Synthesising and Assessing the Public Health Risks of SARS-CoV-2 in Animals

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ABSTRACT

Background: SARS-CoV-2 is zoonotic in origin and has retained the capacity to infect animals. If susceptible animal species can readily transmit the virus to other animals or humans, this could extend the pandemic. To assess animal host susceptibility and the potential outcomes of animal-human interactions, I had the following objectives: 1) identify which animal species are susceptible to SARS-CoV-2, 2) determine the risks of SARS-CoV-2 exposure to humans from infected wildlife in North America; and 3) describe how the risks of SARS-CoV-2 in wildlife could be effectively communicated.

Methods: For objective 1), a scoping review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews, which identified animal families considered highly susceptible to SARS-CoV-2. For objective 2, a rapid qualitative risk assessment using the World Organization for Animal Health framework was applied to assess risks of human exposure to SARS-CoV-2 from selected taxonomic families of wildlife in North America. For objective 3, positive and negative instances of risk communication were identified from personal experiences, and suggestions for communicating risks were provided.

Results: The scoping review identified 97 source manuscripts investigating 649 animal species from eight different classes. Four different methods were used to evaluate susceptibility: *in silico, in vitro, in vivo*, and epidemiological analyses. From the identified sources, animal species varied in their evaluated susceptibilities. The risk assessment identified four families that pose a risk to humans: cervids, cricetid rodents, felids, and mustelids. While the likelihood of a human becoming exposed to a wild animal currently shedding SARS-CoV-2 was minimal, the consequences of such an event could be severe. Risk communication can be improved by understanding the characteristics of the target audience and the context in which they will perceive the information.

Conclusions: This thesis identified animal families that posed higher risk to humans, and critically evaluated different methods of determining animal susceptibility, emphasizing the importance of epidemiological and *in vivo* studies. Finally, this thesis emphasized the need for careful and effective communication to lessen confusion and misinformation

surrounding SARS-CoV-2, remaining uncertainties, and the need for additional research regarding SARS-CoV-2 in animals.

ACKNOWLEDGEMENTS

With humility and gratitude, I extend a huge thank you to my supervisor Dr. Emily Jenkins for her consistent and unwavering guidance. I am deeply indebted to members of my committee, Dr. Lori Bradford and Dr. Tasha Epp, who provided timely and effective feedback in everything that they reviewed. Lastly, I would like to thank my family and friends who provided unending support and encouragement.

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LIST OF ABBREVIATIONS

ACE2	Angiotensin-Converting Enzyme 2
CBPR	Community based participatory research
CoV	Coronavirus
hACE2	Human ACE2
SARS-CoV-1	Severe Acute Respiratory Syndrome
MERS	Middle Eastern Respiratory Syndrome
MPH	Master of Public Health
OIE	World Organization for Animal Health
ORFs	Open reading frames
PPE	Personal protective equipment
PRISMA-ScR	Preferred Reporting Items for Systematic Reviews and Meta-Analysis Extension for Scoping Reviews
RBD	Receptor Binding Domain
RH	Relative humidity
S	Spike
SES	Socio economic status
U.S.A	United States of America
WHO	World Health Organization
VoC	Variants of Concern

CHAPTER 1

1.1 Preamble

This is a SARS-CoV-2 thesis. It is so, not only in content, but also because it began and ended during the pandemic. Although there have been challenges with this thesis, I do recognize that other research projects have been substantially impacted due to the virus/pandemic. When I started my masters, (Master of Public Health (MPH)) I did not plan on completing a thesis as the MPH program is primarily course-based. There is, however, an option to enrol in the thesis stream when partially through the program. In the end, I elected to enrol in the thesis stream as I believed it would provide me with more valuable experience beyond the course-based option and would allow for the pursuit of a PhD if I so choose. When first discussing thesis project ideas and topics with my supervisor, Dr. Emily Jenkins, we focused on *Toxoplasma gondii* in country foods and ringed seal health, and, almost in passing, discussed how Inuit harvesters had expressed concern over this new virus, SARS-CoV-2, that had recently been making headlines. With these themes decided on, the thesis was named "Blending Traditional Knowledge and Western Sciences to Assess the Risks of Microbiological Hazards in Country Foods". There were three objectives for this project: 1) to determine the risks of SARS-CoV-2 from contact with wildlife 2) review literature of *Toxoplasma gondii* in foods of animal origin, and 3) survey seal hunters in Nunavik on their concerns about seal hunting and seal health.

Based on the title of my thesis, "Synthesising and Assessing the Public Health Risks of SARS-CoV-2 in Animals" and the abstract, it is obvious that the original project was altered. This alteration occurred due to the SARS-CoV-2 pandemic. While we attempted to continue with original objectives early on, modifications became necessary due to travel restrictions preventing community-based work in Canada's North. Nonetheless, we began to make progress, obtaining ethics certification to participate in a conference call with Inuit harvesters and to develop survey questions about parasites and the health of harvested seals. Plans to work with the community to develop and administer this survey changed again, however, when it became apparent that it remained unsafe to travel to Nunavik to attend a workshop in November 2020. Due to safety concerns with SARS-CoV-2, the workshop was

postponed, and then ultimately switched to a conference call (March 2021). Due to the delays and the growing importance of SARS-CoV-2, it became apparent that the ringed seal objective was not achievable at that point. Even though my involvement in the ringed seal project ceased, I was still able to participate in the conference call with Inuit elders, which was quite valuable. With the objectives on *Toxoplasma gondii* and ringed seal health coming to an end, I fully shifted to focus on SARS-CoV-2 in animals for my thesis research.

1.2 Introduction

For this introductory chapter, information on zoonoses, SARS-CoV-2, the Nunavik ringed seal project, risk communication, and misinformation and confusion will be discussed, followed by the research goals and objectives.

1.2.1 Zoonoses

Zoonoses, as defined by the World Health Organization (WHO), are diseases or infections that are naturally transmissible from vertebrate animals to humans or vice versa (1). Zoonotic pathogens account for just over 60% of infectious diseases in humans (2). Zoonotic diseases are caused by a wide range of pathogens including bacteria, viruses, parasites, fungi, prions, and pathogenic agents (3). Bacteria are the most common zoonotic agent and mammals are the most commonly infected class (3,4). Zoonotic diseases can be transmitted through various pathways such as close or physical contact (bites, scratches, or respiratory droplets), contact with fomites (dust), vectors (mosquitos), ingestion (fecal-oral or contaminated meat), or inhalation (aerosolized pathogens) (3,4).

Zoonotic diseases are classified as emerging or endemic. An emerging zoonotic disease is one that is novel or recently evolved, has increased in incidence in an area where it was previously endemic, or has spread to a new host or geographical region (2,3,5). An endemic zoonotic disease is one where the disease is regularly found or occurs in a specific area or region (2,3,5). Emerging zoonotic pathogens account for slightly over 60% of the total emerging pathogens that affect humans, with 75% of these emerging pathogens, originating from wild animals (3,6). Zoonotic diseases can arise due to a multitude of factors which are not mutually exclusive; these can broadly be classified into external or internal factors (4). External factors include habitat encroachment by agricultural, resource extraction, or

construction needs, and the consumption of bush meat or use of wet markets (2,4,5). Intrinsic factors include changes in pathogen transmission modes, evolutionary changes such as antimicrobial resistance, and behavioural changes in humans (2,4,5).

Zoonotic diseases represent a major health and economic burden, causing an estimated billion cases of illness and resulting in millions of deaths per year (2). The economic burden from zoonotic diseases can be profound; when bovine spongiform encephalitis (mad cow disease) was first detected in Britain and later Canada and the U.S.A, import bans and the culling of the cattle caused substantial economic hardship (3). Furthermore, the economic burden of Severe Acute Respiratory Syndrome (SARS-CoV-1) was over 60 billion dollars (US) globally (3). Compared to the economic crises by these diseases, SARS-CoV-2 has had a larger effect, causing a world economic decline of over 3%, and has caused recessions in various countries (7). The entire cost of human life and livelihoods of the pandemic is still unknown (7).

1.2.2 SARS-CoV-2 a snapshot

As an extensive SARS-CoV-2 literature review was conducted in Chapter 2, we felt that it was unnecessary to restate the findings, instead electing to give only a brief description.

SARS-CoV-2, the virus responsible for the disease COVID-19, has been a global threat for the last two years. After first emerging in December 2019, and first detected in early January 2020, the WHO labeled the virus a public health emergency of international concern on January 30th and then declared a pandemic on March 11th (8). The SARS-CoV-2 pandemic has demonstrated the importance of public health interventions, such as preventative measures like face masks, vaccinations, lockdowns, contact tracing, and the use of effective communication to help reduce the spread of misinformation.

SARS-CoV-2 is a Coronavirus (CoV) which are RNA viruses with a positive sense single stranded genome (9,10). CoVs are classified into one of four genera, *Alphacoronaviruses*, *Betacoronaviruses*, *Deltacoronaviruses*, and *Gammacoronaviruses* (11). CoVs from all four genera infect animal species; however, *Alphacoronaviruses* and *Betacoronaviruses* are only found in mammals, predominately bats (11). CoVs in the *Deltacoronaviruses* and *Gammacoronaviruses* genera are found in birds with the exception of a *Deltacoronavirus* and a *Gammacoronavirus* which were detected in pigs and cetaceans respectively (11,12). Certain

CoVs that infect animals have also shown the capacity to impact animal health; for example, Porcine Epidemic Diarrhea Virus in pigs can cause severe gastroenteritis and death, and in chickens, Infectious Bronchitis CoV affects the respiratory and urogenital tract (11,13).

There are seven CoVs which have the capacity to infect humans, HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV-1, Middle Eastern Respiratory Syndrome (MERS), and SARS-CoV-2 (3,14). Of these CoVs, SARS-CoV-1, MERS, and SARS-CoV-2 are the most severe, with the other four being endemic with symptoms similar to the common cold (14). Human CoVs are found in both the *Alphacoronavirus* and *Betacoronavirus* genera, with SARS-CoV-2 belonging to the *Betacoronavirus* genera (11,15). All human CoVs are zoonotic in origin, with HCoV-229E, HCoV-NL63, SARS-CoV-1 and MERS originating from bats, while HCoV-OC43 and HCoV-NL63 originated from rats, before being transmitted to people by an intermediate or bridging animal host (3,14,16). SARS-CoV-2 origin has been predicted to be a bat and pangolin CoV which underwent homologous recombination (16).

The host tropism for SARS-CoV-2 is dependent on its spike (S) protein, which binds to and facilitates entry into host cells; the S protein is comprised of two domains. The S1 domain binds to the host receptor Angiotensin-Converting Enzyme 2 (ACE2) through its receptor binding domain (RBD), after which the S2 domain facilitates viral fusion and entry, which is primed by the protease TMPRSS2 (17–20). Compared to SARS-CoV-1 and MERS, SARS-CoV-2 has a much broader host range. For SARS-CoV-1, horseshoe bats, lesser-field rice rat, palm civets, pigs, Chinese ferret badger, golden syrian hamsters, red fox, raccoon dogs, ferrets, cats, rhesus and cynomolgus macaques, African green monkeys, and marmosets have had detectable antibodies, viral RNA, and/or transmission (21–27). For MERS, rhesus macaques, llamas, dromedary camels, alpacas, pig, cattle, sheep, goats, donkeys, horses, Egyptian tomb bats, straw-coloured fruit bat, common-wing bent bat, Japanese pipistrelle, and Asian particolored bats have had detectable antibodies, viral RNA, and/or transmission (21–27). Unlike SARS-CoV-1, dormant since 2004, there are still ongoing cases of MERS due to establishment of the virus in a reservoir population (dromedary camels) that frequently interact with humans (28,29). For SARS-CoV-2, animal species that have had detectable antibodies, viral RNA, and/or transmission include but are not limited to, white-tailed deer, dogs, cats, ferrets, syrian hamsters, golden hamsters, dwarf hamsters, large cats, gorillas,

deer mice, racoon dogs, African green monkeys, Asian small-clawed otters, rabbits, rhesus macaques, tree shrews, cattle, and minks; additional species are included in Chapter 2 (21–27,30,31). The potential host range for SARS-CoV-2 is large and still expanding, and the rise of variants is further increasing the host range. Similar to MERS, there is potential for a reservoir species to be established and spillover into human populations, as reports demonstrate SARS-CoV-2 has already established in certain wildlife species. Therefore, besides understanding what species are susceptible to SARS-CoV-2 and have the potential to become an intermediate host, more understanding on how to prevent or reduce the likelihood of SARS-CoV-2 transmission from animals to humans is needed.

To combat SARS-CoV-2, understanding which animal species have the capacity to become infected and transmit the virus is a priority. This will demonstrate which animals serve as an intermediate or reservoir host. Animals that are susceptible to the virus can be used for vaccine and therapeutic research, which can lead to reducing the spread and likelihood of SARS-CoV-2 transmission (32,33). If SARS-CoV-2, like other zoonotic pathogens, does become established in local animal populations, there is the risk of spillover from the reservoir species to the human population. This can have potentially dangerous complications, such as the virus having increased infectivity, pathogenesis, or transmissibility in humans (32,33). Furthermore, if the viral strain is a unique enough variant from the strain infecting humans or does not match the vaccines, the immunity acquired through vaccination campaigns would be ineffective, potentially leading to a new pandemic cycle (32,33). Captive and wild animal species may also succumb to SARS-CoV-2, such as mink on the fur farms in the Netherlands and three snow leopards in a Nebraska zoo who died due to complications with SARS-CoV-2 infection (34). If SARS-CoV-2 is deadly for certain species and spreads rapidly, it can lead to population declines and contribute to endangering animal species of conservation concern. SARS-CoV-2 could affect animal populations if it can be readily vertically transmitted (mother to fetus), leading to spontaneous abortion or stillbirths. This possibility has been identified in white-tailed deer, where pregnant white-tailed deer were inoculated resulting in unviable fetuses (35). Further, if these animals are relied on by humans for economic, agricultural, or sustenance, this can contribute to economic loss and food shortages.

After identifying the susceptible animal species, understanding factors that increase the risk of these species becoming infected is logical. The identification of relevant risks of zoonotic transmission includes both likelihood and consequences for human health. Once risks have been characterized and prioritized, measures to mitigate or prevent transmission can be established and communicated with stakeholders. When communicating these risks and mitigation strategies, tailoring the response to the specific group or organization is important. This ensures the strategies will be implemented and followed to the highest degree possible.

