mink farmworker surveillance system, provided surveillance reports, liaised with MAFF veterinarian-epidemiologist</p> <p>Laboratory staff—Provided laboratory services including processing of weekly real-time polymerase chain reaction SARS-CoV-2 tests and completion of genomic sequencing of human cases, provided interpretation of genomic sequencing data</p> <p>Coordinated transportation of animal samples to the National Microbiology Laboratory for processing and sequencing</p>
Ministry of Agriculture, Food and Fisheries	Responsible for health and well-being of animals, outbreak management in agricultural settings; performed testing of animals, provided guidance on risk reduction procedures
WorkSafeBC	Regulated safety of farmworkers, supported development of protocols and standards for occupational safety on farms
Ministry of Forests, Lands, Natural Resource Operations and Rural Development	Supported wildlife surveillance surrounding farms
Canadian Food Inspection Agency	Provided expert consultation and scientific advice
Ministry of the Environment	Regulated environmental discharges, including manure

Abbreviations: BC, British Columbia; COVID-19, coronavirus disease 2019; MAFF, Ministry of Agriculture, Food and Fisheries; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2



## Results

### Farm 1 outbreak

There were 11 cases among 12 farmworkers (8 confirmed cases; 3 epidemiologically linked cases) associated with the Farm 1 outbreak (Table 2). Cases had tightly clustered symptom onset November 25–December 4, 2020 (Figure 1). A large mink die-off followed the symptom onset of the 2 index cases by 8 days. Notably, 2 of 4 co-housed migrant workers were asymptomatic with high Ct values, suggesting remote infection and potentially earlier onset than reported to PH.

**Table 2: Demographics and symptoms of confirmed and epidemiologically linked cases associated with mink farm COVID-19 outbreaks December 2020–October 31, 2021**

Case demographics and symptoms	Overall		Farm 1		Farm 3	
	n	%	n	%	n	%
Number of cases	17	17	11	11	6	6
<b>Case type</b>						
Confirmed case	14	82.4	8	72.7	6	100
Epidemiologically linked case	3	17.6	3	27.3	0	0
<b>Age group (years)</b>						
20–39	7	41.2	5	45.5	2	33.3
40–79	10	58.8	6	54.5	4	66.7
<b>Vaccination status</b>						
No vaccination	11	64.7	11	100	0	0
Within 14 days of first dose	1	5.9	0	0	1	16.7
Two valid doses	5	29.4	0	0	5	83.3
<b>Symptoms</b>						
Asymptomatic	5	29.4	2	18.2	3	50.0
Chills	3	17.6	3	27.3	0	0
Fever	3	17.6	2	18.2	1	16.7
Runny nose	3	17.6	1	9.1	2	33.3
Sore throat	3	17.6	2	18.2	1	16.7
Cough	2	11.8	2	18.2	0	0
Fatigue	2	11.8	2	18.2	0	0
Myalgia	2	11.8	2	18.2	0	0
Nasal congestion	2	11.8	2	18.2	0	0

Abbreviation: COVID-19, coronavirus disease 2019

Environmental health inspection on December 5, 2020, identified weak infection control practices. Relevant findings included the use of cloth masks, a single handwashing station with a reusable towel and no log of entrance screening questions or cleaning, which may have facilitated mink-to-human transmission.

Phylogenetic analysis for Farm 1 included 8/11 farmworkers, 6 close contacts and 151 mink samples that generated high-quality sequence data. All Farm 1 samples clustered within 1 distinct genetic clade (Clade 1) within the AW.1 lineage, a lineage circulating locally in October 2020 (Figure 2). Four farmworker samples and a sequenced household contact of an index case were genetically identical or nearly identical to one another and mink samples (Clade 1.2.1). The other sequenced index case clustered on a divergent branch of the tree (Clade 1.3.2.2.1). Mink isolates acted as genetic intermediaries between human sequences (Clade 1.2.4 and 1.3.2.1). Notably, community cases related to Clade 1.3.2.2.1 with onset after December 3, 2020, included clusters among vulnerable populations such as long-term care. Clades 1.3.2.2.1 and 1.2.1 were not detected through routine WGS surveillance in the community prior to the outbreak.

### Farm 3 outbreak

The epidemic curve of the Farm 3 outbreak resembles one expected from an intermittent source, spanned from April–October 2021, and had fewer human cases compared to Farm 1 (six confirmed cases) despite a similar workforce size (Table 2, Figure 3). Other than the index case, new human cases were associated with high levels of mink-human contact. The two confirmed cases with onset in July were associated with a personal protective equipment breach reported during a heat event in June 2021; while the three cases in October had onset after a period of intense animal relocation. When compared to Farm 1, the outbreak on Farm 3 had a greater proportion of asymptomatic cases (Farm 3=50.0%; Farm 1=18.2%, Table 2) and lower attack rates among close contacts (Farm 3=12.5%; Farm 1=29.4%).

Five of six human samples and 79 mink samples generated high-quality sequence data and were included in the phylogenetic analysis (Figure 3). Human and mink samples clustered tightly together on trees that diverged from the B.1.618 lineage. Mink sequences were genetically intermediate between human sequences (Clade 1.4.1 and Clade 3.7.1) and no human samples shared the same clade. While the B.1.618 lineage was circulating in the region at the time of the first human case (Clade 1.1), a more than 80% sequencing coverage of community cases during the time of subsequent human cases on Farm 3 detected no background community circulation of this strain. No community transmission of the Farm 3 strains was detected.



Figure 1: Gantt chart of confirmed and epidemiologically linked cases among mink farmworkers on Farm 1 in British Columbia, Canada

