1 2	Communicating with Northerners on the absence of SARS-CoV-2 in migratory snow geese
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19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36	* Corresponding author: Pierre Legagneux; pierre.legagneux@bio.ulaval.ca
37 38 39	ABSTRACT The COVID-19 pandemic has raised many concerns among the population including Indigenous communities that wanted to get firm answers about the potential virus transmission risks from wild

birds. Snow geese contribute significantly to food security in an Indigenous context, which is already precarious in several communities. The risk for geese hunters is very unlikely since coronaviruses found in birds are from different genera than that of SARS-CoV-2, which is the etiologic agent responsible for COVID-19. Little is currently known on the host tropism range of SARS-CoV-2. To address the concerns raised by Northern communities, we captured 500 snow geese in May 2020 at their stopover along the St Lawrence estuary. We took oropharyngeal and cloacal samples before releasing the birds. All samples were processed within one week and were found to be PCR-negative for SARS-CoV-2, allowing us to communicate rapidly with Northern communities. The current pandemic has shown us the importance of understanding animals as potential viral reservoirs, and a better understanding of these viruses will better prepare us for future spillover events. This project also shows that research can be quickly and efficiently mobilized to respond to concerns from Indigenous communities.

<u>RÉSUMÉ</u>

La pandémie de COVID-19 a soulevé de nombreuses inquiétudes incluant chez les communautés autochtones qui souhaitaient obtenir des réponses claires sur les risques potentiels d'une transmission du virus par les oiseaux sauvages. L'oie des neiges contribue de façon importante à la sécurité alimentaire en contexte autochtone, qui est déjà précaire dans plusieurs communautés. Le risque de transmission pour les chasseurs et consommateurs d'oies est très faible car les coronavirus retrouvés chez les oiseaux et le coronavirus SARS-CoV-2, responsable de la COVID-19 appartiennent à des familles différentes. Toutefois, nos connaissances sur le SARS-COV2 restent limitées notamment sur la diversité des hôtes potentiels. Pour répondre aux inquiétudes des communautés nordiques, nous avons capturés 500 oies sauvages durant leur halte migratoire le long de l'estuaire du St Laurent en mai 2020. Des écouvillons oropharyngiaux et cloacaux ont été réalisés sur l'ensemble des oiseaux avant d'être bagués et relâchés. Tous les échantillons oropharyngiaux ont été traités en une semaine et ont montré l'absence de de SARS-CoV-2 permettant de communiquer rapidement ces résultats auprès des communautés nordiques. Cette pandémie montre l'importance de mieux comprendre le rôle joué par les animaux comme réservoirs viraux afin de se préparer aux futures contagions. Ce projet est également un exemple de la capacité du milieu de la recherche à répondre rapidement à des enjeux importants aux communautés autochtones.

KEYWORDS (2-6)

Greater Snow Geese; SARS-CoV-2; Indigenous food security; Country food; Coronaviruses; animal reservoirs

INTRODUCTION

The transmission of disease-causing agents to humans can occur via multiple pathways. Wild animals and vectors carrying pathogens are two of the most common pathways viruses can spillover to humans (Jones et al. 2008). 75% of all emerging pathogens over the past decade are from a zoonotic origin (Organization 2006). Interestingly, at least half of these pathogens have the ability to infect more than one animal species and pose important risks of emerging and reemerging diseases either in humans, domestic and wildlife animals (Haydon et al. 2002).

Zoonoses spread from animal reservoirs to humans or other animal species. Certainly, migratory birds

play a substantial role in introducing viruses to different regions of the world (Haydon et al. 2002). For example, migratory birds are known to harbor avian influenza viruses and can transmit such viruses through their migratory routes, as evidenced recently with H5N8 (Lycett et al. 2016). Indeed, birds interacting at the same resting areas can transmit the virus between each other via oral or fecal material (Bui et al. 2017; Shi et al. 2018), with a possibility for recombination to generate potentially more dangerous pathogens depending on the identity of the agent (Venkatesh et al. 2018).