1.2.3 Nunavik ringed seal project

At the start of my Master's thesis project, I had the opportunity to participate in a ringed seal surveillance project based in Nunavik. Working with Inuit harvesters and members of the Inuit owned Makivik Corporation, we discussed microbiological hazards that can be found in ringed seals. The project was part of a broader community-based participatory research initiative, the Ringed Seal Monitoring Program in Nunavik, which began after Inuit harvesters expressed concerns for ringed seal health to the Makivik corporation.

Although much of the surveillance project was delayed and had to be adapted due to SARS-CoV-2, I was able to participate in a conference call. During this conference call, results from the surveillance project were presented and I gave a presentation on microbiological hazards that could be found in ringed seals using infographics (Appendix A). There were three infographics, with two focusing on the parasites *Toxoplasma gondii* and *Trichinella* spp. as these were an important topic for the surveillance project. Within these infographics, I gave a brief description of the parasite including life cycle, transmission, and prevention. The third infographic was a collection of other parasites that could be found in ringed seals. After these presentations, a round table discussion followed where concerns regarding ringed seal health were discussed by the Indigenous harvesters. From this, survey questions were developed and sent to the members of the Makivik Corporation.

This project incorporated both traditional knowledge and western sciences. Traditional knowledge is knowledge that has been developed over generations and passed on within a community (36). This knowledge can also be incorporated into the spiritual and

cultural identity of the community, whereas western science refers to knowledge that is analytical and quantitative in approach and presented from an academic point of view (36). Although the time I spent on this project was brief, due to safety concerns surrounding SARS-CoV-2, I experienced different knowledge systems and how two-way risk communication can be conducted.

1.2.4 Risk communication

The WHO defines risk communication as "the exchange of real-time information, advice and opinions between experts and people facing threats to their health, economic, or social well-being" (37). The goal of risk communication is to provide the audience the knowledge required to make an informed decision regarding the risk (37,38). Risk communication is complex and there are different models for how to execute it effectively. A few examples include the three phase model consisting of risk appraisal, situational analysis, and source analysis, the Health Belief Model, the Mental Models, and the Anger Activism Model (39–42).

While these different models exist, there are concepts and considerations a risk communicator should be aware of to ensure the communication is effective with their intended audience. These include knowing who a knowledge user is, and their social and cultural traits. The knowledge user community may consist of the public-at-large, special interest groups, media outlets, public health professionals, or private organizations (38,42). The methods for communication should be tailored to the knowledge user based on their characteristics. For instance, press releases, public consultations, publications, or meetings and workshops are not uniformly effective with different communities (42). Each method has benefits and limitations for communicating risks (42). The characteristics of knowledge users can include their knowledge, language, socio-demographic factors, culture, and previous experiences with the risk (38,42,43).

Other things to be aware of for risk communication is the impact of trust between the knowledge presenter and the knowledge user. If there is no established trust between the two parties, the knowledge user may disregard the information (42,43). Trust can be fostered by explaining the uncertainties about the risk (44). There has been a greater movement for risk communicators to provide information about uncertainties instead of shielding the

public by providing an over-simplified explanation and assuming that knowledge users are incapable of hearing the risks and responding rationally (44). Even in times of high uncertainty when accurate estimates are unavailable, being transparent to the public can help allay fears and encourage active participation in risk management (37,43–45). Another way to cultivate trust is to be knowledgeable, accurate, and understanding of the publics' needs (43). This includes making risk messages easily comprehensible (42,43). If the message is technical or laden with jargon, individuals unfamiliar with the technicalities of the risk may misunderstand or ignore the message entirely (42,43). The latter can occur especially when the message is lost in the technical jargon and does not seem to address the knowledge users' genuine concerns (42,43). How the public will perceive the risk compared to the risk communicators is also something to be aware of (43,45,46); some risks are inherently less tolerable or create more outrage than others (risk heuristics). Promoting two-way communication between the knowledge presenter and the user allows both parties to understand the how the risk is perceived by the other, allowing for better communication and reception (38,45,47).

Even with different models, strategies, and guidelines available, poor risk communication still occurs. Poor risk communication can result in the presentation of inaccurate information, conflicting information from similar sources or experts, risks being over or underestimated, or poor execution of the risk message (42). A study which interviewed Métis and First Nations people in Manitoba after the H1N1 pandemic found the risk communication employed by the Canadian government to be poor (48). During the interviews, the Métis and First Nations people described how public health messaging had left them feeling less valued, themselves a risk factor, and tactlessly classified as "other" (48). Besides these negative outcomes, poor risk communication can also result in misinformation and confusion.

1.2.5 Misinformation and confusion

The SARS-CoV-2 pandemic has made evident the importance of effective and timely communication. Sharing of misinformation can lead to disastrous consequences, such as reports of people consuming cleaning products, not following public health guidelines, developing increased vaccine hesitancy, and believing conspiracy theories on the origins of

SARS-CoV-2 (49). The collection of misinformation about SARS-CoV-2 has been referred to as an infodemic (50). The major platforms for the sharing of misleading and incorrect information are social media channels. Here, incorrect and unmoderated information can be shared, spreading to various individuals and groups (49,50). Studies investigating the rampant misinformation that occurs on social media have found that, of the top coronavirus YouTube videos, 25% contained some source of misinformation, and 40% of 112 million posts shared on social media were from unreliable sources. From January to April 2020 social media channels allowed the spread of over 600 fabricated stories on COVID-19/SARS-CoV-2, and, misinformation about COVID-19 vaccines were viewed over 4.5 billion times in the span of one month (49,50).

In addition to social media channels, scientific articles recently published or available on preprint servers can also be a source of misinformation (51). Articles on preprint servers have not gone through extensive peer-review processes but can be readily accessed by the public or news outlets and social media sites (51). If the data and results are incorrect, taken out of context, or misinformed, it could result in dangerous and/or life threatening situations (51,52). Further, articles published after going through the peer review processes have also been redacted. While this occurs infrequently in the scientific literature, this is particularly problematic for SARS-CoV-2 due to the rush to produce novel information on SARS-CoV-2, and the expedited review process for articles discussing SARS-CoV-2 (51). As a result of the pandemic, new topics of research and innovation have occurred, and the pandemic will be a learning opportunity for public health and government officials in order to address misinformation for years to come.

1.2.6 Research objectives

The overarching goal for this thesis was to determine the role of animals in transmission of SARS-CoV-2 post-emergence – i.e. not to explore the original source but the current situation - and the risks this may pose for human health. A secondary goal is to examine transferable lessons from risk communications on diseases in animals to present this information in an accurate manner to reduce misinformation and confusion. To complete these goals, this thesis addressed the following questions.

What animal species are susceptible to SARS-CoV-2?

To answer this question, a scoping review was conducted where sources of literature describing an animal species susceptibility to SARS-CoV-2 were collected and critically evaluated for relevance. From this scoping review, animal species which could play a role as an intermediate or reservoir host and the methods that can be used to evaluate susceptibility were identified. After determining what animal families were the most susceptible, the second research question was addressed.

What are the risks of SARS-CoV-2 transmission from wildlife to people in North America?

To answer this question, a rapid qualitative risk assessment was conducted. This risk assessment utilized the findings from the scoping review to identify which animal species are susceptible to SARS-CoV-2. The risk assessment focused on North American wildlife as a manageable scope, in light of the global nature of SARS-CoV-2, its broad host range, and marked regional differences in fauna worldwide.

How should the risks of SARS-CoV-2 transmission in animals be communicated to reduce confusion and misinformation?

Throughout my MPH thesis work, I have identified and witnessed instances where misinformation and confusion have occurred. From these experiences, I conducted a thought exercise based on my own views for areas to be aware of when undertaking risk communication.

In Figure 1.1, I illustrate how the objectives relate to each other and the research goals, along with the probing questions I used to deepen learning. The top text box represents the research goals and the text boxes from left to right represent the scoping review, risk assessment, and risk communication exercise. In each Chapter, a section of the diagram will be highlighted in red, giving an indication of what will be discussed.

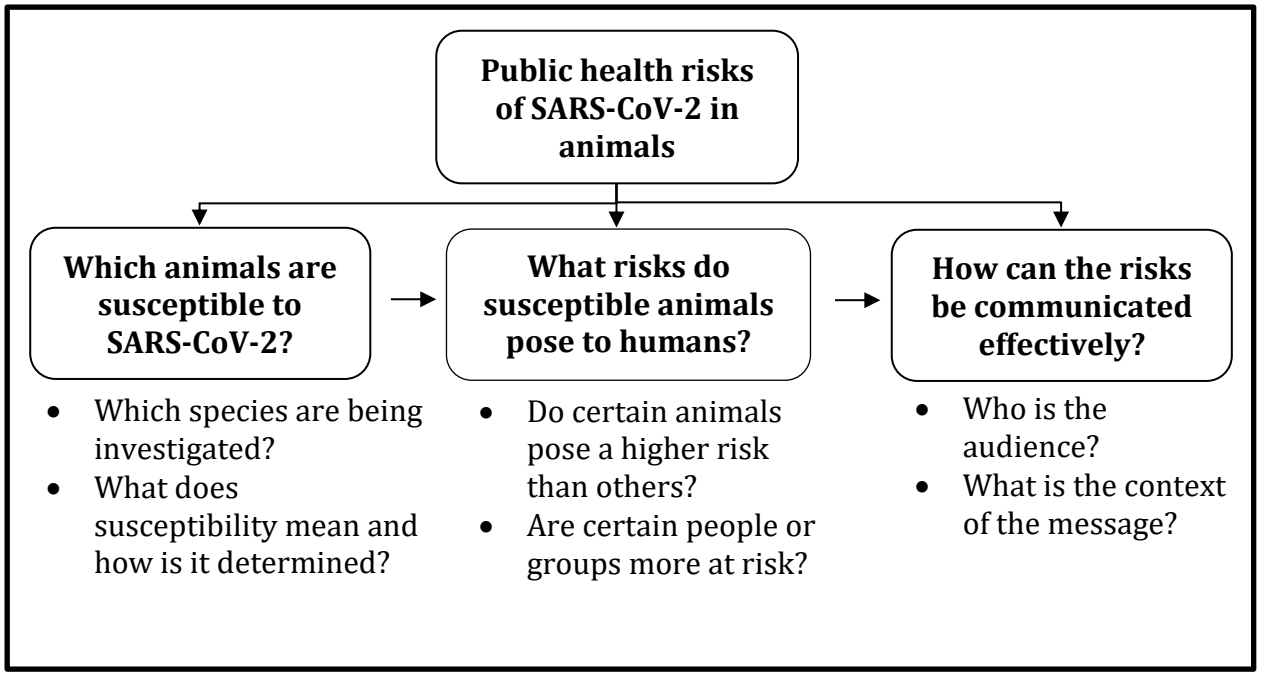


Figure 1.1. Flow chart outlining the research question (top), the three research objectives for this thesis, and probing questions for each objective.

Due to the pandemic, this thesis primarily consisted of desk research. The use of other methodologies to complement and further expand on the findings in the subsequent chapters would have been helpful but were not possible under the circumstances. These other methodologies would have included longitudinal studies of wild animal populations, interviews and discussions with experts and Indigenous groups about SARS-CoV-2 and the potential risks to wildlife, and/or discussions with individuals who have had positive and negative experiences with risk communication. Even without these other methodologies, the findings in this thesis are capable of standing on their own and provide new knowledge.

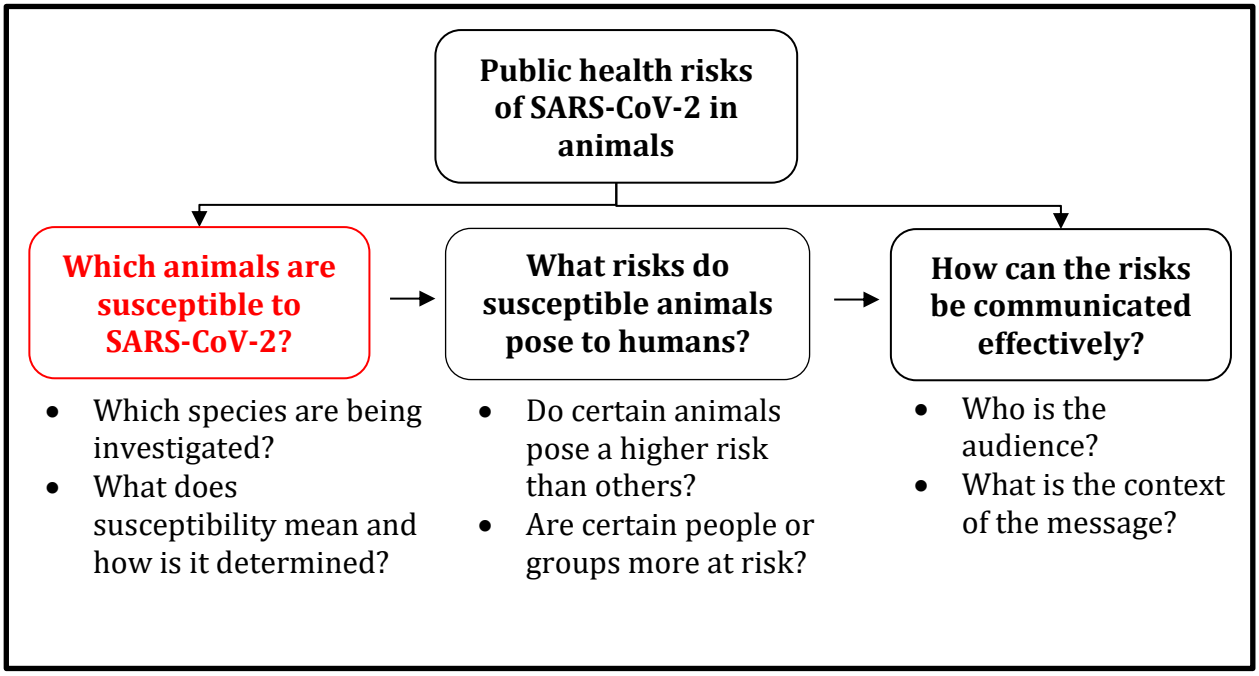
In this thesis, the chapters include the following:

Chapter 2: the scoping review on SARS-CoV-2 in animals, which identifies the animals investigated, the methods used to evaluate susceptibility, and the contrasting susceptibility evaluations from various sources;

Chapter 3: risk assessment focusing on 4 taxonomic families containing free ranging North American wildlife deemed to pose the most risk to humans;

Chapter 4: discusses the key results from Chapters 2 and 3, and the importance of risk communication, then summarizes the conclusions, limitations, reflections, and future directions for this work.