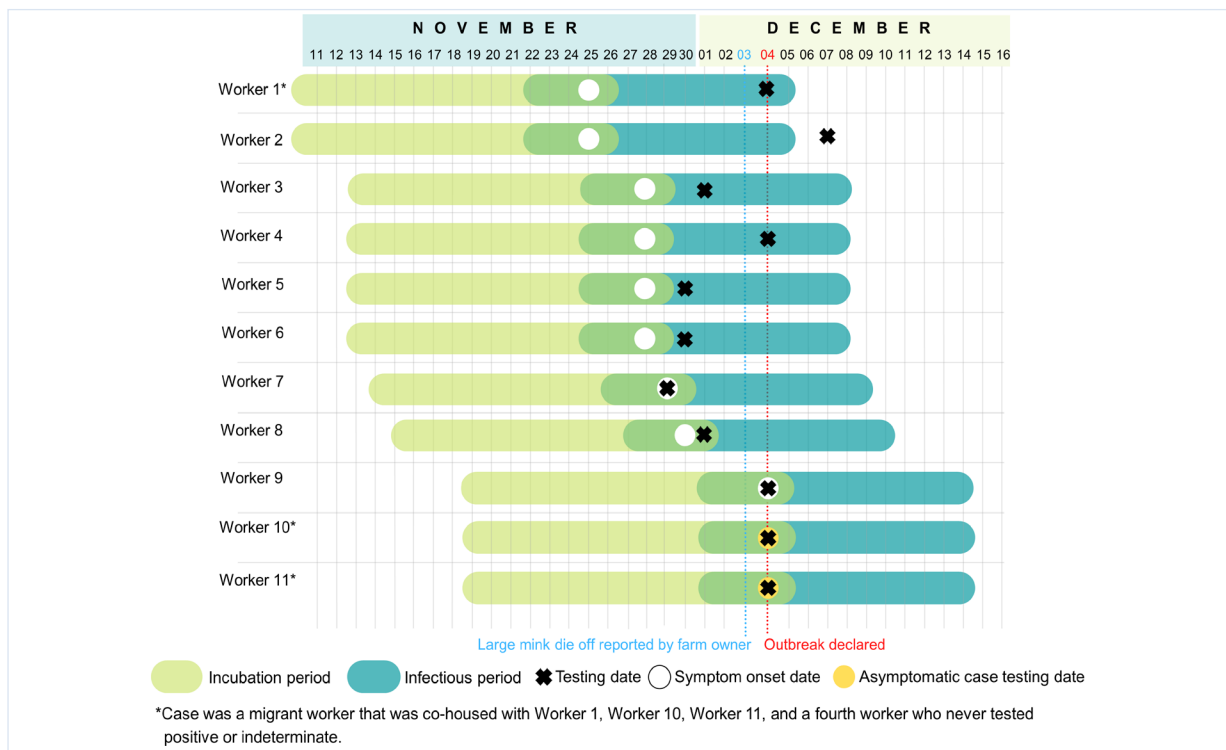
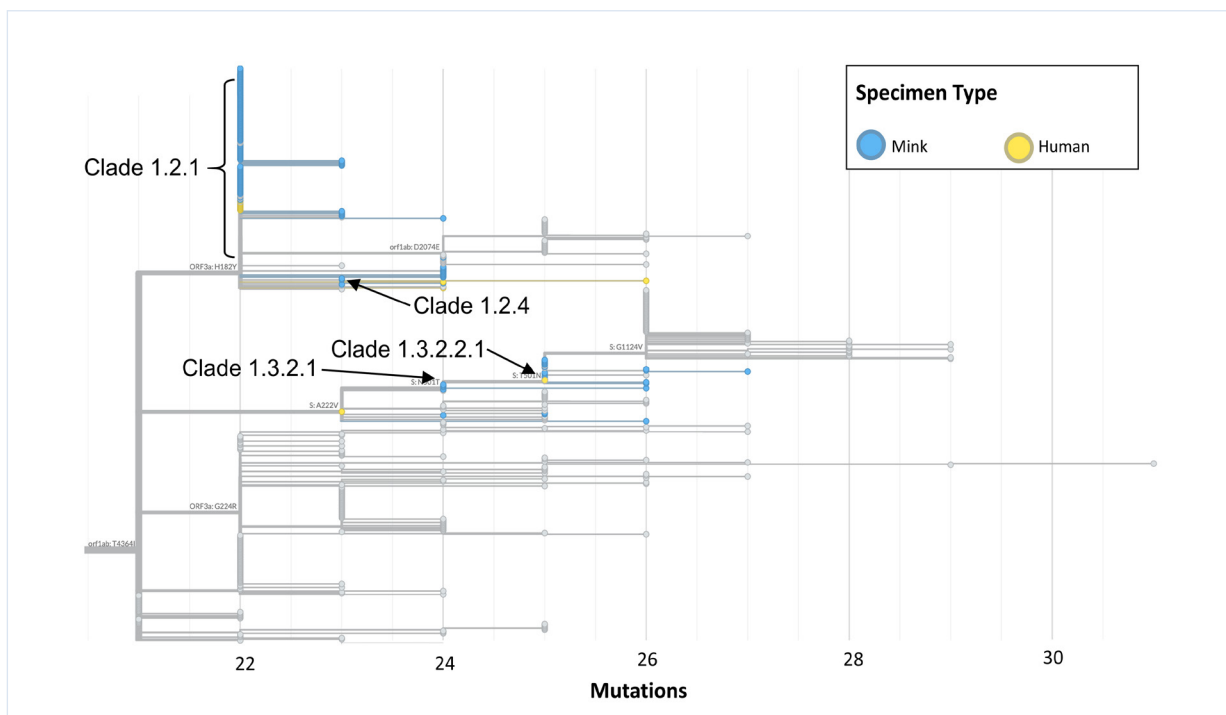


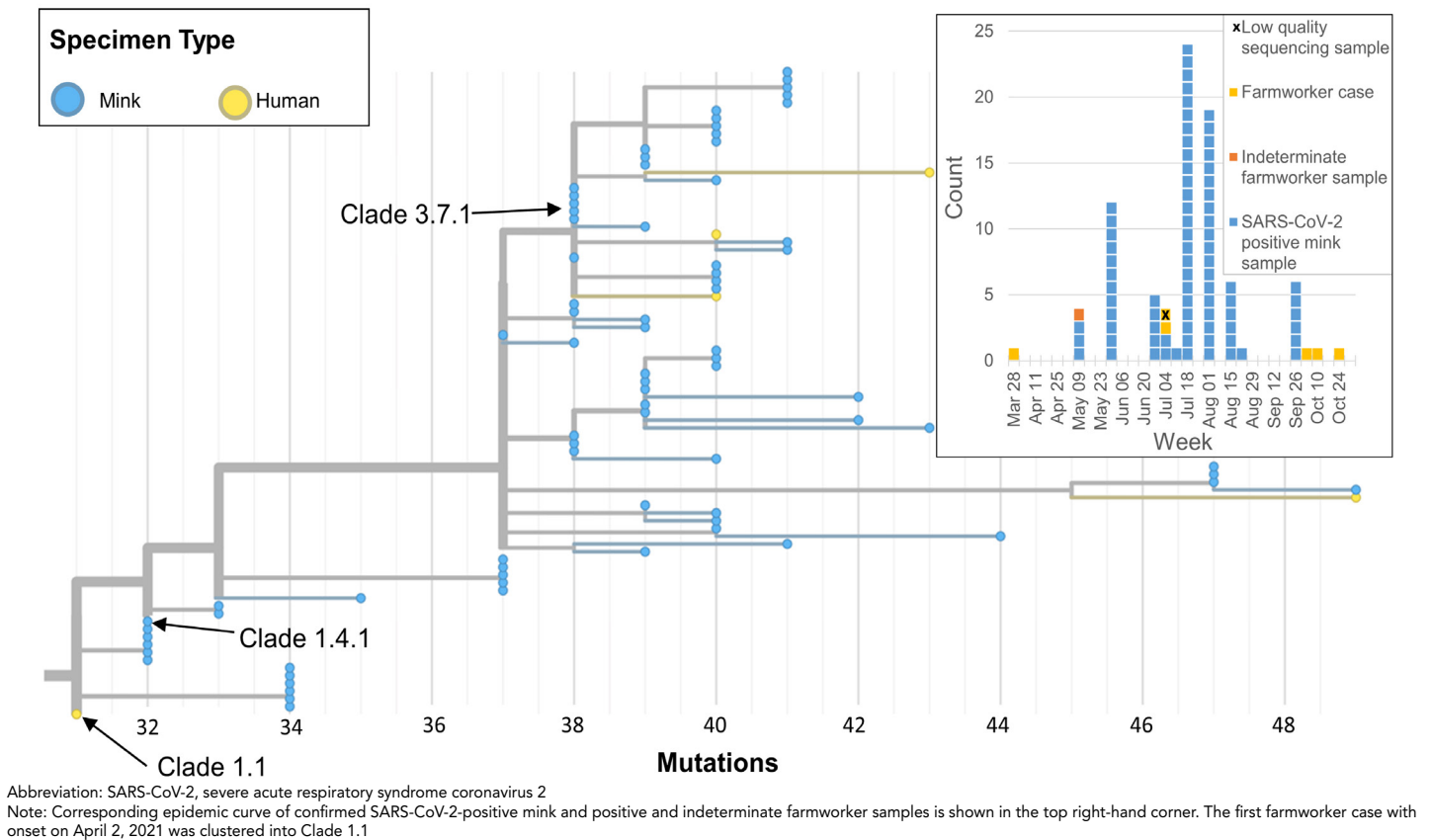
Figure 2: Farm 1 and a subset of community cases<sup>a</sup> of SARS-CoV-2 identified in British Columbia, Canada, November 10, 2020–December 19, 2020



Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

<sup>a</sup>This tree contains only community cases classified as belonging to the SARS-CoV-2 PANGOLIN AW.1. There were additional lineages circulating at this time in British Columbia; however, these were not considered genetically related to Farm 1 cases and were subsequently excluded from this custom phylogenetic tree. The corresponding epidemic curve of confirmed SARS-CoV-2-positive mink and human samples is shown in the top right-hand corner. The index farmworker case with high quality sequencing data and onset on November 25, 2020 clustered into Clade 1.3.2.2.1. A household contact of the index case with low quality sequencing data clustered with four farmworker samples in Clade 1.2.1

**Figure 3: Farm 3 human and mink samples of SARS-CoV-2 identified in British Columbia, Canada, April 2, 2021–October 31, 2021**



## Discussion

This report summarizes two COVID-19 outbreaks among mink farmers and their livestock in Canada. On both farms, symptoms were present in staff before detection in the minks, viral sequences from mink and human-derived isolates were closely related, and human cases predominantly arose during periods of higher human-mink contact (i.e. harvesting and animal relocation). These findings point to a likely anthropogenic introduction of SARS-CoV-2 into farmed mink by farm staff, viral evolution in the mink host and then reintroduction into human hosts. Variations in the length and transmission patterns observed between outbreaks may be attributable to the different PH measures in place at each outbreak's onset.

The rapid transmission observed on Farm 1 may be credited to late detection, the absence of natural or vaccine-mediated immunity among farmworkers and mink and ineffective biosecurity control measures. Factors resulting in late outbreak detection included self-initiated community testing and early spread among the co-housed migrant workforce, a population well recognized for limited access to health services (15,16). Alternatively, the short course of the outbreak can be attributed to the exhaustion of the susceptible farmworker population

(91.6% attack rate) and pelting of the mink herd eliminating the outbreak's source.

The late detection of the Farm 1 outbreak makes it difficult to establish the date of introduction onto the farm and chain of transmission, which could plausibly have begun weeks earlier than the index case. While it is challenging to establish directionality of transmission for all cases, the WGS analysis strongly suggests two human cases acquired SARS-CoV-2 from mink. Mink-to-human transmission is further supported by the rapid genetic divergence observed on Farm 1, which is beyond the approximate one mutation per two weeks expected through human-to-human transmission alone (12).

The absence of related co-circulating community strains, dispersal of human cases in time, epidemiology and WGS pointed to multiple transmissions from mink to fully vaccinated humans over seven months at Farm 3. Although other outbreak reports have suggested outbreaks in farmed mink can run their course quickly (2,6), Farm 3's outbreak timeline demonstrated that a herd outbreak can persist for months and function as an intermittent source of SARS-CoV-2. This complements existing evidence that mink can function as long-standing reservoirs of SARS-CoV-2 (6,17). While vaccination and enhanced biosecurity practises were able to reduce transmission risk, as displayed





by the high proportion of asymptomatic cases (Farm 1=18.2%; Farm 3=50.0%) and month-long periods between farmworker cases in Farm 3 as compared to Farm 1, they were unable to eliminate mink-to-human transmission from an established mink reservoir. Research on farm-related factors contributing to prolonged infection in mink is needed to inform future prevention efforts.

The absence of spillover into the community stemming from Farm 3 illustrates the potential importance of provincial policies enacted in BC that led to the development of early outbreak detection systems, enhanced biosecurity measures and early vaccination of mink farmers and their households (9). Specifically, early detection of cases through biweekly worker surveillance and vaccinations may explain the absence of farmworker-to-farmworker transmission and the low attack rate among farmworkers' close contacts on Farm 3 compared to Farm 1 (Farm 3=12.5%; Farm 1=29.4%). Simultaneously, the spillover of SARS-CoV-2 identified at Farm 1 into high-risk populations in the community, similar to previous reports in Denmark (4) and the Netherlands (6), illustrates the risk of late detection of infection in the mink farm setting. Alternatively, this difference may reflect the Farm 3 variant's introduction into a highly vaccinated population at a time of high community prevalence of the Delta variant, the dominant lineage from July 4, 2021, onwards (18,19).

The strengths of this outbreak investigation included the adoption of a One Health approach that integrated multiple agencies to respond to a pathogen with demonstrated capacity for human spillovers. Comprehensive, frequent testing of staff during outbreaks makes the likelihood of undetected human intermediary cases remote.

## Limitations

There are several limitations to this report. Data on symptoms and close contacts were self-reported and vulnerable to social desirability and recall bias. Due to concerns about economic, logistical and reputational impacts, farmworkers and owners may have been hesitant to report a large number of contacts. It is difficult to ascertain a causal relationship between individual initiatives and their effects on controlling each outbreak given the application of multiple interventions. Despite these limitations, this report adds to the literature of the emerging threat of SARS-CoV-2 in mink reservoirs and describes the actions that led to the interruption of the chain of transmission of mink variants in the largest health authority in British Columbia.

## Conclusion

These outbreaks provide additional evidence of zoonotic transmission of SARS-CoV-2 from mink to humans and the potential for subsequent community spread. The second outbreak at Farm 3 demonstrated that the risk of human acquisition can persist for months during longer outbreaks in mink herds. Biosecurity requirements, staff vaccination and an ongoing surveillance system contributed to reducing the spillover of mink variants into the general community; however, these

measures were unable to eliminate the risk of mink-to-human transmission during persistent herd infections. These outcomes provide evidence for other jurisdictions of the importance of active surveillance to support timely response to SARS-CoV-2 in these high-risk settings. A One Health approach is needed to successfully respond to outbreaks involving humans and animals, as experts in various fields must collaborate to limit disease spread.