Coronaviruses have been identified in multiple wildlife and domestic animal species (Van der Hoek et al. 2004). Recent studies confirmed the existence of numerous coronaviruses in various bird species, mainly waterfowl such as ducks, geese and shorebirds (Chamings et al. 2018; Paim et al. 2019). Phylogenetic evidence has shown that bats and rodents serve as reservoirs of most α -and β -CoVs, while birds are the main reservoir of γ - and δ -CoV (Su et al. 2016; Ye et al. 2020).

Recently, the porcine coronavirus (δ-CoV) has been found in North American waterfowl species such as snow geese (Paim et al. 2019) where 5.8% of individuals have been reported to be carriers. Snow geese are closely associated with human activities and infrastructure: they feed on agricultural fields and often rely on city drinking water reservoirs or wastewater treatment basins. Greater snow geese can be found all over Eastern North America: they spend the winter along the coast from North Carolina to New Jersey, stopover in Québec during both spring and fall and breed in the high Arctic (Lefebvre et al. 2017). In 2020, the greater snow goose population transited through the state of New York, where 10% of all the global positive cases were identified at the time; https://covidtracking.com). This occurred during the snow goose migration towards Eastern Canada from mid-March to early May 2020.

The COVID-19 pandemic is a major concern among both Indigenous and non-Indigenous groups. This was especially true in the Canadian North including the Inuit Nunangat (homeland of the Inuit), where socioeconomic factors, overcrowding, prevalent food insecurity and limited capacity of health services contribute to the vulnerability of communities to infectious diseases and respiratory illnesses. The response to the COVID-19 pandemic by Northern authorities in Canada (Council 2020) consisted in drastically restricting travel within and outside each territory. In Nunavut, no cases of COVID-19 were detected during spring 2020 and at that time, the arrival of migratory birds was raising concerns. The snow goose is indeed a particularly prized game both in Quebec and several Indigenous communities in the North (Cree, Innu, Naskapi, Inuit), where it is also an important part of the traditional country food diet (Leonard J.

S. Tsuji and Liberda 2020), contributing to food security. If a positive case was detected in geese migrating North, this would pose potential health risks, in particular for hunters, as well as for those who handle and consume these geese, especially raw.

Although the types of CoVs are different (β vs. δ -CoV) and the possibility of transmission of SARS-CoV-2, the virus involved in the COVID-19 pandemic, to birds is very unlikely (Chamings et al. 2018; Paim et al. 2019; Su et al. 2016; Ye et al. 2020), the great resistance of the virus outside his primarily host (Van Doremalen et al. 2020) was raising concerns about possible transportation of the virus through migratory birds through Indigenous communities. The response of health agencies at that time was that the risk of transmission was very low but could not be ruled as null given that the role of wild animals in the transmission of the SARS-CoV-2 virus was and still remains unclear (Malik et al. 2020). In the current context of food security in northern communities and to respond to concerns raised by Indigenous groups in Canada, it was important to rapidly provide clear answers on the potential presence of the SARS-CoV-2 virus in the greater snow goose population.

MATERIAL AND METHODS

157 <u>Field procedures</u>

Geese were captured in the spring during the migratory staging period at Île-aux-Oies (IAO; 47°08N 70°29W), a small agricultural island in the Saint Lawrence River estuary, 60 km northeast

of Québec City (Canada). Baited canon-nets were placed in the fields to capture geese between May 03-19, 2020 (Morez et al. 2000). Adults were aged based on plumage examination and sexed according to cloacal examination. All birds were marked with metal bands. Birds were weighed to the nearest gram with an electronic balance and tarsus length was measured to the nearest 0.1mm using calipers.

For each captured individual, we collected oral and fecal swabs. The swabs were placed in sterile falcons containing 1mL of the Universal Transport Media (UTM) and/or in 1mL of Dulbecco's Modified Eagle Medium (DMEM) with 1% of Penicillin/Streptomycin. Samples were frozen at -20°C after all birds from a catch were processed. Geese were released together either immediately after handling or the following morning when manipulations ended after sunset to avoid bird disorientation if released during the night.

Personal protective equipment (PPE) including gloves, N-95 masks, scrubs and gowns was used when handling birds to avoid any cross-contamination between human and geese. All fieldworkers were trained on how to properly wear PPE and were tested prior, during and after the field season for SARS-CoV-2. Oral and oropharyngeal swabs taken from the 7 field crew members were analyzed at the Infectious Disease Research Center (IDRC) laboratory in the Centre Hospitalier de l'Université Laval (CHUL) using the same methodology that was used for goose samples (see below). Results (all negative) for the fieldworkers were obtained within 36h.