CHAPTER 2


Public health risks of SARS-CoV-2 in animals
Which animals are susceptible to SARS-CoV-2?
What risks do susceptible animals pose to humans?
How can the risks be communicated effectively?
• Which species are being investigated?
• What does susceptibility mean and how is it determined?
• Do certain animals pose a higher risk than others?
• Are certain people or groups more at risk?
• Who is the audience?
• What is the context of the message?

ABSTRACT

Background: In the early stages of response to the SARS-CoV-2 pandemic, it was imperative for researchers to rapidly determine what animal species may be susceptible to the virus, under low knowledge and high uncertainty conditions. Methods for assessing species susceptibility to SARS-CoV-2 infection include *in silico, in vitro, in vivo,* and epidemiological approaches.

Methods: In this scoping review, the animal species being evaluated for SARS-CoV-2 susceptibility, the methods used to evaluate susceptibility, and comparing the evaluations between different studies were conducted. Using the PRISMA-ScR methodology, publications and reports from peer-reviewed and grey literature sources were collected from databases, Google Scholar, reports from the World Organization for Animal Health (OIE), snowballing, and recommendations from experts. Inclusion and relevance criteria were applied, and information was subsequently extracted, categorized, summarized, and analyzed.

Results: Ninety-seven sources (publications and reports) were identified for the scoping review, 81 from databases and Google Scholar and 16 from snowballing, reports from OIE, and expert recommendations. There were 649 animal species investigated from eight different classes: Mammalia (431), Aves (88), Actinopterygii (87), Reptilia (28), Amphibia (6), Insecta (6), Chondrichthyes (2), and Coelacanthimorpha (1). Sources used four different methods, *in silico* (46), *in vitro* (21)*, in vivo* (36), and epidemiological analysis (12). Along with using the different methods, how each source described "susceptibility" and evaluated the susceptibility of different animal species to SARS-CoV-2 varied, with conflicting susceptibility evaluations evident between different sources, especially for *in silico* methods.

Conclusions: Early in the pandemic, *in silico* methods were used the most to predict animal species susceptibility to SARS-CoV-2 and helped guide more costly and intensive studies using *in vivo* or epidemiological analyses. However, the limitations of all methods must be recognized, and evaluations made by *in silico* and *in vitro* should be re-evaluated when more information becomes available, such as demonstrated susceptibility through *in vivo* and epidemiological analysis.

INVESTIGATING SARS-COV-2 SUSCEPITBILITY IN ANIMALS: A SCOPING REVIEW

2.1 Introduction

To first understand which animal species were susceptible to SARS-CoV-2, a scoping review was conducted. A scoping review was chosen instead of other literature reviews such as a systematic or narrative due to the novel nature of SARS-CoV-2. A scoping review is designed to survey and describe the existing literature without arriving at discrete answers regarding the literature identified (53,54). Furthermore, a scoping review can source a broader amount of literature that uses different methods and study designs. This is where the scoping review and the systematic review contrast; that is, systematic reviews may require specific study designs, critically evaluate the data for bias, and arrive at pinpoint conclusions based on the sources selected (53,54). For the purposes of this thesis, I wanted to investigate sources of literature regardless of method, which focused on an animal species' evaluated susceptibility to SARS-CoV-2. Furthermore, various studies evaluating SARS-CoV-2 were being produced at a rapid rate over the initial pandemic years, therefore, using a methodology that could include pre-prints was warranted.

Before conducting the scoping review four different methods which can be used to evaluate an animals' susceptibility were identified: *in silico, in vitro, in vivo,* and epidemiological analyses (32). In general, *in silico* analysis refers to using computer modeling or simulations to evaluate receptor binding; *in vitro* analysis refers to investigating receptor binding or viral entry in cell lines; *in vivo* analysis refers to testing for antibodies, viral RNA, infectious virus, transmission, or pathogenesis in experimentally exposed live animals; and epidemiological analysis refers to testing for the presence antibodies, viral RNA, infectious virus, transmission, or pathogenesis in naturally infected animals (32,55–58,60–63).

For this scoping review, the goals were to determine which animal species were being investigated, the methods used to evaluate susceptibility, the conclusions regarding the evaluated susceptibility, and if contrasting evaluations between sources, the reasons why.

These results would help identify targets for ongoing surveillance, epidemiological studies, and other forms of analyses. Criteria that can be applied for weighing evidence of animal susceptibility to an emerging zoonoses, even for a novel pathogen under high scientific uncertainty is also suggested.

2.2 Methods

The framework for the scoping review was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Extension for Scoping Reviews (PRISMA-ScR) (64).

Search strategy

Sources (publications or reports) were collected between July 9th - 13th, 2020 and December 30th - January 2nd, 2021, from established databases (Medline, Scopus, Web of Science, PubMed, Global Health, and Public Health Database), and the first 100 results from Google Scholar collected on a single day in both time frames. For the databases and Google Scholar, search terms were drafted and then reviewed by a university librarian and an interdisciplinary research team (epidemiologist, microbiologist, and social scientist) for input and modification. Additional sources were added through investigating cited references in the selected sources (snowballing), from the recommendations of expert researchers, and the World Organization for Animal Health (OIE) (30). For OIE, sources were gathered on April 30, 2021 and were found by accessing the *COVID-19 Events in Animals* webpage (30). All sources were imported into Zotero software and duplicates were removed manually (65). An example of the search strategy is shown in Appendix B, Figure B.1.

Eligibility criteria

Eligible sources consisted of peer-reviewed or grey literature (pre-prints or non-peer reviewed articles) that investigated or reported on an animal species' susceptibility to SARS-CoV-2. Articles that were excluded include, self-described review articles, studies using animal models to evaluate SARS-CoV-2 therapeutics or vaccines, studies using lab specific or transgenic animals, articles not in English, or duplicate studies reporting on the same

naturally infected animals in time and space such as the SARS-CoV-2 outbreaks on the mink fur farms, in which case the formal report to the OIE took precedence.

Selection of sources

After duplicates were removed, sources were sorted by two researchers in two rounds, in which irrelevant sources were removed (Figure 2.1). The first round consisted of reading the title and abstract of each source. If no abstract was provided, the title and keywords were used. The next round comprised of reading the source material. After both rounds, the researchers then compared their results, and any disagreements (n=555) were settled through consensus. In a scoping review, settling disagreements through consensus has shown to be an effective method as described by Peterson et al. (66). After the second round, the sources selected underwent snowballing. Sources based on recommendations from researchers (often seminal or novel findings) were added throughout the scoping review process, and subsequently underwent snowballing. Additionally, after the second round, results from animals naturally infected with SARS-CoV-2 were compiled from OIE.

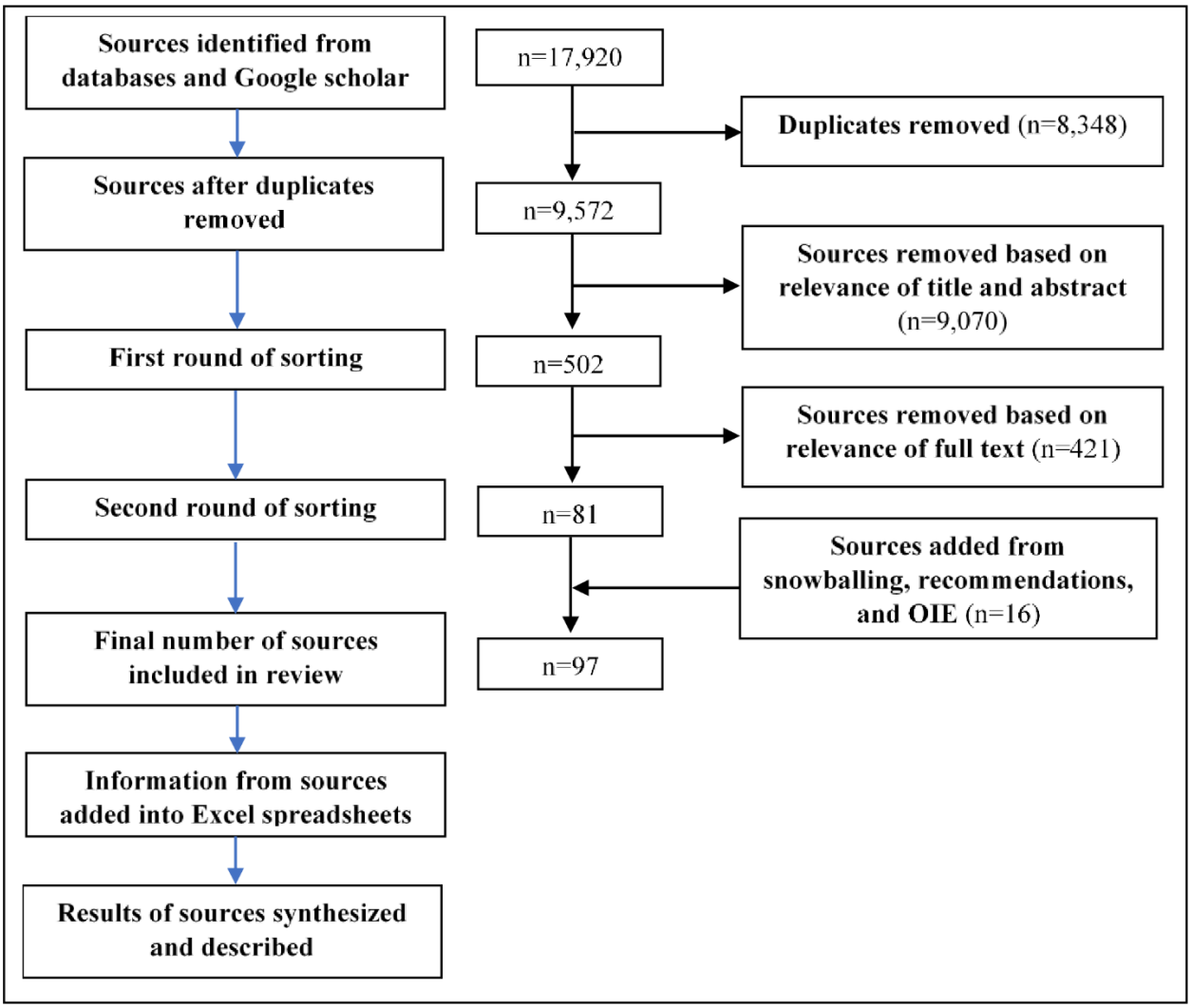


Figure 2.1. Flow chart demonstrating the methods used for source gathering, selection, and synthesis for the scoping review.

Data charting

Once the selected sources were finalized, corresponding information from each source was entered into predetermined categories in two Excel spreadsheets. The first Excel spreadsheet categories were: author, title of source, date published / uploaded, source type (self-described by source, including dispatches, letters, articles, reports, etc.), country of first author, method used to evaluate susceptibility, overview of the methods, number of animal species evaluated, and overview of findings. The second spreadsheet contained a list of all animal species investigated with the animal's taxonomic class, scientific and common name, which were matched with the investigating source.

The scientific and common name were identified through an accession number or sequence ID provided from the source linking to a public database such as the National Centre for Biotechnology Information (67). The taxonomic class, if not already provided by the source, was found through the Integrated Taxonomic Information System (68). If no sequence ID was provided, the scientific name and common name in the source were used. If the common name and scientific name did not match, the common name took priority i.e. *in vivo* studies citing *Canis lupus* were presumed to be using dogs, vs wolves. If only the common name was provided it was matched to its representative scientific name, where possible. This was dependent upon the common name being linked to a single species, such as cats or dogs (*Felis catus* and *Canis lupus domesticus*). If the common name was too general and could not be matched to a specific species, then all animal species which shared the similar common name were identified in the Excel spreadsheet and the unstated species was assumed to be the species most commonly investigated by the other sources. For example, if the common name listed was "bear", and there were 4 studies on American black bears, 11 on brown bears, and 12 on polar bears, a source using only the common name "bear" was entered as polar bear (*Ursus maritimus*). As the location where the source study occurred was not considered, this is an acknowledged limitation of the scoping review. Subspecies were removed, recording only the genus and species. For example, if a source investigated related subspecies such as *Sus scrofa* and *Sus scrofa domesticus,* only *Sus scrofa* would have been recorded and that source would be considered to have investigated only one species. Only certain subspecies were included, namely *Canis lupus familiaris* (dog) and *Canis lupus*

dingo (dingo), and *Mustela putorius furo* (Ferret) and *Mustela lutreola biedermanni* (Mink) as there were a large number of sources that investigated these animals and made clear distinctions among subspecies. Humans were not included in the animal species list and were not counted.

Synthesis of results

Descriptive statistics summarizing source characteristics, animal species and their corresponding class, the methods used for evaluating an animal's susceptibility, the conclusion of the source regarding susceptibility of certain animal species, and the cross-referencing of animal species with the different methods of analysis are described and summarized in both tables and figures. The reasons for the contradictions among different sources regarding the evaluated susceptibility of an animal species were also explored.

2.3 Results

Sources selected

After removal of duplicates, 3,306 and 6,266 sources were identified in the first and second rounds of source gathering, respectively. After the two sorting rounds and with the addition of sources through snowballing, expert recommendations, and the compilation of case reports from OIE, 97 sources were included in the scoping review (Figure 2.1).

Characteristics of the included sources

Most sources were published or made available in 2020. There were 19 different countries in which the studies occurred, with China, then the USA, having the highest counts. There were nine different source types as self-described by the sources, the most common being journal articles. The number of animal species investigated per source ranged from 1 to over 300, with ≤ 10 animal species investigated in most sources. *In silico* was the most common method used to evaluate a species susceptibility to SARS-CoV-2. Certain sources used multiple analysis methods; therefore, the total for this category does not equal 97 (Table 2.1).

Table 2.1. Characteristics of the literature sources selected for the scoping review.

Characteristics of studies	N (%)
Year	
2020	86 (88.66)
2021†	11 (11.34)
Country	
Australia	1 (1.03)
Bangladesh	1 (1.03)
Brazil	1 (1.03)
Canada	5 (5.15)
China	37 (38.14)
France	3 (3.09)
Germany	5 (5.15)
India	3 (3.09)
Iran	1 (1.03)
Italy	3 (3.09)
Japan	1 (1.03)
Malaysia	1 (1.03)
Mexico	1 (1.03)
Morocco	1 (1.03)
Netherlands	3 (3.09)
Republic of Korea	1 (1.03)
Spain	3 (3.09)
UK	4 (4.12)
USA	22 (22.68)
Source type (self-described by source)	
Communications	9 (9.28)
Correspondences	2 (2.06)
Dispatches	2 (2.06)
Essay and Perspectives	1 (1.03)
Journal articles	68 (70.10)
Letters	5 (5.15)
Preprints	8 (8.25)
Reports	1 (1.03)
Webpage	1 (1.03)
Study design‡	
In silico	46
In vitro	21
In vivo	36
Epidemiological	12
Number of animal species investigated per source	
≤10	59 (60.82)
11 - 50	25 (25.77)

Table 2.1 Cont'd	
Characteristics of studies	**N (%)**
51 - 100	5 (5.15)
101 -150	3 (3.09)
151 - 200	1 (1.03)
201 - 250	1 (1.03)
250 -300	2 (2.06)
408	1 (1.03)

Note.†For the year 2021, sources were collected up to April 30th ‡Total number does not equal 97 as some sources used more than one method of analysis.