## Authors' statement

The first author named is lead and corresponding author. All other authors are listed in alphabetical order.

AP — Conceptualization, validation, data curation, formal analysis, writing—original draft, writing—review and editing, visualization

EC — Data curation, writing—review and editing

EF — Resources, writing—review and editing

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NP — Investigation, resources, formal analysis, visualization, writing—review and editing

SR — Investigation, resources, formal analysis, visualization, writing—review and editing

VC — Conceptualization, validation, writing—review and editing

## Competing interests

None.

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# A multi-provincial *Salmonella* Typhimurium outbreak in Canada associated with exposure to pet hedgehogs, 2017–2020

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## Abstract

**Background:** In October 2020, an investigation began in Canada on an outbreak of *Salmonella* Typhimurium infections of the same strain as a concomitant outbreak in the United States (US) that was linked to pet hedgehogs. The objective of this article is to identify the source of the outbreak, determine if there was a link between the Canadian and US outbreaks and identify risk factors for infection to inform public health interventions.

**Methods:** Cases were identified through whole genome sequencing of *S. Typhimurium* isolates. Information was collected on case exposures, including animal contact. Hedgehog and environmental specimens were tested for *S. Typhimurium* and a trace back investigation was conducted.

**Results:** There were 31 cases in six provinces, with illness onset dates from June 1, 2017, to October 15, 2020. Median case age was 20 years and 52% were female. Isolates grouped together between 0–46 whole genome multi locus sequence typing allele differences. Of 23 cases with available exposure information, 19 (83%) reported contact with hedgehogs in the seven days prior to symptoms; 15/18 (83%) reported direct contact and 3/18 (17%) reported indirect contact. Trace back investigation did not identify a common source of hedgehogs but uncovered an industry with a complex distribution network. The outbreak strain was detected in samples collected from a hedgehog in one case's home and from a hedgehog in a Québec zoo.

**Conclusion:** Direct and indirect contact with hedgehogs was identified as the source of this *S. Typhimurium* outbreak. Public health communications aimed to increase awareness about the risks of zoonoses from hedgehogs and shared key hygienic practices to reduce disease transmission.

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**Keywords:** salmonella, *S. Typhimurium*, hedgehog, zoonotic, enteric, outbreak

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## Introduction

*Salmonella* remains a leading cause of human enteric illness in Canada. Symptoms of salmonellosis typically begin 6 to 72 hours after exposure, and can include fever, chills, diarrhea, abdominal cramps, headache, nausea and vomiting that usually end within 4–7 days (1). Although many infections are linked to consumption of contaminated foods, an estimated 13%–19% are associated with animal contact (2–4). *Salmonella* bacteria colonize the gastrointestinal tract of a wide range of



host species; animals can experience clinical disease following infection, but most often no clinical signs are observed, with intermittent fecal shedding and carriage (5). Many *Salmonella* Typhimurium outbreaks in the United States (US) and Canada have been linked to direct or indirect contact with a variety of pets and their foods, including rodents and other small mammals (mice, rats, guinea pigs, hedgehogs), reptiles and amphibians (frogs, turtles, snakes) and dogs and cats (6–8).

Hedgehogs have gained popularity as pets in recent decades, with the African pygmy hedgehog (*Atelerix albiventris*) the species most often sold in the North American pet trade (9–11). Captive breeding is in place in Canada and the US, as importation directly from Africa is prohibited due to their potential to carry serious diseases including foot-and-mouth disease (10–12). Hedgehogs can be a source of several zoonotic diseases, including salmonellosis (11,13,14). *Salmonella* infections in hedgehogs can result in clinical illness; however, many remain asymptomatic carriers with prevalence of *Salmonella* carriage in wild hedgehog populations ranging from 0% to 96% (10,11,14–16).

A number of *Salmonella* outbreaks and individual cases linked to pet or wild hedgehogs have been reported since the 1990s (11), involving different serotypes including *S. Tilene* (17–19), *S. Typhimurium* (10,16,19,20), *S. Enteritidis* (21,22) and *S. Stanley* (23). In Canada in 1995–1997, there was a multi-provincial outbreak of 10 cases of *S. Tilene* associated with pet hedgehogs and sugar gliders (18). The US Centers for Disease Control and Prevention (CDC) investigated three multistate outbreaks of *S. Typhimurium* infections linked to pet hedgehogs that occurred during 2011–2013, 2018–2019 and July 2020 (10,24–27). These outbreaks were caused by a genetically similar strain of *S. Typhimurium*, as determined by whole genome sequencing (WGS), suggesting wide dissemination throughout the US pet hedgehog industry (25–27).

In October 2020, a Canadian investigation was initiated by the Public Health Agency of Canada (PHAC) and provincial public health officials when *S. Typhimurium* isolates from humans identified were genetically related by WGS to the US pet hedgehog outbreak (26). The objectives of the investigation are to identify the source of illness and risk factors for infection, determine if there is an epidemiologic link between the US and Canadian outbreaks, and implement public health interventions, including education and awareness activities.

## Methods

### Overview

Following notification by the CDC on September 19, 2020, about an outbreak of *S. Typhimurium* infections linked to contact with pet hedgehogs (25), genetically related Canadian isolates were identified through PulseNet Canada (PNC) (28). The Canadian

outbreak investigation began on October 28, 2020, with the objective of describing *S. Typhimurium* outbreak cases, and identifying and tracing the source of the outbreak.

### Outbreak detection and case identification

Since salmonellosis is a notifiable disease in Canada, clinical laboratories send *Salmonella* spp. isolates to provincial public health laboratories or to the National Microbiology Laboratory for WGS-based subtyping (implemented in 2017) (29). The PNC national database team at the National Microbiology Laboratory analyzes all Canadian WGS data in a centralized BioNumerics v7.6 (Applied Maths) database (30). Multi-jurisdictional clusters of *S. Typhimurium* were identified using a threshold of at least three *S. Typhimurium* isolates related within 0–10 whole genome multi-locus sequence typing (wgMLST) allele differences where two of three isolates are within five wgMLST alleles. All three isolates must have isolation dates within the last 60 days and at least one must be clinical. Allele ranges may expand during an investigation based on available laboratory, epidemiologic and other relevant evidence. Once a cluster is identified, PNC assigns a cluster code, and isolates subsequently identified as genetically related are added to the WGS cluster. Epidemiologists at CDC and PHAC regularly communicate regarding investigations of interest to both countries. As a result, representative isolates from the US investigation were used to search for matching Canadian isolates in the PNC database.

### Case definition

The case definition included Canadian residents or visitors to Canada with laboratory confirmation of *S. Typhimurium* matching the outbreak cluster by WGS with symptom onset, specimen collection, or isolation date on or after December 1, 2019. Cases were related within 0–46 wgMLST allele differences, which was supported by both epidemiologic and trace back data. As the investigation progressed, genetically related historical clinical isolates from cases with a symptom onset, specimen collection, or isolation date on or after June 1, 2017, were added to the investigation.