Laboratory procedures

Samples from geese were transferred to the IDRC-CHUL at the end of the field season and stored in a -80°C freezer until processing. Due to the high number of samples and speed-up the analyses, pools of 4 samples per tube were prepared to proceed with the extraction. Viral RNA was extracted using the QiAmp Viral RNA Mini Kit (QIAGEN), following manufacturer instructions. Samples were handled under a laminar flow hood during the inactivation period of each sample. A one-step real-time quantitative polymerase chain reaction (RT-qPCR) program was used to perform a rapid screening using a high speed and high-performance magnetic induction cycler (MIC) machine. Specific primer probes were used to target the ORF1b gene that is used as a reference gene because it is a conserved gene for coronaviruses (Deming et al. 2007). The selected primers targeted a gene only present in the β-CoV genera. To perform this rapid test, 0,5uL of primer mix forward (20uM) CoV ORF1b For (5' TGG GGY TTT ACR GGT AAC CT 3') and reverse (20uM) CoV ORF1b Rev (5' AAC RCG CTT AAC AAA GCA CTC 3') were added to a PCR tube with 0,5uL of ORF1b probe (5' TAG TTG TGA TGC WAT CAT GAC TAG 3'), 5uL of TagMan Fast Virus 1-step Master Mix and 10 uL of water. In each tube, 4uL of the previously extracted RNA was added (Biosystems; Kilic et al. 2020). PCR amplification profiles were presented with a reverse transcription at 50°C 5 mins followed by an inactivation and initial denaturation at 95°C 20s, denaturation and annealing/extension are repeated at 40 cycles of (95°C 5s, 60°C 30s) respectively (Biosystems).

RESULTS

A total of 500 birds, 391 adults (200 females and 191 males) and 109 juveniles (43 females and 66 males) were captured, marked, measured, swabbed and released (Fig. 1). Juveniles are 9 months old, non-sexually mature birds easily identified through plumage examination. Since the results on the presence/absence of SARS-CoV-2 had to be available quickly to inform Northern communities, and because coronaviruses are preferentially detected in the upper respiratory tract, oropharyngeal swabs were prioritized in the analyses. Screening of all 500 oropharyngeal swab samples from the snow geese collected on the field yielded only negative results. Theoretically, assuming a 1% prevalence and a 1% margin error, at least 380 samples would be needed to have 95% confidence to detect the presence of the virus. Our study with 500 sampled individuals thus fulfills this requirement.

DISCUSSION

Our study brought together scientists in biology, virology, and public health to rapidly address concerns from Indigenous communities that largely depend on wildlife (including migratory birds)

for cultural activities and to sustain food security. Traditional foods are indeed essential to health, nutrition, social cohesion, identity and cultural continuity in Indigenous ((ITK) and (ICC) 2012; King and Furgal 2014; Kuhnlein 2017) communities. Furthermore, the price of market foods is much greater in isolated and northern communities than in southern Canada (St-Germain et al. 2019). Snow geese are a prized game in southern Quebec, and particularly among Cree, Innu, Naskapi and Inuit communities in Northern Quebec, Labrador and Nunavut in late spring and summer. Moreover, snow geese contribute in a major way to food sovereignty and food security in Indigenous settings, which is precarious in many communities (Academies 2014). The COVID-19 pandemic is adding another degree of complexity in an ongoing changing Arctic where disease transmission is also affected by global warming through the increase of vector-borne transmission and zoonosis (Waits et al. 2018).

outbreaks in Indigenous communities.