Results of individual sources of evidence

The full data charting table containing the author, title of source, date published / uploaded, source type, country of first author, susceptibility evaluating method, overview of the methods, number of animal species evaluated, and overview of findings for each source can be found in the attached Excel document, ES B.1. The animal species evaluated by each source, along with the taxonomic class and scientific and common names, can be found in the attached Excel document, ES B.2.

Synthesis of results

Animal species evaluated

Six-hundred and forty-nine animal species from eight classes were investigated in the 97 sources (Figure 2.2). Within the individual methods of evaluating susceptibility, mammalian species were the most studied class with 45 *in silico*, 20 *in vitro*, 33 *in vivo*, and 11 epidemiological studies. Aves was the second most investigated class in all methods except for epidemiological analysis, where there was a tie with Insecta. The *in silico* method investigated the most classes (n=7) and was utilized by the most sources (Figure 2.3 & Appendix B Table B.1.).

In the total number of animal species investigated for each of the methods used to evaluate susceptibility, *in silico* dominated, investigating 633 out of the possible 649 species, followed by *in vitro* (129 species), epidemiological (42 species), and then *in vivo* (27 species). As a percentage for investigating the total number of each animal species in the different classes, the *in silico* method investigated 98% of the Mammalia, 99% of the Aves, and 100%

of the Reptilia, Actinopterygii, Amphibia, Chondrichthyes, and Coelacanthimorpha species. Again, the Mammalia class had the most species investigated for each analysis method (Table 2.2).

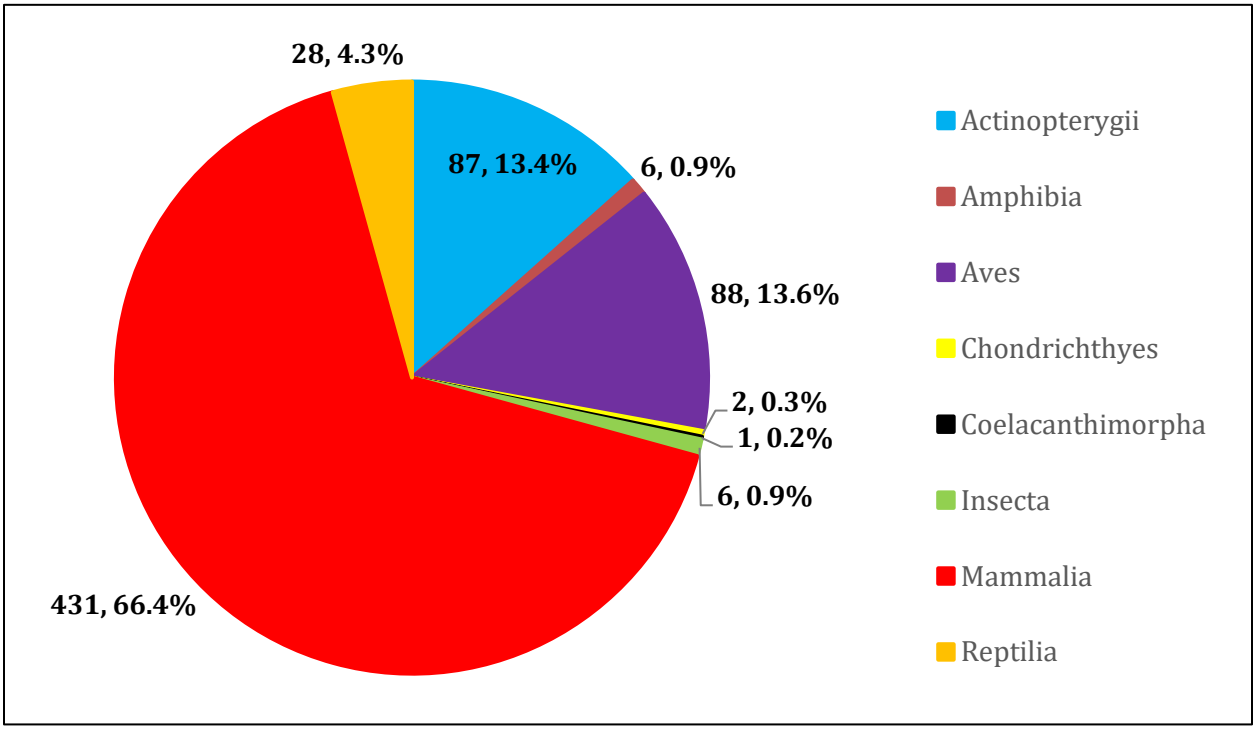


Figure 2.2. Total number of animal species (by taxonomic class) investigated in the sources chosen for the scoping review.
Note. A total of 649 animal species belonging to eight different classes were investigated by the 97 sources selected for the scoping review.

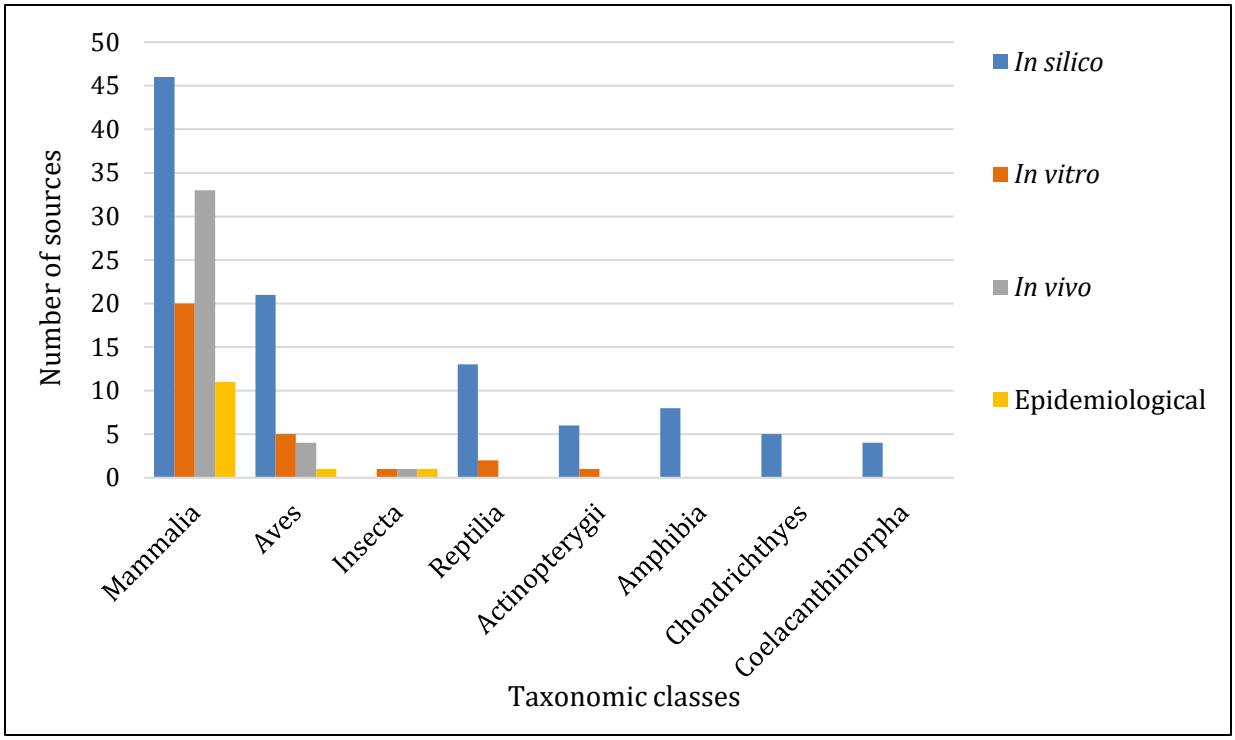


Figure 2.3. The number of sources identified in the scoping review that investigated each taxonomic class of animals for susceptibility to SARS-CoV-2, sorted by evaluation method. *Note.* For each class, the number of sources along with the method used to determine susceptibility is shown. The corresponding numbers for the figure can be found in Appendix B Table B.1.

Table 2.2. Total number of animal species investigated in the literature (based on taxonomic class) by each of the four susceptibility predicting methods.

Class	***In silico***	***In vitro***	***In vivo***	**Epidemiological**
Mammalia	422	118	19	35
Aves	87	4	5	5
Insecta	0	3	3	2
Reptilia	28	3	0	0
Actinopterygii	87	1	0	0
Amphibia	6	0	0	0
Chondrichthyes	2	0	0	0
Coelacanthimorpha	1	0	0	0
Total	**633**	**129**	**27**	**42**

Methods used to describe and evaluate susceptibility

How an animal species susceptibility to SARS-CoV-2 was evaluated varied among the different analysis methods, ultimately contributing to different meanings of "susceptibility" among the different sources.

For *in silico* analysis, an animal species' susceptibility to SARS-CoV-2 was commonly evaluated through investigating the binding potential of an animal species ACE2 receptor to the SARS-CoV-2 RBD. By comparing the homology of the human ACE2 (hACE2) receptor to the ACE2 receptor of different animal species, binding potential could be assessed through: 1) evaluating the homology to the entire hACE2 sequence, 2) selecting critical residues utilized by the hACE2 receptor when binding to the SARS-CoV-2 RBD, 3) evaluating residues that are in close proximity and may alter binding, or 4) creating homology models where the hACE2 binding to the SARS-CoV-2 RBD was used as a template to model an animal species ACE2 receptor binding to the SARS-CoV-2 RBD. Based on the homology, susceptibility scores were created or, if the ACE2 residues of the animal species differed from the hACE2 critical residues, the effects of those mutations on binding could be explored (18,19,69–96). With homology modeling, the interactions between the ACE2 receptor and the SARS-CoV-2 RBD could be further examined through analyzing binding affinities, molecular dynamics, adaptation indexes, or docking simulations (17,19,70,72–75,85,86,90,94,97–108). Other *in silico* methods used to predict susceptibility include: 1) investigating the relative synonymous codon usage, which compares the codons of the viral genome to the codons used in different animal species; 2) comparing the homology of the human TMPRSS2 sequence to animal species; 3) creating statistical models or learning algorithms to predict susceptibility based on the characteristics of the ACE2 receptor, CoVs, or animal species; 4) investigating ACE2 isoforms and gene expression; or 5) comparing the ACE2 receptor sequence of different animal species (19,74,78,84,94,98,109–111).

The methods used by *in vitro* analysis to evaluate and describe susceptibility investigated ACE2 receptor binding or cellular entry of SARS-CoV-2 in cell culture. Viral binding methods included expressing the ACE2 receptor of various animal species combined with the SARS-CoV-2 RBD expressed on cells or as an Fc fusion protein. Binding was determined through surface plasmon resonance, ELISA, flow cytometry, or

immunofluorescence (69,81,83,92,93,95,103,112). Viral entry methods included expressing the ACE2 receptor of different animals on cells not permissive to SARS-CoV-2 entry, or infecting cell lines from animal species with a SARS-CoV-2 pseudo or live virus. Viral entry was determined by immunofluorescence, cytopathic effects, or isolation of viral RNA or infectious virus from the exposed cells (17,69,81,88,92,95,96,103,112–121). Finally, some *in vitro* methods investigated the location and concentration of an animal species ACE2 receptor or TMPRSS2 protease (18,114,120).

In vivo methods demonstrated susceptibility to SARS-CoV-2 infection through the experimental exposure of an animal species, usually a mammal. Animal species were inoculated through various routes including intranasal, intratracheal, oral, aerosolization, ocular, or intragastric with doses of SARS-CoV-2 ranging from 10^2 - $7x10^6$ TCID50 or 10^2 - $1.1x10^6$ PFU. After an animal was inoculated, susceptibility to SARS-CoV-2 infection or disease was determined through the analysis of clinical signs, pathogenesis, detection of viral RNA, infectious virus, or antibodies, or direct or indirect contact transmission. For direct contact transmission, the inoculated animal was placed in the same cage or pen as a naïve animal, while for indirect contact, the inoculated animal and naïve animal were separated by a barrier although air was exchanged between the animals (99,113,115–118,122–151).

Epidemiological studies involved evaluating domestic, zoo, or wild animals naturally exposed to SARS-CoV-2 for clinical signs, pathogenesis, viral RNA, infectious virus, antibodies, or transmission (10,30,119,152–160).

For each method, any limitations specified by the authors were recorded (Appendix B, Table B.2).

Contrasting susceptibility evaluations

Contrasting results from the different methods used to evaluate an animal species susceptibility to SARS-CoV-2 were identified in the scoping review. Using the top six investigated species, *Felis catus* (cats), *Canis lupus familiaris* (dogs), *Sus scrofa* (pigs), *Mus musculus* (house mice), *Mustela putorius furo* (ferrets), and *Oryctolagus cuniculus* (European rabbits), the susceptibility of each species to SARS-CoV-2 as evaluated by the sources is listed and compared in Table 2.3. Results for *in silico* analysis had the most variability, whereas results for *in vivo* and epidemiological analysis were more consistent. The contrasting results

were more prevalent in dogs and pigs, whereas susceptibility evaluations were more consistent for cats, house mice, and European rabbits.

Table 2.3. Evaluation of susceptibility for the top six animal species investigated as described by the selected sources, sorted by method of evaluation.