### Epidemiologic and trace back investigation

Cases with laboratory-confirmed *Salmonella* infections were routinely interviewed by local or regional public health authorities in most jurisdictions. The questionnaires captured exposure information for the seven days prior to symptom onset and generally cover clinical, travel, food and other risk factors including animal exposures. Consent for future follow-up was gathered at the time of interview.

Information was collected from initial interviews, and cases were re-interviewed by PHAC or individual provinces with a questionnaire focused on hedgehog exposures, which included the following queries:





- Where hedgehog exposure occurred (i.e. home, relative/friend residence, pet store)
- Where and when hedgehogs were purchased
- Type of contact with the hedgehog (i.e. direct contact such as holding, kissing and feeding the hedgehog, or indirect contact, such as being in a household where hedgehogs are kept, or contact with the hedgehog environment and/or enclosure)
- Type of food the hedgehog consumed
- If the hedgehog appeared sick
- Cleaning practices (i.e. bathing the hedgehog and cleaning supplies)
- Other animal husbandry practices implemented (i.e. disinfection, hand washing and isolation of sick or newly obtained hedgehogs)

Interviews with identified hedgehog suppliers (which included pet stores, wholesalers and breeders) collected details on facility husbandry practices, herd health history, *Salmonella* precaution protocols and client education practices. Data collection also allowed to determine if a common supplier was associated with outbreak cases.

## Epidemiologic and statistical analyses

The proportions of sick people who reported any animal contact and contact with hedgehogs specifically were compared with corresponding reference values from the Foodbook study, a population-based study of Canadians' exposure to food, animals and water over a seven-day period (31). Exact probability testing was used to measure the statistical significance of the proportion of cases who reported animal contact compared to Foodbook reference values.

## Laboratory investigation

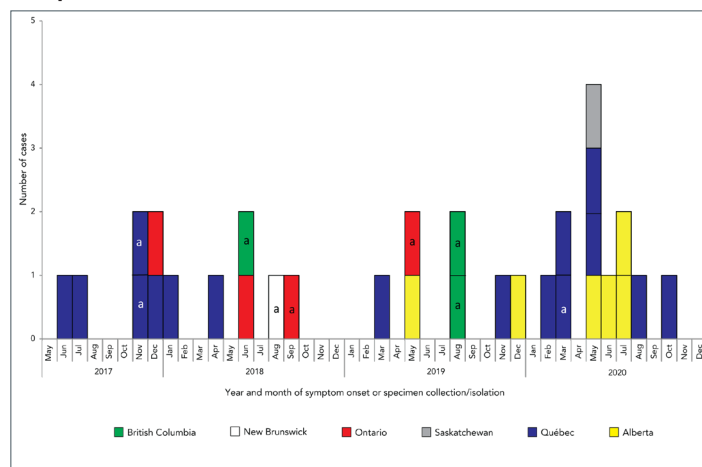
Environmental and hedgehog fecal samples were collected from cases' homes and hedgehog suppliers' premises. Samples were submitted to provincial public health laboratories for WGS, which was performed according to the current PNC protocol. Briefly, genomic DNA was extracted using the DNeasy blood and tissue kit (Qiagen) or Epicentre MasterPure Complete DNA and RNA Purification Kit (Mandel). Libraries were prepared using the Nextera XT library prep kit (Illumina) and sequenced using the Illumina MiSeq platform (Illumina), using either V2 or V3 chemistry to achieve an average genome coverage of greater than or equal to 40x. The analysis of WGS data was done using the *Salmonella* wgMLST schema within the BioNumerics v7.6 (BioMerieux) platform. A dendrogram was constructed with BioNumerics v7.6 using a categorical (values) similarity coefficient and an unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm. The UPGMA is a hierarchical clustering method used to generate a dendrogram to visualize isolate relatedness; it allows for analyses to be rapidly updated as isolates are added during the course of an investigation.

## Results

### Epidemiological investigation

A total of 31 cases were identified in six provinces (British Columbia [BC]=3, Alberta [AB]=6, Saskatchewan [SK]=1, Ontario [ON]=4, Québec [QC]=16 and New Brunswick [NB]=1). Symptom onset or specimen collection or isolation dates ranged from June 1, 2017, to October 15, 2020 (Figure 1).

**Figure 1: Number of cases with the outbreak strain of *Salmonella* Typhimurium by province and illness onset or specimen collection date (n=31)**



Note: The a indicates isolate for which only specimen collection or isolation date was available

Cases ranged in age from four months to 79 years with a median of 20 years. Thirty-two percent (n=10/31) were children aged 10 years of age or younger; of these, seven (70%) were two years of age or younger. Fifty-two percent of cases were female. Four of eight (50%) cases with available information were hospitalized and no deaths were reported (Table 1).

**Table 1: Characteristics of persons infected with the outbreak strain of *Salmonella* Typhimurium (n=31)**

Characteristics	Number of cases	Total cases	%
<b>Age</b>			
2 years of age or younger	7	31	23
3–10 years	3	31	10
11–20 years	6	31	19
21–50 years	9	31	29
Older than 50 years	6	31	19
<b>Sex</b>			
Female	16	31	52
<b>Outcome</b>			
Hospitalizations	4	8	50
Death	0	31	0



Animal exposure information was available for 26 of 31 (84%) cases. The proportion of cases who reported animal or pocket pet contact was significantly higher ( $p < 0.001$ ) than the general population when compared using the Foodbook study (Table 2). Nineteen cases reported exposure to pocket pets, all of which were hedgehogs. Fifteen reported direct contact with a hedgehog and three reported indirect contact (Table 3). Most cases reported bathing their hedgehog and cleaning their supplies in a sink or tub also used for other purposes, and three cases reported allowing their hedgehog to roam free in the home; all potential routes of indirect transmission. No commonalities were observed among hedgehog diets.