It was thus crucial for us to communicate the objectives of the present project to Public health organizations rapidly prior to sampling geese in Southern Quebec and communicate our negative results within two weeks after sample collection in May 2020 (See the infographic in 3 languages in Fig. 2). Thanks to this great collaborative effort with Southern and Northern partners, the information was relayed in a timely manner to several communities by email, press release and social media (university and community Facebook pages). Thus, this intersectoral surveillance project rapidly set in place to address Indigenous concerns contributed to reassure community members, to ensure they could continue safely hunting and eating geese in the coming months and that travel restrictions and social distancing remained the best approaches to prevent COVID-19

Viral respiratory infections typically spread by direct contact, such as touching an infected patient, surfaces or fomites, in which the virus can remain stable for days (Van Doremalen et al. 2020). Therefore, the main precautions for preventing SARS-CoV-2 infection include frequent handwashing and maintaining social distance of at least 1 meter in length. However, there is evidence that SARS-CoV-2 can also be transported by virus-containing particles in the air. In infected Syrian golden hamsters, the presence of the virus was detected in the nasal mucosa, bronchioles and other areas of the lung, with nasal washes being positive for SARS-CoV-2 for 14 day after inoculation. The virus was found to spread efficiently from inoculated to naive hamsters by both direct contact and aerosols (Sia et al. 2020). In addition, ferrets that were infected with SARS-CoV-2 shed live viruses in nasal washes, saliva, urine, and feces for 8 days postinfection. SARS-CoV-2 could be detected by 2 days post-contact in naive direct contact animals, whereas a subset of naive indirect contact animals was also positive for SARS-CoV-2, strongly implying airborne transmission for this virus (Kim et al. 2020). As SARS-CoV-2 is primarily a respiratory disease, oronasal sampling is the most efficient method for detection of this virus from our samples in this study. Analysis of samples from cloacal swabs, blood and feathers will provide a more complete picture as to the spectrum of pathogens (aside from SARS-CoV-2) that snow geese carry, and these will be investigated in-depth in a subsequent study.

Coronaviruses circulate in animals, and some cause zoonotic infections (SARS-CoV, MERS-CoV) (Andersen et al.; Li et al. 2020). The novel SARS-CoV-2 virus was unknown prior to late December 2019, and likely evolved in an animal reservoir from related SARS-CoV genomes (Li et al. 2020). The COVID-19 pandemic has underscored the importance of better understanding the

transmission of viruses by animals, and more broadly, of paying closer attention to evolution of viruses.

Finally, collecting these samples will give us the opportunity to organize further research such as metagenomics, 16S amplicon-based sequencing and many more. These assays can help characterize targeted or novel pathogens, study how they can possibly protect themselves against future infections. But more importantly, processing the acquired samples will contribute to increase surveillance efforts of viruses that may potentially one day cause zoonoses in humans.

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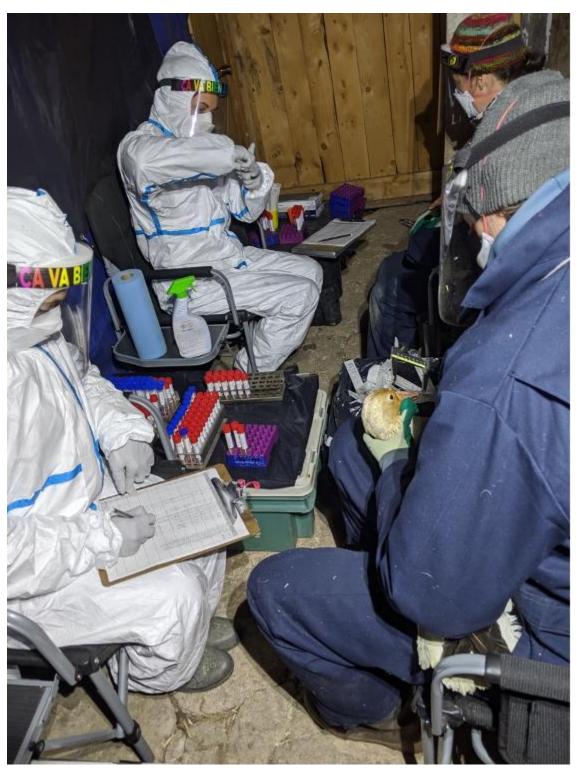


FIGURE 1 Greater snow geese being sampled for the presence of SARS-CoV-2 in spring 2020 in Southern Québec, Canada. (photo credit: Pierre Legagneux)

472 FIGURE 2 Infographics delivered to indigenous communities and public health organizations

473 (credit: Ali Langweider)