Species	Source ranking†	*In silico*	*In vitro*	*In vivo*	Epidemiological
Cats N=47	Not Susceptible	N=1			N=1
	Very low susceptibility				N=1
	Low susceptibility				
	Medium / Intermediate susceptibility	N=3			
	Potentially susceptible	N=4			
	Susceptible	N=13 (6) †	N=1 (6)	N=2	N=8
	High susceptibility	N=5		N=2	
Dogs N=39	Not Susceptible	N=4	N=1		N=1
	Very low susceptibility			N=1	N=1
	Low susceptibility	N=4		N=1	
	Medium / Intermediate susceptibility	N=1			
	Potentially susceptible	N=3			
	Susceptible	N=10 (6)	N=1 (6)		N=5
	High susceptibility				
Pigs N=31	Not Susceptible	N=4	N=1 (2)	N=1 (2)	N=1
	Very low susceptibility				
	Low susceptibility	N=2			
	Medium / Intermediate susceptibility	N=1			
	Potentially susceptible	N=2			
	Susceptible	N=9 (5)	N=2 (5)	N=1	
	High susceptibility				
House mice N=31	Not Susceptible	N=14 (7)	N=2 (7)		N=1
	Very low susceptibility	N=1			
	Low susceptibility	N=5			
	Medium / Intermediate susceptibility				
	Potentially susceptible	N=1			
	Susceptible				
	High susceptibility				

Table 2.3 Cont'd					
Species	**Source ranking†**	***In silico***	***In vitro***	***In vivo***	**Epidemiological**
Ferrets N=24	Not Susceptible	N=1			N=1
	Very low susceptibility	N=1			
	Low susceptibility	N=1			
	Medium / Intermediate susceptibility	N=1			
	Potentially susceptible	N=2			
	Susceptible	N=9 (1)	N=1 (2)	N=2 (1)	N=1
	High susceptibility	N=1		N=1	
European rabbits N=24	Not Susceptible	N=2			N=1
	Very low susceptibility				
	Low susceptibility				
	Medium / Intermediate susceptibility	N=1			
	Potentially susceptible	N=1			
	Susceptible	N=10 (5)	N=1 (6)	N= (1)	
	High susceptibility	N=2			

N refers to the number of sources. Sources that did not give a susceptibility classification were omitted from this table but can be found in Excel document ES B.1. References for Table 2.3 can be found in Appendix B Table B.3. †Numbers in parentheses represent sources that used more than one method of analysis and are shared between different analysis methods.

2.4 Discussion

The literature on susceptibility of animal species to SARS-CoV-2 is growing at a rapid speed, reflecting the urgency of identifying animal reservoirs and potential animal models for vaccines and drug therapies. With many studies investigating various animal species using different methods, this scoping review identifies areas of consensus, including a focus on mammals (versus other classes of animals), as well as areas of and reasons for contrast, with different sources reporting different species' susceptibility depending on methods and definitions. In addition, an early preponderance of studies relying on *in silico* methods, appropriate to early response, which served as useful guides to target species for further *in vivo* and epidemiological studies were identified.

SARS-CoV-2 literature is expanding

While 97 sources where chosen, a high number of sources were first identified from the search terms used. When comparing the number of sources identified from the two different search dates, literature focusing on SARS-CoV-2 almost doubled between July 2020 and January 2021. Further, the number of sources selected from the first and second round was 31 and 50 respectively. Based on the large number of sources removed from the first sorting round, the search terms were not sufficiently specific, as irrelevant sources such as those with a focus on dentistry and oral health or physical activity were captured (161–164). To combat this, a more specific search strategy would be needed.

The sources identified were comprised of both peer reviewed and grey literature, including preprints. This more relaxed approach to sources was necessary to gain as much information as quickly as possible due to the novelty of SARS-CoV-2. It is recognized that limitations exist for preprints / grey literature, which have not gone through an extensive peer-review process. During the peer-review process, inaccuracies with the methodology or the results are identified, resulting in the source being updated or rejected by the journal. It is important to note that published articles can also have inaccuracies; articles have been redacted after the peer-review and publication process, possibly due to rapid peer-review or pressure on reviewers and editors to fast-track papers. Interestingly, on preprint servers and through social media sites like Twitter or Facebook, readers can make comments which can influence authors to rework their methodology / results or remove the manuscript from the preprint server, a less formal type of peer review. With preprints / grey literature, information is readily accessible, in contrast to peer-reviewed articles, in which the time until publication can vary considerably. The additional time required can potentially delay research in the respective field, especially a rapidly emerging one like SARS-CoV-2 in 2020-21 (165,166).

Source characteristics

Sources were uploaded or published either in 2020 or early 2021, as SARS-CoV-2 was detected in late 2019. Sources that were published or uploaded after the last round of source gathering were either expert recommendations or preprints which are now published.

Journal articles comprised most of the source types, which is reassuring, as peer-review presumably critically evaluated methodology and interpretation of results evaluating an animal's susceptibility to SARS-CoV-2. However, with the novel nature of the pathogen, the high levels of uncertainty in the early pandemic, and the rapidly expanding literature on SARS-CoV-2, conflicting reports and disagreements between published articles are inevitable. For example, the paper by Ji et al. which used relative synonymous codon usage, concluded snakes were possible intermediate hosts for SARS-CoV-2; however, this was refuted in subsequent papers (109,167).

Sources originated from 19 different countries, reflecting the fact that SARS-CoV-2 is a global concern but also because different animal species are geographically bounded, requiring regional knowledge of fauna. China produced the greatest number of sources included in this review, most likely due to SARS-CoV-2 first being detected in China. In addition, CoV research was occurring in China before the global spread of the virus.

Most sources investigated 10 or fewer animal species; sources which investigated more than 10 primarily used *in silico* or *in vitro* analysis. These larger studies helped target species for more costly (in terms of time, resources, and animal use) investigations involving experimental infections, transmission, re-challenging, or necropsies (168,169). For example, early findings allowed researchers to target animals with a legitimate potential for successful infection (such as mammals), versus animals with little to low susceptibility (such as fish).

Animal species investigated

Early *in silico* and *in vitro* findings steered investigation towards animal species belonging to Class Mammalia, which is supported by subsequent findings that mammals have been successfully infected with SARS-CoV-2, both experimentally and naturally. Although unlikely, it is important to note that this bias might lead to missing some unusual potential animal hosts. Aves was the second most investigated class, and previous work has shown that Aves are commonly infected with delta and gamma CoVs. Although the CoVs that

infect Mammalian and Aves species belong to different genera, exploring all avenues for susceptible animals, especially those known to be infected with CoVs, is essential (11,13). For the other classes investigated, the species were either chosen since they are classified as vertebrates and express the ACE2 receptor, or to test a specific purpose, such as if mosquitos could carry SARS-CoV-2 (88,119,133).

Evaluating methods and animal species

The *in silico* method was employed the most and across the highest number of animal species and classes. This method is advantageous as it can cover a large swath of animal species in a relatively short period and at comparatively lower cost than other methods. Its efficiency demonstrates the utility of *in silico* methods to rapidly pre-screen numerous species, narrowing the focus on species and classes that are more likely to be susceptible for follow-up investigation using more resource-intensive methods (170). It is important to note that i*n silico* results are not necessarily supported by the other methods. Encouragingly, as the results of *in vivo* and epidemiological analysis were published, many sources used these results to refine the accuracy of their *in silico* models (87,100).

Somewhat surprisingly, more sources used *in vivo* versus *in vitro* methods, perhaps because this was thought to provide stronger evidence to determine animal models for SARS-CoV-2. Furthermore, many common laboratory animals were readily available (especially as non-SARS-CoV-2 research was paused) before *in vitro* cell lines could be made. The first *in vitro* study was available February 3rd, 2020, before any *in vivo* studies; then, prior to publication of the second *in vitro* study on May 13th, 2020, six *in vivo* studies became available (99,120–122,138,140,147,149) Four *in vivo* studies investigating Syrian hamsters, a common lab animal, were available before the first *in vitro* study became available (18,99,134,137,142) (Appendix B, ES B.1).

More species and classes were investigated using *in vitro* compared to *in vivo* methods. Thus, with *in vitro* methods, a greater diversity of species can be investigated, including the many potentially susceptible animal species that cannot be cultivated in the laboratory, such as cetaceans and large ungulates. Additionally, *in vitro* methods allow for investigation of species of high conservation concern.

Epidemiological studies in naturally exposed animals appeared less often due to the low occurrence of SARS-CoV-2 in domestic and wild animals in the early stages of the pandemic, and because OIE reports were combined into one source. The number of species investigated in epidemiological studies, however, was higher than *in vivo*. This is largely due to the impact of a single source, Deng et al., which investigated serological response in 35 potentially naturally exposed animal species; if removed, only 13 animal species would have been investigated (153). This may also reflect lag times in securing animal research ethics approval for experimental exposure of captive animals, and responsible animal use.

Variations among studies evaluating susceptibility

The term "susceptibility" was used variably depending on the methods used. For *in silico* and *in vitro* analysis, susceptibility meant that animal species potentially could, or have, the capacity to become infected, with SARS-CoV-2. Whereas for *in vivo* and epidemiological analysis, susceptible hosts were those in which the virus can replicate and transmit to other hosts. These differences demonstrate how susceptibility can be a subjective term, possibly resulting in misunderstandings when interpreting the results if the audience is unfamiliar with the capabilities of each method.

Depending on the species, sources reported different results for susceptibility to SARS-CoV-2, even when using similar methods, this was evident for both dogs and pigs.

Overall, *in silico* analysis had the most variable susceptibility evaluations among the different analysis methods, followed by *in vitro* analysis. *In vivo* and epidemiological analysis were more consistent in their susceptibility evaluations. For *in silico,* the variance in susceptibility predictions were in part due to the ranging methods used to predict susceptibility, from comparing certain hACE2 critical residues to the ACE2 residues of select animals to more in-depth analysis such as homology modeling with follow up analysis including binding affinities or docking simulations. In addition, simulated modeling and the infection of a single cell may not translate to the real world, where additional characteristics will impact whether an animal becomes infected or ill, and/or is capable of transmission (168,170,171). These additional characteristics include the concentration and location of the ACE2 receptor, viral avoidance of host immune response, the potential for ACE2 isoforms that inhibit cellular entry, and/or the acquisition of cellular components for replication

(18,78,79,86,92,102). If SARS-CoV-2 fails in any of these regards, chances of an established infection decrease, which demonstrates the importance of follow-up *in vivo* and epidemiological analyses.

Differences in susceptibility derived from experimental infection in *in vivo* studies and natural infection in epidemiological studies also require careful interpretation. Results from *in vivo* testing are dependent on the dose, route of inoculation, and monitoring indicators such as detectable viral RNA, infectious virus, and antibodies (168,169). If conspecific animals receive different doses of SARS-CoV-2, and the animal with the higher dose is deemed infected but the animal that received the lower dose is negative, whether the animal species should be considered susceptible under natural circumstances depends greatly on how closely the experimental conditions mimic natural transmission and infective doses. In pigs inoculated with 1x10^5 or 1x10^6 TCID, three sources determined pigs were not susceptible, while the fourth determined pigs to be susceptible based on observation of ocular discharge, detection of viral RNA from nasal washes in two pigs and a communal chew rope, recovery of infectious virus from a submandibular lymph node in one pig, and detection of neutralizing antibodies in two other pigs (115,118,122,151).

In both *in vivo* and epidemiological studies, interpretation of susceptibility should also consider the indicators used to determine infection status: i.e. antibodies, detection of viral RNA, recovery of live virus, transmission, and the timeframe. Virus or RNA is detected in animals before antibodies are present. Conversely, detection of antibodies does not necessarily equate to the animal being truly infected or competent for transmission, only that the animal was previously exposed to SARS-CoV-2 (172). Therefore, detection of viral RNA and, especially, infectious virus are more definitive indicators of infection status; however, there may be biosafety reasons why recovery of live virus is not feasible. Assessing transmission is also valuable as it shows that an animal species cannot only become infected but also infect other animals, making it an ideal intermediate and possible reservoir host (173). In dogs, the contrasting susceptibility predictions between *in vivo* and epidemiological analysis stems from epidemiological analysis determining dogs were susceptible through the detection of antibodies or viral RNA, while *in vivo* analysis, which used more specific indicators for SARS-CoV-2 susceptibility, such as transmission, determined dogs had a lower susceptibility (10,30,122,124,152,154,156). The latter is also borne out by observations that

dogs only rarely become infected or ill with SARS-CoV-2, generally in households with close, prolonged contact with infected people (30,159,174).

The genetics of the animal can also affect the outcome. Most laboratory strains of animals are genetically engineered, pathogen free, and kept in artificial husbandry conditions, which does not mimic the real world, where domestic and wild animals are genetically diverse, may experience nutritional stress, and are subject to a barrage of other pathogens (169,175). Epidemiological analyses of domestic animals should also consider animal co-morbidities (chronic disease, immunosuppression) as we have observed in human populations, where severe disease associated with SARS-CoV-2 is frequently linked to other risk factors (169,175).

2.5 Conclusions and limitations

For the different methods used to evaluate an animal's susceptibility to SARS-CoV-2 (and other emerging zoonoses), it would be optimal to use *in silico* and *in vitro* to screen multiple animal species in a rapid and inexpensive fashion early in a pandemic, followed by *in vivo* or epidemiological analysis, with a preference for detecting infectious virus and/or viral RNA. Antibody testing could also be used as a secondary screening tool to prioritize animal species to determine reservoir and bridging hosts for SARS-CoV-2. This integrated approach has demonstrated success in different areas of research including toxicology and virulence (168,176–178).

Based on the results from the sources included in this scoping review, susceptible mammals with a peridomestic or commensal relationship with humans could be closely monitored as a potential reservoir species (179,180). Although not an exhaustive list, species that could be monitored are found within the mustelid, cricetid, and cervid families. Ferrets and minks (mustelids), have both demonstrated a high susceptibility to SARS-CoV-2 infection through *in vivo* and epidemiological analysis (30,34,118,122,149,181–183). Also, in the U.S.A. and Italy, viral RNA was detected in wild minks, and in a pet ferret (30,181,182). Deer mice, Syrian hamsters, and dwarf hamsters, in the cricetid family, have shown high susceptibility through *in vivo* analysis (infectious virus, viral RNA, antibodies, and transmission detected) (127,130,134,137,184). Although not susceptible to the initial SARS-

CoV-2 variant, Old World rodent species have demonstrated increased susceptibility to SARS-CoV-2 variants (185). White-tailed deer (cervids) were experimentally infected with SARS-CoV-2. Viral RNA, infectious virus, antibodies, and transmission were subsequently detected. Epidemiological analysis also revealed antibodies in 40% of tested wild deer in the U.S., indicating some form of natural exposure (117,186).