**Table 2: Summary of animal contact and pocket pet exposures among persons infected with the outbreak strain of *Salmonella* Typhimurium (n=26), compared with population-based reference values<sup>a</sup>**

Exposure	Number of cases	% of cases	Reference value (%) (Canada)	p-value
Animal contact	26/26	100	63.4	<0.001
Pocket pets <sup>b</sup>	19/26	73	3.4	<0.001

<sup>a</sup> Murray R et al. Canadian consumer food safety practices and knowledge: Foodbook study. J Food Protect. 2017;80(10):1711-8

<sup>b</sup> Pocket pets include mice, rats, gerbils, hamsters, guinea pigs, ferrets and hedgehogs

**Table 3: Description of hedgehog-related exposures and interactions among persons infected with the outbreak strain of *Salmonella* Typhimurium**

Exposures or interactions	Number of cases n/N <sup>a</sup>	% of cases
<b>Type of hedgehog exposure</b>		
Direct contact	15/18	83
Touching and/or holding	10/15	67
Indirect contact	3/18	17
<b>History of hedgehog illness</b>		
Ill prior to case symptom onset	3/16	19
<b>Length of hedgehog ownership prior to case illness</b>		
One month or less	7/15	47
Two to three months	6/15	40
Approximately one year	2/15	13
<b>Hedgehog hygiene practices</b>		
Allowed to roam free around the house	3/16	19
Bathed and cleaned supplies in case's home tub or sink in the kitchen, bathroom, or laundry	11/14	79
Bathed and cleaned supplies in case's home in sink or bin designated for this purpose	3/14	21
<b>Hedgehog diet<sup>b</sup></b>		
Kitten/cat kibble	19/26	73
Mealworms	26/26	100
Fruits/vegetables	19/26	73

<sup>a</sup> Denominators differ as a result of missing data

<sup>b</sup> Categories not mutually exclusive

## Traceback investigation

Hedgehog suppliers were identified for 21/23 (91%) cases: 4 pet stores; 5 wholesalers; and 12 breeders (Figure 2). Although no single source was identified, there were common suppliers reported and one direct link identified between the Canadian and US outbreak investigations, as one breeder located in the US was reported in both investigations (Figure 2). Six suppliers were interviewed and all reported being aware that hedgehogs can carry *Salmonella* and take precautions to prevent zoonotic transmission.

## Laboratory investigation

Environmental samples from hedgehog habitats and fecal samples were collected from three cases' homes, one wholesaler and two breeders. One hedgehog stool sample collected from a case's home in QC tested positive and was genetically related to the outbreak strain based on WGS. All other samples were negative for *Salmonella*. An additional hedgehog stool isolate genetically related to the outbreak by WGS was identified from a sample collected in July 2020 during routine quarantine exams at a QC zoo; however, the supplier of this hedgehog was a breeder in QC with no identified connection to the hedgehog suppliers reported by cases (personal communication Ministère des Forêts, de la Faune et des Parcs).

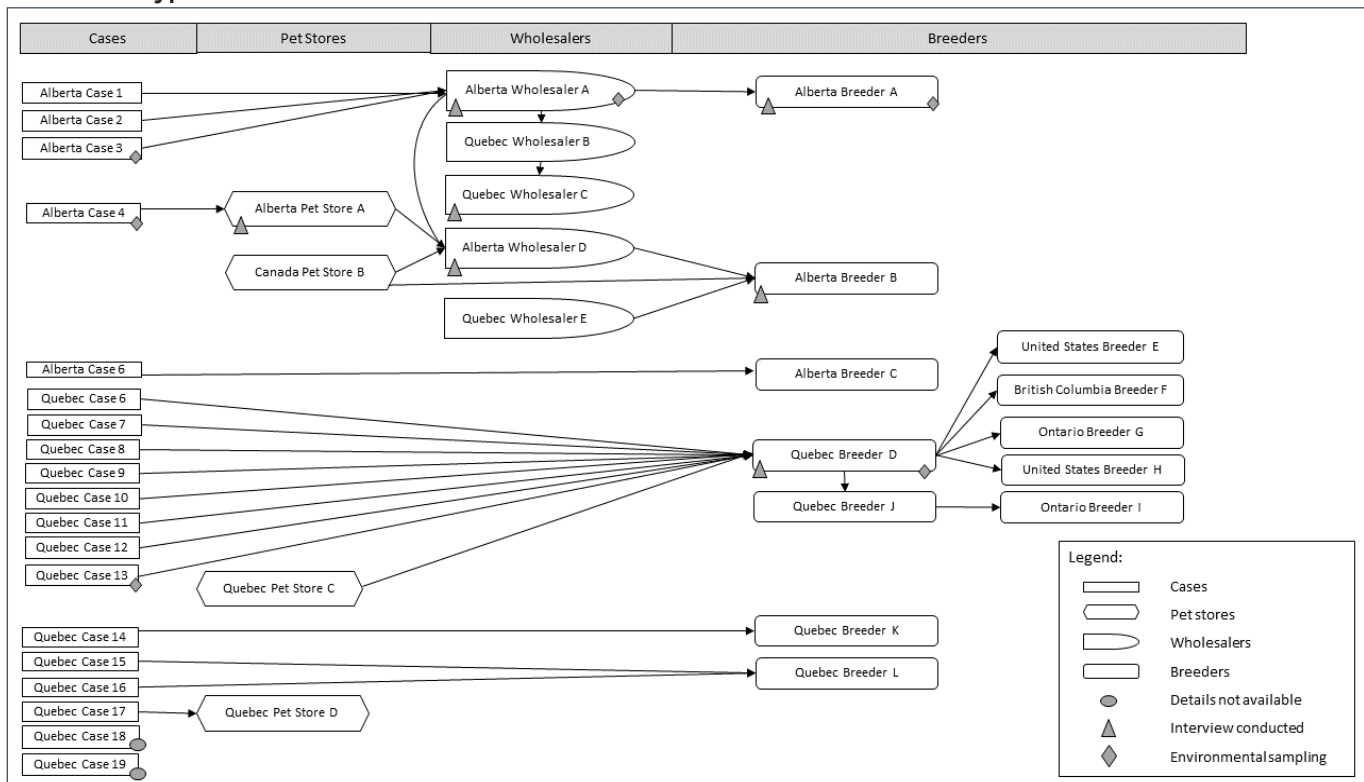
The 33 isolates grouped together with 0–46 wgMLST allele differences, and were genetically related to a concurrent US investigation associated with hedgehogs. In the US investigation, isolates were grouped into three clades based on their genetic profiles; Canadian isolates were genetically related to all three clades from the US (Figure 3) (26,27). Notably, nine isolates from QC (including one animal) grouped together in clade 1 and were linked to a specific breeder. A pairwise comparison between the isolate of QC case 13 and their hedgehog's isolate showed they were within three wgMLST allele differences of each other. Four AB isolates were also in clade 1, and grouped more tightly with isolates from SK, NB and ON than the QC isolates. Nine isolates from QC (including one animal), along with isolates from ON and BC, were in clade 2, and two isolates from AB were in clade 3.

## Public health response and interventions

A Public Health Notice was issued by PHAC on November 6, 2020, to notify the public about the outbreak and share prevention tips on how to safely interact with pet hedgehogs (7,24). Teleconferences were held by PHAC and CDC with Canadian and US hedgehog industry members to notify them about the outbreak and provide key prevention principles to help reduce the risk of disease transmission from hedgehogs to humans (13,14).

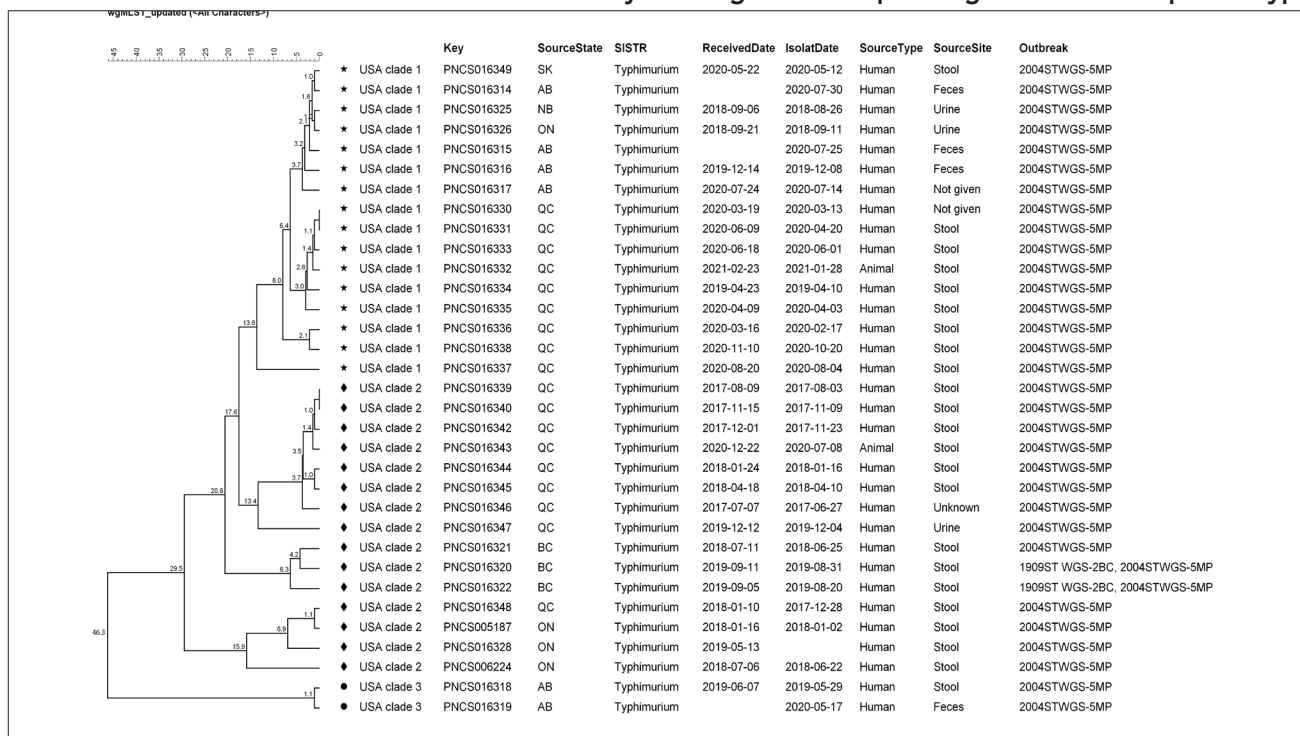


**Figure 2: Traceback network diagram of hedgehogs associated with sick persons infected with the outbreak strain of *Salmonella* Typhimurium<sup>a</sup>**



<sup>a</sup> All cases with hedgehog exposures are shown in rectangles. All pet stores (depicted by hexagons) and wholesalers (depicted by half circles) in this diagram were located in Alberta and Québec, Canada. The breeders (depicted by rounded rectangles) were located in Alberta, Québec, British Columbia and Ontario, Canada and in the United States. Filled circles depict cases where further hedgehog information was not available. The triangles depict the location of hedgehog suppliers that were interviewed, and the stars depict the locations where environmental sampling was performed. Arrows illustrate the reported links by cases or suppliers

**Figure 3: Relatedness of outbreak-associated isolates by whole genome sequencing multi-locus sequence typing<sup>a</sup>**



<sup>a</sup> Unweighted pair group method with arithmetic mean (UPGMA) dendrogram of whole genome multilocus sequence typing (wgMLST) results for human and animal isolates included in the investigation, generated using BioNumerics v7.6 (Applied Maths)



## Discussion

This is the second *Salmonella* outbreak linked to pet hedgehogs in Canada, and the first caused by *S. Typhimurium* (18). The investigation identified 31 cases in six provinces, from June 2017 to October 2020. With 73% of cases reporting exposure to hedgehogs, the epidemiologic information provided strong evidence to the source of the outbreak, further strengthened by laboratory and trace back investigations. The investigation revealed a large, interconnected network of hedgehog suppliers, with some cases' hedgehogs linked to common suppliers, but no single source of the infections. Results of this outbreak investigation emphasize the risk of *Salmonella* transmission from pet hedgehogs to humans, as previously described (10,18).

As was the case in this outbreak, children are often disproportionately affected in pet-related outbreaks (26,32–37). Young children have higher risk of developing more severe salmonellosis, are more likely to get tested, and often more likely to be exposed through both increased contact with pets and less vigilant hand washing (5,34,35,38–41). Although most cases reported direct contact, only indirect contact was reported by 17% of cases, including two one-year-old children. This speaks to the difficulty in preventing cross-contamination in homes. It is not recommended to keep hedgehogs in households with children younger than or five years old and strict hygiene practices should be adopted around these pets (7).

The WGS analysis, epidemiologic and trace back evidence helped inform the case definition and characterize distribution of the outbreak strain of *S. Typhimurium*. The search for highly related cases in previous years was limited because WGS analysis of *Salmonella* isolates began in 2017. Nonetheless, cases from 2017 to 2019 were identified, indicating the presence of this strain in Canada since at least 2017. This strain also caused reoccurring outbreaks of human infections linked to contact with pet hedgehogs in the US as far back as 2011–2013, suggesting its persistence in the hedgehog industry (6,26,27). One direct link to the concurrent US outbreak was identified during the trace back investigation. A hedgehog breeder in the US was connected to QC "Breeder D", identified as a common source by eight cases, including QC case 13 whose hedgehog's isolate was genetically related to the outbreak. This same US breeder was also linked to other US suppliers identified as sources of hedgehogs of cases in the US investigation (26).

The expansion of the case definition to include older samples from 2017 to 2019 helped to demonstrate the ongoing persistence of this strain in hedgehogs in Canada. The older samples may also reflect a baseline of sporadic infections for this *S. Typhimurium* strain, of 6–7 cases per year, with 0–2 cases per month and 0–5 months between cases. The original outbreak case definition, which includes cases from December 1, 2019, or after, would therefore be more accurate, since between then and October 2020 the number and frequency of cases exceeded the baseline incidence. Cases matching the outbreak strain

then decreased to expected monthly baseline incidence, and the outbreak was declared over on December 18, 2020. Since this strain is an ongoing issue in hedgehogs in the US (26), and based on epidemiologic information gathered through this outbreak, it can be confirmed that sporadic cases occurred and might continue to occur in Canada with an occasional increase in incidence, potentially signalling an outbreak event. The use of WGS will be useful to distinguish between outbreak-associated and sporadic illnesses. In this outbreak, the US reporting on their outbreak and associated early signal of hedgehog contact also resulted in a strengthened rationale for additional epidemiological follow-up on genetically related Canadian cases and highlighted a potential source for the illnesses identified.