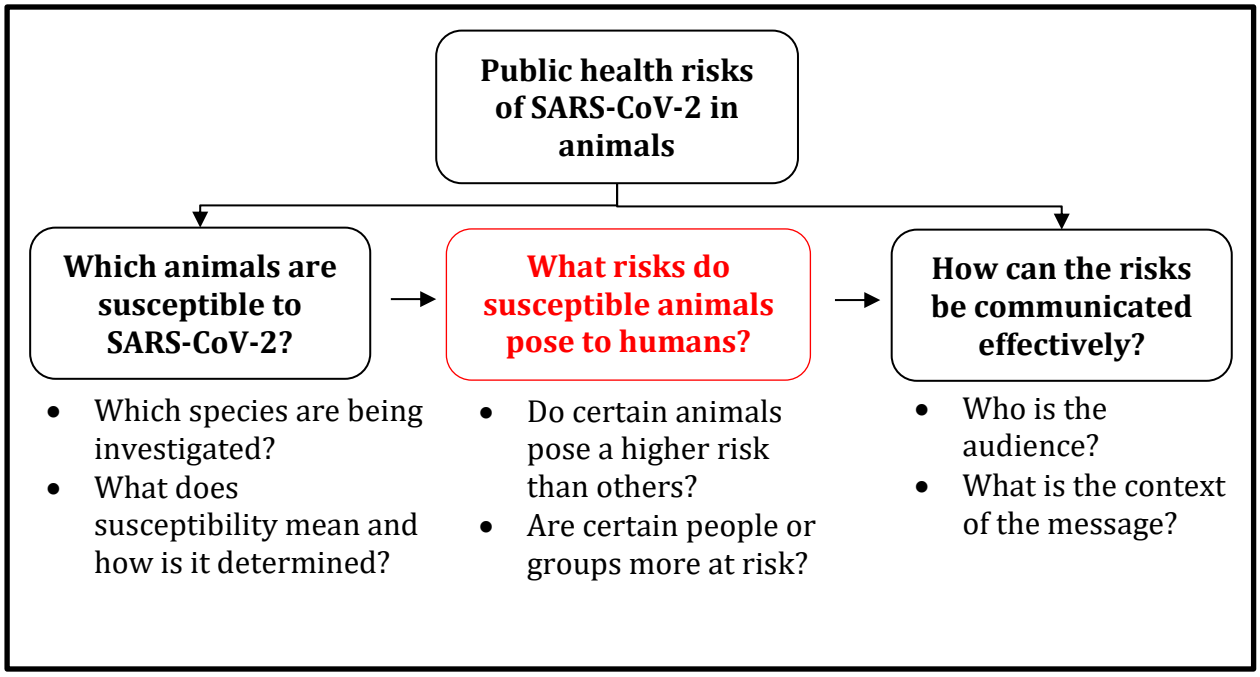
Next steps could include further scoping reviews with up-to-date sources, conducting systematic reviews where the different methods of evaluating susceptibility are evaluated and ranked, and/or meta-analyses for combining the results of select animal species based on their evaluated susceptibility. Of the species determined to be susceptible from *in vivo* methods, assessing them for natural exposure is a critical next step in determining their potential to become reservoir species, increasingly important as the pandemic becomes better managed in humans and the rise of variants threatens the efficacy of existing diagnostic assays and vaccinations. The breadth of information surrounding an animal species' susceptibility to SARS-CoV-2 is extensive and increasing. This scoping review demonstrated the utility and limitations of the rapidly expanding (and often overwhelming) literature evaluating susceptibility of animals to an emerging, global zoonoses, which can be helpful in planning and surveillance in the existing pandemic, and in preparing for future emerging disease events.

The limitations for this scoping review include the exclusion of non-English sources and missing relevant sources due to the sheer volume of literature. Moreover, as the last search for sources occurred in January 2021, there are likely new sources available that include animal species not presently included in this scoping review. Even with these limitations, this scoping review is important for those designing studies to determine animal susceptibility to a novel pathogen, and to efficiently target surveillance for potential animal reservoirs for SARS-CoV-2.

2.6 Acknowledgements

We would like to thank Susan Murphy, the University librarian who provided guidance on search terms and databases.

CHAPTER 3


Public health risks of SARS-CoV-2 in animals
Which animals are susceptible to SARS-CoV-2?
What risks do susceptible animals pose to humans?
How can the risks be communicated effectively?
Which species are being investigated?
What does susceptibility mean and how is it determined?
Do certain animals pose a higher risk than others?
Are certain people or groups more at risk?
Who is the audience?
What is the context of the message?

ABSTRACT

Background: Evidence continues to emerge on the susceptibility of wild animals, such as mink and white-tailed deer, to SARS-CoV-2. Wild animals with SARS-CoV-2 may expose humans, either as a bridging host, where SARS-CoV-2 infects a wild animal which then subsequently infects a human, or where SARS-CoV-2 becomes established in an animal population which then acts as a reservoir host, in which the virus establishes, evolves, and transmits among animals.

Methods: To determine the overall risk of an infected wild animal transmitting SARS-CoV-2 to humans, a qualitative risk assessment was developed with a focus on North American wildlife. This risk assessment followed the framework outlined by the World Organization for Animal Health (OIE) consisting of the following components: hazard identification, entry assessment, exposure assessment, consequence assessment, and risk estimation. In conjunction with the OIE framework, a qualitative scoring method was used to determine the likelihood of a specific event occurring. Information on wild animal susceptibility to SARS-CoV-2 was sourced from a recently completed scoping review and a literature search using the databases: PubMed, Global Health, and Web of Science.

Results: Four taxonomic families of North American wildlife (cervids, cricetid rodents, felids, and mustelids) demonstrated both susceptibility to SARS-CoV-2 (i.e. hosts in which virus actively replicates) and the capability of transmitting the virus to other animals. It was determined that the likelihood for a human to be exposed to SARS-CoV-2 from an infected species belonging to the four identified taxonomic families varied from moderate in cervids (largely because entry of the virus into wild populations has already occurred) to low or very low in the other families. Since consequences are highly dependent on human risk factors which could lead to variable disease outcomes, overall risk estimates ranged from very low to high for cervids, negligible to low for cricetid rodents, and negligible to moderate for felids and mustelids. Levels of uncertainty were medium to high in light of the emergent nature of the pandemic.

Conclusion: As SARS-CoV-2 is still novel, there are many unknowns which provide a high degree of uncertainty. In addition, if the virus mutates within a wildlife species, it may develop greater zoonotic potential, severity of human disease, or vaccine escape, increasing

the risk. Additional research into the infection and transmission of SARS-CoV-2 in wild animals is needed to address these uncertainties.

A RAPID QUALITATIVE RISK ASSESSMENT FOR HUMAN HEALTH RISKS POSED BY SARS-COV-2 IN NORTH AMERICAN WILDLIFE

3.1 Introduction

In the previous chapter, various animal species were identified as susceptible to SARS-CoV-2. Using these results, a subsequent risk assessment will address both the likelihood and consequences of exposure of SARS-CoV-2 from the most susceptible wildlife to humans.

A risk assessment is an iterative process where hazards or risks are identified and then evaluated on their potential to cause harm (187). Risk assessments can either be quantitative or qualitative in approach, depending on the risk in question, time frame, available data, level of uncertainty, and mandate (188). For a qualitative risk assessment, the risks are categorical instead of numerical as in a quantitative risk assessment (187). A qualitative risk assessment was chosen due to the novel nature and insufficient information surrounding SARS-CoV-2, especially with respect to animal exposure leading to human infection.

As described previously in the scoping review, species can be evaluated for their susceptibility to SARS-CoV-2 through four different methods. *In vivo* and epidemiological analysis have shown the most accurate representation of evaluating an animal species susceptibility to SARS-CoV-2, compared to *in silico* and *in vitro* analysis which are more suited to screening candidates for subsequent *in vivo* and epidemiological analyses. Therefore, only results from *in vivo* and epidemiological analysis were used to ensure accuracy.

The focus for this risk assessment is on North American wild animal species from taxonomic families which have demonstrated susceptibility to SARS-CoV-2. Susceptible animals present in North America have been identified through the scoping review, and wild animals (e.g. white-tailed deer and mink) in North America have tested positive for SARS-

CoV-2 viral RNA and/or antibodies. The knowledge of risk posed by SARS-CoV-2 infected wildlife is also relevant for subsistence harvesting and trading economies such as First Nations, Métis, Sahtú Dene, and Inuit ethnic groups in North America, who still rely on up to half of their food intake from wild animals (189,190). Therefore, assessing the risk (likelihood and severity) of SARS-CoV-2 exposure in humans due to a spillover event or from an established reservoir of wild animal species in North America is critically needed. In part, this may allay current community fears that wildlife are not safe to handle or harvest, as well as determine knowledge gaps for an emerging pathogen with high uncertainty. Through this rapid qualitative risk assessment, taxonomic families which have SARS-CoV-2 susceptible wild animal species inhabiting North America were focused on as they are most likely to maintain and transmit SARS-CoV-2 to humans.

3.2 Materials and methods

Risk assessment outline

The risk assessment began with hazard identification, which provides background information on the pathogenic agent responsible for causing disease, in this case SARS-CoV-2 and its variants (191). A rapid qualitative risk assessment that followed the framework outlined by the OIE was chosen (191). Although this framework is based on the importation of animals and animal products, the categories for this framework can be generically applied (191). The OIE risk assessment framework is comprised of four sections: 1) entry assessment, which is the entry of SARS-CoV-2 into a wild animal species; in this case, representatives of four identified taxonomic families in North America; 2) exposure assessment, which corresponds to the interaction and exposure of SARS-CoV-2 from a wild animal to a human; 3) consequence assessment, which assess the severity of SARS-CoV-2 infection in humans; and 4) risk estimation, a combination of the results from entry assessment, exposure assessment, and consequence assessment, to determine the overall risk of humans becoming exposed to SARS-CoV-2 from four different families of North American wildlife. In this risk assessment, risk is defined as, “the likelihood of the occurrence and the likely magnitude of the biological consequences of an adverse event or effect to human health” (191); hazard as, “a biological agent in an animal with the potential to cause

an adverse health effect in a human" (191); and exposure as, "having come into contact with a cause of, or possessing a characteristic that is a determinant of a particular health problem" (192).

Selecting families for the risk assessment

Taxonomic families were selected if the following two conditions were met: 1) At least one species found within the family was susceptible to SARS-CoV-2 infection (i.e. viral RNA or infectious virus has been isolated from the species and it is capable of transmitting SARS-CoV-2 in *in vivo* experimental infection studies or epidemiological analyses of naturally infected animals), and 2) The susceptible species is a wild animal naturally present and free-ranging in North America. For this risk assessment, a wild animal is one that does not rely on humans to provide food, shelter, or other needs in its daily life. After a family was identified, the known susceptible specie(s) were discussed, and other North American wildlife species belonging to that family were listed to identify other species that could serve as a susceptible host, given the high level of uncertainty for an emerging pathogen.

Likelihood and uncertainty categories

For each taxonomic family, likelihood categories were assigned for entry assessment and animal-human exposure assessment as per Table 3.1 (adapted from Rinchen et al., and Dejyong et al.,) (193,194). For each taxonomic family, the likelihood of entry and animal-human exposure were combined to create an overall likelihood of exposure which followed the matrix outlined by Dufour et al., and Dejyong et al. (188,194). Briefly, when two likelihood scores are combined, the new likelihood score can only be as high as the lowest score (188,194). For consequence assessment, a qualitative scale detailing the severity of SARS-CoV-2 infection in a human was developed (see consequence assessment section). For risk estimation, the levels of risk were determined through the matrix outlined by Dejyong et al., which was a combination of the likelihood categories for overall exposure and the varying severity levels (Table 3.3) (194). The corresponding level of risk categories are also defined in Table 3.1.

For each of the categories (entry assessment, exposure assessment, and consequence assessment), there is a corresponding, qualitative, uncertainty level assigned. This uncertainty level determines the confidence of that likelihood scale to prevent

misinterpretation, given the current literature and early stage of emergence of SARS-CoV-2 (Table 3.4). The uncertainty level for risk estimation was determined by taking the highest uncertainty level between the three assessment categories for each family.

Table 3.1. Likelihood categories for entry assessment, exposure assessment, and risk estimation for the risk assessment adapted from (193,194).

Categories	Definitions
Negligible	Likelihood of the event occurring is so rare that it does not merit consideration
Very low	Likelihood of the event occurring is extremely rare but cannot be excluded
Low	Likelihood of the event occurring is rare
Moderate	Likelihood of the event occurring is occasional
High	Likelihood of the event occurring is regular

Table 3.2. Matrix rules for determining overall exposure for the risk assessment (188,194)**.**

Likelihood of SARS-CoV-2 entry		**Likelihood of human becoming exposed**				
		Negligible	**Very low**	**Low**	**Moderate**	**High**
	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
	Very low	Negligible	Very low	Very low	Very low	Very low
	Low	Negligible	Very low	Low	Low	Low
	Moderate	Negligible	Very low	Low	Moderate	Moderate
	High	Negligible	Very low	Low	Moderate	High

Table 3.3. Matrix rules for determining estimate of risk for the risk assessment (194).

Overall likelihood of SARS-CoV-2 exposure		Severity of SARS-CoV-2 infection in a human			
		Very low	**Low**	**Moderate**	**High**
	Negligible	Negligible	Negligible	Negligible	Negligible
	Very low	Negligible	Negligible	Very low	Low
	Low	Negligible	Very low	Low	Moderate
	Moderate	Very low	Low	Moderate	High
	High	Very low	Low	Moderate	High

Table 3.4. Uncertainty categories used for the risk assessment.

Categories	Definitions
High	Evidence is sparse and/or contradictory and outside factors have a high chance of occurring and influencing the outcome
Moderate	Some evidence available with minor contradictions and outside factors may occur and influence the outcome
Low	Current and strong evidence available, outside factors unlikely to occur and influence the outcome

Data collection

Data for the risk assessment was identified through the analysis of peer reviewed and grey literature (non-peer reviewed or preprints), describing *in vivo* or epidemiological analyses. Literature from December 2019 - January 1st, 2021, was provided in Chapter 2 and literature between January 1st - July 17th, 2021, was sourced from a search of three databases (PubMed, Global Health, and Web of Science). The following search terms were used: SARS-CoV-2 OR COVID-19, and Experiment* OR Natural OR "in vivo" and Wildlife OR "Wild animal*" OR Animal*. Citations from review papers and reports of animals naturally infected from OIE were also investigated (195).

3.3 Results

Hazard identification

CoVs are classified in the order *Nidovirales*, family *Coronaviridae*, subfamily *Orthoconovirinae*, and then subsequently divided into four genera: *Alphacoronavirus, Betacoronaviruses, Deltacoronaviruses,* and *Gammacoronavirus* (15). All CoVs share four structural proteins: the spike (S), envelope, membrane, and nucleocapsid protein (9,196). The function of the S protein is to enter hosts cells through binding to a specific receptor; the envelope protein serves multiple functions including viral replication, assembly, and pathogenesis; the membrane protein is involved in formation of the virus particles; and the nucleocapsid protein surrounds the viral RNA and provides protection (9,196).