Isolates from cases whose hedgehogs were traced back to a common source were found to be closely related genetically. For example, isolates from the eight cases and one hedgehog associated with QC "Breeder D" differed by 16 wgMLST alleles or fewer, and the four isolates from cases associated with AB "Wholesaler A" were within four allele differences, compared with 46 alleles difference for all outbreak-associated isolates. Other outbreak-associated isolates were closely related genetically too but could not be traced to a common hedgehog source, with cases' residences spread geographically across Canada and illness onset dates spanning a wide temporal range. The proportion of cases by province also varied over time: cases from QC (52% of all cases) were observed throughout 2017–2020 while cases from AB were observed in 2019–2020, suggesting a more recent introduction of the outbreak strain in AB. These findings might be explained by the interconnected and dynamic hedgehog distribution network, but would require further investigation to elucidate.

## Limitations

Limitations to the investigation include 1) the inability to re-interview all cases with the focused questionnaire as some were retrospectively linked through WGS and 2) the absence of hedgehog exposure reported by some cases. For the latter, it is possible these cases had unknown indirect exposure to hedgehogs. The inability to interview more hedgehog suppliers also limited full understanding of the interconnectedness in the supplier network which could have provided more details of potential transmission pathways.

## Conclusion

This investigation benefited from strong collaboration between Canadian partners in public and animal health at the provincial and federal level, the pet industry including Pet Industry Joint Advisory Council of Canada and the CDC. Communication between these groups and to the public aimed to increase awareness and provided education regarding the risk of *Salmonella* infection from hedgehogs and proper hygienic practices, with the goal of preventing further disease transmission.





Although the carriage rates and transmission dynamics in the pet hedgehog industry are not well characterized, extrapolation from rodent models indicates that *Salmonella* carriage may be persistent and heterogeneous, with the majority of transmission occurring through heavily infected super spreaders (42). During this investigation, members of the hedgehog industry expressed knowledge of *Salmonella* transmission prevention, yet one breeder reported treating all their hedgehogs with antibiotics upon hearing of the outbreak. Antibiotic-induced alterations in the intestinal microbiota are thought to increase the likelihood of colonization and shedding; antibiotic treatment is therefore contraindicated in non-clinical cases (14,42,43). Collaboration with the pet industry is needed to better understand transmission dynamics and target interventions to reduce levels of infection and transmission rates. The industry and its clients should be educated on the harms of indiscriminate antibiotic use, which potentially leads to more transmission, and selection for antibiotic resistant strains.

The high proportion of young children among cases in this outbreak emphasizes the importance of providing potential small pet owners the educational materials necessary to make informed decisions about pet choices and to implement safety precautions. Anecdotal reports suggest an increase in pet ownership during the coronavirus disease 2019 pandemic (44,45), which may include small pets like hedgehogs. While recognizing the benefits of having a pet, this outbreak of *S. Typhimurium* is a timely reminder of the importance of *Salmonella* awareness and education among suppliers and owners of small pets, to prevent disease transmission.

## Authors' statement

KFG — Conceptualization, analysis and interpretation of data, drafting the paper

LD — Analysis and interpretation of data, drafting the paper, visualization

JT — Conceptualization, interpretation of data, drafting and reviewing the paper

RJ — Investigation, reviewing the paper

OVO — Investigation, reviewing the paper

AK — Investigation, methodology, reviewing the paper

CM — Investigation, methodology, reviewing the paper

CN — Investigation, reviewing the paper

CG — Investigation, reviewing the paper

VM — Investigation, reviewing the paper

RS — Investigation, reviewing the paper

MW — Investigation, reviewing the paper

BA — Investigation, reviewing the paper

HS — Investigation, reviewing the paper

AML — Conceptualization, analysis and interpretation of data, drafting and reviewing the paper, supervision

## Competing interests

No conflicts of interests to declare.

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# Monkeypox brief, June 2022

**Source:** Public Health Agency of Canada. [Update on monkeypox in Canada, May 26, 2022](https://www.canada.ca/en/public-health/news/2022/05/update-on-monkeypox-in-canada.html). <https://www.canada.ca/en/public-health/news/2022/05/update-on-monkeypox-in-canada.html>

Monkeypox is a rare infectious disease caused by the monkeypox virus (genus *Orthopoxvirus*). Monkeypox virus is related to, but distinct from, the viruses that cause smallpox (variola virus) and cowpox. Human cases and outbreaks of monkeypox are regularly reported in central and western Africa and cases outside of the endemic geographic area are typically linked to travel. Monkeypox can cause serious illness, but human-to-human transmission is typically very limited, and the West African clade of the virus is associated with a relatively low case fatality (1%). On May 13, 2022, the World Health Organization (WHO) was notified about a cluster of laboratory-confirmed human cases of monkeypox in the United Kingdom that were identified as the West African clade, and clusters have been reported in several European countries. On May 19, the Public Health Agency of Canada (PHAC) reported the first two human cases of monkeypox in Canada and, as of June 1, 54 confirmed cases have been identified in Québec and Ontario. As of May 31, a cumulative total of 557 laboratory-confirmed cases have been reported globally from non-endemic countries, with the majority (n=310) in the United Kingdom and Europe. Most cases in Canada, and in other countries, are not associated with travel to an endemic region and have been identified through primary care and sexual health services.

On May 27, PHAC convened a meeting of provincial partners from affected jurisdictions, together with external experts, to assess the emerging outbreak and develop consensus on public health management and guidance. An expanded expert panel gathered on June 1 to refine public health guidance and to identify knowledge gaps and opportunities for implementation and clinical research in the Canadian outbreak. Input from the June 1 meeting has informed Canadian contributions to the WHO R&D Blueprint sessions, which were held on June 2–3. The expert panel will re-convene on June 7 to assess priorities in applying the global research agenda to Canadian knowledge needs.

The McMaster Health Forum recently completed the first edition of a living evidence profile looking at the best available evidence, as of May 27, 2022, related to the monkeypox outbreak. Evidence and experiences were identified from 11 countries (Australia, Belgium, France, Germany, Italy, Netherlands, Portugal, Spain, Sweden, the United Kingdom and the United States) and from all Canadian provinces and territories. This living evidence profile will be updated every two weeks. In the May 27, 2022 profile, 22 highly relevant evidence documents were found: two systematic reviews; four non-systematic reviews; and 16 single studies. Findings were presented according to the organizing framework: biology; epidemiology (including transmission); prevention and control; clinical presentation; diagnosis; prognosis; and treatment. Full details are available in the living evidence profile.

## For more information:

Please [click here](#) to access the McMaster Health Forum Living Evidence Profile #6.1: What is the best-available evidence related to the monkeypox outbreak?

Please [click here](#) to access the Monkeypox Outbreak Update.

Please [click here](#) to access the Interim guidance on infection prevention and control for suspect, probable or confirmed monkeypox within Healthcare settings, May 27, 2022.





# CCDR

## CANADA COMMUNICABLE DISEASE REPORT

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