The SARS-COV-2 genome is comprised of 12 open reading frames (ORFs) which encode different proteins (197–199). ORFs 1a and 1b encode 12 non-structural proteins such as an RNA dependent RNA polymerase and helicase; the other ORFs encode structural proteins and various accessory proteins (197–199).

To enter a cell, SARS-CoV-2 uses the S protein to bind to the host cellular receptor angiotensin converting enzyme 2 (ACE2). The S protein is comprised of two subunits, S1 and S2. S1 contains the receptor-binding domain (RBD) which binds to ACE2, and S2 is involved in viral entry. Within the S2 protein, there is a furin-cleavage site which is cleaved by the host protease, e.g. TMPRSS2, after which viral entry can occur (199). After viral entry, the positive sense RNA genome will be released into the cell cytoplasm, where the replication machinery of the host cell will translate the genome at ORFs 1a and 1b, creating two polyproteins. These polyproteins will then be processed into individual non-structural proteins. These non-structural proteins are involved in replicating the SARS-CoV-2 genome to the negative sense strand. The negative sense strand will then be either replicated back to the positive sense SARS-CoV-2 RNA genome or be transcribed through discontinuous transcription, then translated to form various proteins. Following translation, the genome and proteins will be used to create new virions, which will be released through exocytosis and subsequently infect new cells (196,200).

SARS-CoV-2, like many RNA viruses, is prone to mutations in its genome. These mutations can occur either through viral replication or homologous recombination. During

replication, as SARS-CoV-2 lacks proof reading machinery, any errors made during replication will result in the production of mutated viral progeny. Homologous recombination occurs when two different CoVs infect the same host cell and their genomes become mixed, resulting in a novel virus. Both mutations can either be deleterious or beneficial. If deleterious, the progeny virus will be unable to replicate in cells and die, whereas if the mutation is beneficial, the progeny virus could have increased virulence, transmissibility, or an extended host tropism (200). Beneficial mutations that have occurred during the SARS-CoV-2 pandemic include the rise of variants of concern (VoC) like the Alpha, Beta, Gamma, and Delta variants (201). Variants also arose in SARS-CoV-2 infected animals. During the SARS-CoV-2 outbreak at the Netherland mink fur farms, the virus mutated from the strain originally introduced to the farm (202). For purposes of this risk assessment, all SARS-CoV-2 variants were grouped together, although recognizing that this is an oversimplification.

Entry assessment

For this section, taxonomic families which have free ranging representatives in North America that are susceptible to SARS-CoV-2 were investigated.

Taxonomic families identified

There were four families identified which met our selection criteria. These families were cervids, cricetid rodents, felids, and mustelids. An overview of the results for *in vivo* and epidemiological analysis for each of the families are presented in Tables 3.5 and 3.6.

Table 3.5. Overview of *in vivo* evidence for susceptibility to SARS-CoV-2 for the most susceptible animal families (experimental exposure).

Family	Species	Viral RNA	Infectious virus	Antibodies	Transmission	Pathogenesis	Sources
Cervidae	White-tailed deer	Detected in nasal, oral, and rectal swabs; nasal cavities, secretions, washes and turbinates; fecal samples, palatine tonsil, spleen, bronchi, lung lobes, various tissues, and lymph nodes. Viral RNA also detected in various deer fetal tissue	Detected in nasal, oral, and rectal swabs; nasal secretions and washes; bronchoalveolar lavage fluid, bronchi, and trachea	Detected through an indirect ELISA and a neutralization and Luminex assay	Antibodies, viral RNA, and infectious virus detected in direct and indirect contact deer	Microscopic changes resembling SARS-CoV-2 infection in human lungs, rhinitis, and mild to moderate lung damage. Fetuses in some deer became unviable	(35,117)
Cricetidae	North American deer mouse	Detected in oral, oropharyngeal, and rectal swabs, lungs, small intestine, colon, nasal turbinates and washes, urine, feces, brain, and blood	Detected in oropharyngeal and rectal swabs, lungs, nasal turbinates, small intestine, and colon	Detected through ELISA and neutralization assays	Viral RNA, infectious virus, and antibodies detected in direct contact mice	Detected in lungs and various other indicators of pathogenesis	(127,130, 180)
Felidae	Cat	Detected in nasal, oropharyngeal, rectal, and oral swabs, trachea, esophagus, nasal turbinates and washes, respiratory tract, bronchoalveolar lavage fluid, soft palates, tonsils, small intestine, and lungs	Detected in nasal and oral swabs, trachea, esophagus, nasal turbinates, soft palates, tonsil, and lungs	Detected through an ELISA and neutralization assays	Viral RNA, infectious virus, and antibodies in direct contact cats	Includes atelectasis, edema, and congestion in the upper and lower airways, lesions in the lungs and trachea, lymphoplasmacytic rhinitis, tracheitis, and interstitial lymphocytic pneumonia	(122,124, 129,131)
Mustelidae	Mink	Detected in nasal washes, ear and rectal swabs, nasal turbinates, soft palates, tonsils, lung lobes, and submaxillary lymph nodes	Detected in nasal washes, ear and rectal swabs, nasal turbinates, soft palates, tonsils, lung lobes, and submaxillary lymph nodes	Detected through a neutralization assay and ELISA	Viral RNA, infectious virus, and antibodies detected in contact minks	Severe lung lesions, nasal cavities containing mucous and neutrophil debris, and inflammation and necrosis in the respiratory tract, the nasal mucosa, and submucosa	(183)

Table 3.6. Overview of epidemiological evidence for susceptibility to SARS-CoV-2 for the most susceptible animal families (natural exposure).

Family	Species	Countries	Location(s)	Findings	Sources
Cervidae	White-tailed deer	USA	Wild and captive	SARS-CoV-2 antibodies detected in 40% (N=385) and viral RNA detected in 35.8% (N=360) and 33.2% (N=283) of deer	(203–207)
Cricetidae	North American deer mice	USA	Wild	Two mice had SARS-Cov-2 N1 gene detected, however, both N1 and N2 needed for positive confirmation	(182)
Felidae	Cat	Argentina, Belgium, Brazil, Canada, Croatia, Chile, China, France, Germany, Greece, Hong Kong, Italy, Japan, Latvia, Russia, Spain, Switzerland, Thailand, UK, US, and Uruguay	Domestic and feral	Cats naturally exposed to SARS-CoV-2 from humans or cats. SARS-CoV-2 viral RNA, infectious virus, and antibodies detected	(152,154–156,158,160,195,208–210)
	Cougar	US	Conservatory	Cougar positive for viral RNA	(31)
Mustelidae	Mink	Canada, Denmark, France, Greece, Italy, Latvia, Lithuania, Netherlands, Poland, Spain, Sweden, and US	Fur farms and the wild	Viral RNA detected on fur farms and in wild minks. On fur farms, minks succumbed to SARS-CoV-2 infection, antibodies detected, SARS-CoV-2 pathogenesis recorded, and mink to human transmission	(34,181,182,195,211)

Cervidae

Cervids, collectively known as the deer family, are hoofed ruminants (212). Within this family, only one species, the white-tailed deer (Odocoileus virginianus) has been investigated through both in vivo (experimental infection) and epidemiological analysis (natural infection) (117,203–207). Besides white-tailed deer, other wild cervids found in North America include moose (Alces americanus), mule and black-tailed deer (Odocoileus hemionus), caribou (Rangifer tarandus), Fallow deer (Dama dama) and elk, or wapiti, (Cervus elaphus) (212).

In the USA, SARS-CoV-2 antibodies were detected in 40% (154/385) of deer sampled through a surrogate virus neutralization test from four different states: Illinois, Michigan, New York, and Pennsylvania. Positive samples were also detected in 2020 and 2019; however, none were positive between 2011 to 2018 (203,207). In Ohio USA, 36% (129/360) of nasal swabs taken from wild deer in 2021 were positive for SARS-CoV-2 viral RNA (204,206). In Iowa USA, 33% (94/283) of retropharyngeal lymph node swabs taken from wild and captive deer between 2020 and 2021 were positive for SARS-CoV-2 viral RNA (205).

Cricetidae

The *Cricetidae* family belongs to the order *Rodentia* and is one of the largest mammalian families with over 700 species (213). Two cricetid rodent species that are free-ranging have been investigated for SARS-CoV-2 susceptibility, the North American deer mouse (*Peromyscus maniculatus*) and bushy-tailed woodrats (*Neotoma cinerea)*. Other cricetid species in North America include northern collared lemmings (*Dicrostonyx groenlandicus*), Ungava collared lemmings (*Dicrostonyx hudsonius*), Ogilvie mountains collared lemmings (*Dicrostonyx nunatakensis*), Richardson's collared lemmings (*Dicrostonyx richardsoni*), Sagebrush voles (*Lemmiscus curtatus*), Nearctic brown lemmings (*Lemmus trimucronatus*), Rock voles (*Microtus chrotorrhinus*), Long-tailed voles (*Microtus longicaudus*), Singing voles (*Microtus miurus*), Montane voles (*Microtus montanus*), Prairie voles (*Microtus ochrogaster*), Tundra voles (*Micotus oeconomus*), Creeping voles (*Microtus oregoni*), Meadow voles (*Microtus pennsylvanicus*), Woodland voles (*Microtus pinetorum*), North American water voles (*Microtus richardsoni*), Townsend's voles (*Microtus townsendii*), Taiga voles (*Microtus xanthognathus*), Southern red-backed voles (*Myodes gapperi*), Bank

voles (*Myodes glareolus*), Northern red-backed voles (*Myodes rutilus*), Common muskrats (*Ondatra zibethicus*), Heather voles (*Phenacomys intermedius*), Northern bog lemmings (*Synaptomys borealis*), Southern bog lemmings (*Synaptomys cooperi*), Northern grasshopper mice (*Onychomys leucogaster*), Keen's mice (*Peromyscus keeni*), White-footed mice (*Peromyscus leucopus*), and Western harvest mice (*Reithrodontomys megalotis*) (212).

The North American deer mouse (*Peromyscus maniculatus*) has been shown to be susceptible to SARS-CoV-2 infection through *in vivo* experimental exposure (127,130,180). For natural exposure, the SARS-CoV-2 N1 gene was detected in an oral or rectal swab from two wild deer mice; however, for this test to be positive, both the N1 and N2 gene were needed, and neutralizing antibodies were not detected (182). The bushy-tailed woodrats have been determined to be susceptible though *in vivo* analysis; however, transmission was not assessed and therefore were not included in Table 3.5 (180).

Other *Cricetidae* species not found in North America but highly susceptible to SARS-CoV-2, include golden hamsters (*Mesocricetus auratus*) and three dwarf hamsters species, the Roborovski dwarf hamster (*Phodopus robovskii*), Campbell's dwarf hamster (*Phodopus campbelli*), and Djungarian hamster (*Phodopus sungorus*). Golden hamsters presented with detectable clinical signs, viral RNA, infectious virus, increased cytokine and chemokine expression, pathogenesis, antibodies, and transmission (99,134,135,137,142). Golden hamsters have also been utilized as an animal model for SARS-CoV-2, testing vaccines and therapeutics (214–216). In infected dwarf hamsters, clinical signs, viral RNA, infectious virus, and pathogenesis were detected. Of the dwarf hamsters, the Roborovski dwarf hamster was most affected, and had to be euthanized before the set end date for humane reasons.

Felidae

Felids encompass large and domestic cats; free ranging felids in North America include feral or semi wild cats (*Felis catus*), Canadian lynx (*Lynx canadensis*), bobcats (*Lynx rufus*), and cougars (*Puma concolor*) (212). Although there are no reports of wild felids in North America naturally infected with SARS-CoV-2, there is overwhelming evidence of experimental and natural infections in domestic cats and captive large cats world-wide (122,124,129,131) (195).

In Italy, 11 out of 191 domestic and stray/feral cats tested positive for SARS-CoV-2 neutralizing antibodies (156). In Minnesota USA, 19 out of 239 serum samples from domestic cats were positive on an ELISA and 15 were positive on a neutralization assay (208). In Texas, USA, viral RNA/infectious virus was detected in rectal, respiratory, or body swabs from 3 of 16 cats with at least one infected owner, and neutralizing antibodies were detected in seven cats (209). In Wuhan China, antibodies were detected in 2 of 10 cats (ELISA and a neutralization assay); in another study, antibodies were detected in 15 (ELISA) and 11 (neutralization assay) of 102 cats, and viral RNA was detected from nasopharyngeal and rectal swabs from 7 cats (152,160). In France, 8 out of 34 cats living with SARS-CoV-2 positive owners were positive on immunoassay, as compared to 1 of 16 cats living with owners of unknown SARS-CoV2 status (154). In another study in France, antibodies and viral RNA in a rectal swab were detected in 1of 22 cats expressing clinical signs of SARS-CoV-2 (210). In Germany, 920 serum samples from cats were tested, six were positive using ELISA, two were positive through a neutralization assay, and eight were positive through an indirect immunofluorescence assay (155). In Spain, viral RNA was detected in an oropharyngeal swab from one of eight cats (158).

Among the large cats, the most notable outbreak occurred at the Bronx Zoo in New York, US, where five tigers and three lions were positive. Other large cats from around the world have tested positive for antibodies, viral RNA, or infectious virus of SARS-CoV-2 including pumas (Argentina and South Africa), lions (Estonia, Sweden, and USA), tigers (Sweden and USA), snow leopards (USA), and a cougar (USA) (31,195).

Mustelidae

Mustelids are a family of semiaquatic, terrestrial, arboreal, and burrowing carnivorous mammals (212). Mustelid species found in North America include wolverines (*Gulo gulo*), American martens (*Martes americana*), Fishers (Martes *pennanti*), Sea otters (*Enhydra lutris*), North American river otters (*Lontra canadensis*), Ermines (*Mustela erminea*), Long-tailed weasels (*Mustela frenata*), Least weasels (*Mustela nivalis*), Black-footed ferrets (*Mustela* nigripes), American mink (*Neovision vision)*, and American badgers (*Taxidea taxus*) (212). Several species within this family have shown high susceptibility to SARS-CoV-2 infection. American minks (*Neovision vision)* have been one of the more prominent species, with recorded experimental and natural infections. American minks are

free ranging throughout Canada and much of North America. American minks are also bred for their fur, with fur farms located in various areas of the world, including Canada and the USA (195,217).

Natural infection of farmed mink has occurred in 12 different countries (195). The most well-known outbreak of SARS-CoV-2 in mink farms occurred April 2020, in the Netherlands. Here, the mortality was 2.4% on the first farm and 1.2% on the second; during this time period, the average mortality was approximately 0.6%. Clinical signs in the mink included respiratory distress and nasal discharge. Mink that succumbed to SARS-CoV-2 infection had interstitial pneumonia detected during necropsy and viral RNA was detected in the lungs, conchae, throat and rectal swabs, liver, and intestines. Based on serological analysis, SARS-CoV-2 infection was widespread but self-limiting, resolving when most of the mink developed antibodies. Furthermore, at these farms, mink to human transmission occurred (34). In Canada, there have been two outbreaks of SARS-CoV-2 on mink farms (211). In the USA, mink that escaped from a farm tested positive for SARS-CoV-2 antibodies and a subset harbored viral RNA which matched the strain isolated from an outbreak at a nearby mink farm (182,195). In Italy, low levels of viral RNA were detected in mesenteric lymph nodes of 2 of 13 trapped wild mink from November 2020 to January 2021. Both mink were located in different areas, separated by a mountain range, and each roughly 20 km from the nearest mink farm. The authors hypothesized the minks were exposed from virus in fecal waste shed by infected humans (181).

Other susceptible mustelids include ferrets, identified through *in vivo* analysis, detection of viral RNA, infectious virus, or antibodies, and demonstration of both direct and indirect transmission. Natural infection has also occurred where a pet ferret was positive for viral RNA (118,122,139,149,195,218). Another mustelid species infected with SARS-CoV-2, although not found naturally in North America are Asian small-clawed otters (*Aonyx cinereus*) which acquired SARS-CoV-2 naturally in a Georgia aquarium; the otters presented with clinical signs and viral RNA was detected (195,219)

Entry of SARS-CoV-2 into wild animal species and uncertainty scales

For each family, the likelihood of a wild species becoming infected with SARS-CoV-2 is shown (Table 3.7). Cervids were given a High likelihood of SARS-CoV-2 entry based on the evidence of SARS-CoV-2 infection in wild species and species within this family can be found

in herds which increases the likelihood of transmission. Both felids and mustelids received a medium likelihood of SARS-CoV-2 entry. For mustelids, although viral RNA has been detected in wild mink, this appears to be an isolated occurrence associated with an outbreak in captive mink. As seen from the mink farms and *in vivo* studies, mustelids are susceptible and escaped infected farmed mink could expose wild mustelids. SARS-CoV-2 has not been detected in wild or free ranging felids. Yet, these species have been shown to be susceptible and there is evidence of domestic and captive felids becoming infected. Thus, there is potential for wild felids to become infected as well. Cricetid rodents received the lowest likelihood as the evidence for species in this family is limited to a partial PCR positive in a natural population, but SARS-CoV-2 entry could occur. The uncertainty categories ranged from Low to High and were based on available evidence.

Table 3.7. Likelihood of SARS-CoV-2 entry in the four most susceptible animal families.

Family	Likelihood of SARS-CoV-2 entry	Uncertainty
Cervidae	High	Low
Cricetidae	Low	High
Felidae	Moderate	Medium
Mustelidae	Moderate	Medium

Exposure assessment

This section describes the likelihood of an infected wildlife species (from the families identified above) exposing a human to SARS-CoV-2, which could then lead to human infection. Specifically, addressing how SARS-CoV-2 exposure could occur using examples from both humans and animals, the survivability of SARS-CoV-2 in the environment, and the risk factors that could lead to an increased chance of exposure.

SARS-CoV-2 animal-human exposure

SARS-CoV-2 animal-human exposure would occur through an infected animal transmitting infectious virus to a human. This could occur through different pathways like aerosol, direct, or indirect contact (the latter includes droplets). While some of these routes are more speculative, there are some established pathways for SARS-CoV-2 exposure (220).

Aerosol / droplet

By definition, aerosol transmission involves the airborne spread of particles less than 5μm in diameter (221–223). Aerosolized infectious particles remain suspended in the air and can travel long distances (223). While in the air, these particles can then be inhaled by an individual or settle on a surface and be introduced into the body through fomites (220,223). Aerosol transmission of SARS-CoV-2 has been hypothesized in both humans and animals (117,139,142,218,220,223,224). In hospitals, viral RNA has been detected in patient rooms, on air outlets and grates, and isolation rooms. Examples of human infection from potential aerosolized SARS-CoV-2 virions include: 1) groups of people becoming infected in a restaurant separated more than 1m from each other and the infected individual; 2) SARS-CoV-2 spreading to different passengers on a bus; and 3) an individual becoming infected from passing the room of an infected individual with no direct contact (220,223,224). For animals, evidence of potential aerosol transmission of SARS-CoV-2 has been demonstrated in ferrets (139,218), white-tailed deer (117), and hamsters (142).

Exposure to SARS-CoV-2 through droplets is the most common form of SARS-CoV-2 exposure, where droplets are larger than 5μm and can only travel a distance of approximately 1m. Droplets may be inhaled, come into contact with the eyes, or settle on a surface and be transmitted through fomites; for example, viral RNA was detected in 70% of samples from swabbing the floor of an ICU with COVID patients (220,223,225,226). During

the outbreak of SARS-CoV-2 at the mink fur farms in the Netherlands, environmental testing revealed viral RNA laden dust, indicating that exposure through droplets may have contributed to the spread of the virus in ferrets and humans (34).

Fomite

Exposure to SARS-CoV-2 fomites can occur when the virus is transferred from a contaminated surface to the eyes, mouth, or nose (220,225,227). SARS-CoV-2 has been detected on frequently touched surfaces such as door handles, phones, toilet seats, and tables; however, the amount of time the virus can survive on different surfaces varies, with stainless steels and plastics favouring SARS-CoV-2 survival (222,225). Furthermore, on certain surfaces, SARS-CoV-2 can survive longer than in the air (222,225). Exposures that have occurred in public areas may involve fomite as well as respiratory routes (223). In other instances, exposure to fomites has been predicted to be the major source, such as on the Diamond Princess cruise ship (225). Viral RNA but not infectious virus, has been detected on body swabs of cats and dogs (209). Another possible route of direct zoonotic exposure could result from handling infectious tissue or organs from animals. To date, this has not been documented for SARS-CoV-2 but has been described for both SARS-CoV-1 and MERS (228).

Feces

Exposure to SARS-CoV-2 contaminated feces has been suggested as a possible pathway. This has been predicted due to detection of live virus and viral RNA in fecal samples and viral RNA in wastewater (229,230). Furthermore, humans can shed viral RNA and infectious virus through the feces after they have ceased shedding from the respiratory system (220,223). Exposure to SARS-CoV-2 from feces could occur from contaminated hands introducing the virus to the nose, mouth, or eyes, or from the virus becoming aerosolized in fine particles or droplets and contaminating the surrounding area (228). Untreated wastewater was a documented source of infection for SARS-CoV-1, where virus became aerosolized from waste pipes where it was then inhaled by other residents in an apartment complex (228).

Viral RNA has been detected in wild mink and bivalves, where the exposure was hypothesized to have occurred from viral shedding in improperly treated human feces (181,229). To date, there have been no records of humans being infected with SARS-CoV-2 though feces. As SARS-CoV-2 is a relatively fragile, enveloped virus, which would not survive

for an extended period of time, exposure from feces may be less likely to occur, especially in treated wastewater (229,231,232).

Ingestion

Currently, ingestion of SARS-CoV-2 is not believed to be a significant pathway, with no foodborne human cases reported (220,228,233). There have been reports of contamination of food packaging materials with SARS-CoV-2 (233). Three *in vivo* studies have evaluated SARS-CoV-2 infection through ingestion. Two studies evaluated rhesus macaques, with one finding no evidence of infection, whereas the other found SARS-CoV-2 could actively replicate and cause infection. The third study, in Syrian hamsters, found that infection could be established following ingestion; however, disease was less severe compared to intranasally inoculated hamsters (126,135,234).

SARS-CoV-2 survivability in the environment

SARS-CoV-2 is an enveloped virus; therefore, it does not have the same durability as non-enveloped viruses in the environment. Changes in temperature and humidity can drastically reduce survivability and spread, with lower humidity correlating with a 6-fold increase in local SARS-CoV-2 infections (220). Experimentally, there have been mixed results assessing SARS-CoV-2 survival at different temperatures and humidity's. In one study, a mixture of SARS-CoV-2 in nasal mucus or sputum on polypropylene disks at a concentration of 10^5 TCID50 survived longer at 4°C and 40% relative humidity (RH) than at 21°C at 40% RH or 27°C at 85% RH (235). Viral RNA was detected for over 7 days in all conditions but infectious virus had a half life of 3.3 hours in nasal mucus and 5.8 hours in sputum; authors predicted SARS-CoV-2 would remain infectious on surfaces for more than 10 hours in all conditions (235). Another study assessed surface stability of $1.58x10^7$ TCID50 of SARS-CoV-2 at 4°C, room temperature, and 30°C, all at 30-40% RH. One hour after application, the infectivity of the virus was reduced by 100 fold; however, decline became more stable and infectious virus could be isolated after 190 hours (236). The authors found that the half-life for infectious virus was the highest at 30°C (17.9 hours), then 4°C (12.9 hours), and lastly room temperature (9.1 hours) (236). Exposure to UV sunlight is also predicted to reduce the durability of the virus (225,237). With these different conditions affecting SARS-CoV-2, exposure is more likely to occur in an indoor environment.

Risk factors for increased likelihood of exposure from wildlife to people

For most humans in North America, close interactions with wildlife that could lead to zoonotic exposure are relatively uncommon; however, some professions or hobbies may put humans (and animals) at a higher risk. This includes humans who interact with wild animals regularly within 1m distance including researchers in the field, workers in wildlife rehabilitation centres and zoos, individuals who trap or harvest wild animals, those who work with game-ranched cervids or captive mink, or individuals who bait and feed wild animals. Domestic animals may also interact with wild animals, acting as a source of infection (bridging host) between people and wild animals. For individuals like wildlife rehabilitators, exposure to SARS-CoV-2 may also occur in an indoor environment where the survivability of SARS-CoV-2 could be increased, leading to a higher chance of exposure.

Other factors that can impact the likelihood of SARS-CoV-2 exposure include density of the animals, the transmissibility and zoonotic potential of the particular variant involved, and the length of viral shedding. For animal density, animals who are solitary (such as lynx) have a lower likelihood of becoming infected and therefore a lower chance of exposing a human to SARS-CoV-2 compared to animals that are found in groups or herds (32,179,238–240). Some variants have increased likelihood of transmission and/or broader host range, such as the variants from the B.1.351 and P.1 lineages which have demonstrated active replication in BALB/c and C57BL/6 mice, whereas the original strain could not establish in mice (185). From *in vivo* studies, the longest shedding of infectious virus was six days in mink (inoculated and contact), six days (inoculated) and nine days (contact) in deer mice, five days (inoculated) and nine days (contact) in cats, and five days (inoculated and contact) in white-tailed deer (34,35,117,130,131,183).

Likelihood of exposure and uncertainty scales

For each identified family, the likelihood of an infected wild species from that family exposing a human to SARS-CoV-2 was categorized (Table 3.8). When assigning categories, the best available evidence for how SARS-CoV-2 exposure could occur (most likely aerosol and droplet, less likely from feces, fomites, and ingestion) along with environmental survivability of SARS-CoV-2 and potential for human interaction was considered. Species with a peridomestic relationship, such as cervids which are baited, petted, and hunted, were

more likely to interact with humans. Therefore, cervids were determined to have a moderate chance of exposing humans to SARS-CoV-2. For cricetid rodents, although they can live closely with humans, people are unlikely to handle them and therefore the risk may be through shedding infectious virus in their feces, which has been seen previously with hantavirus. This has not been established for SARS-CoV-2; thus, it received a likelihood of Very low. For wild felids, given their solitary behaviour and avoidance of humans, the likelihood for animal-human transmission was deemed low. For mustelids, although they are the only species to date that has exposed humans to SARS-CoV-2, this occurred on a fur farm inside a building and therefore this does not reflect the likelihood of infection from a wild mustelid in an outdoor environment, which was deemed low because both people and mustelids tend to avoid one another.

Uncertainty scores were medium to high due to lack of data on pathways of exposure in the outdoor environment, the potential for differing transmissibility and zoonotic potential of variants, and variations in the behaviour of wildlife species and people when interacting with wild species.

Table 3.8. Likelihood of human exposure to SARS-CoV-2 from the four most susceptible animal families.

Family	Likelihood of human exposure	Uncertainty
Cervidae	Moderate	Medium
Cricetidae	Very low	High
Felidae	Low	Medium
Mustelidae	Low	High

Overall likelihood of exposure

Below (Table 3.9), the overall likelihood of exposure from a species of one of the identified families was determined through combining the likelihood categories from entry and exposure assessment and applying the rules from the matrix outlined in Table 3

Table 3.9. Overall likelihood of exposure of people to SARS-CoV-2 through contact with wild animals from the most susceptible animal families, based on the combined entry and exposure assessment as per the matrix rules (188,194).

Family	Likelihood of SARS-CoV-2 entry (uncertainty)	Likelihood of human exposure (uncertainty)	Overall likelihood of exposure	Uncertainty
Cervidae	High (L)	Moderate (H)	Moderate	High
Cricetidae	Low (H)	Very low (M)	Very low	High
Felidae	Moderate (M)	Low (M)	Low	Medium
Mustelidae	Moderate (M)	Low (H)	Low	High

Consequence assessment

Clinical manifestations of SARS-CoV-2 in humans

An individual is most likely to become infected with SARS-CoV-2 through the inhalation of infectious droplets or aerosols. After inhalation, the virus will bind to the epithelial cells in the lungs and begin to replicate. Humans infected with SARS-CoV-2 can remain asymptomatic or present with varying symptoms. Although variable, between 25 to 45 percent of people infected with SARS-CoV-2 remain asymptomatic. Humans who are younger and without co-morbidities are less likely to present with symptoms. Infected humans will most often present with symptoms between 3 - 14 days after infection. Symptoms of SARS-CoV-2 infection include fatigue, fever, dry cough, anorexia, myalgia, sore throat, nausea, vomiting, potential conjunctivitis, mild pneumonia, the absence of taste or smell, diarrhea, and abdominal pain. In severe outcomes, symptoms may reflect organ failure, severe pneumonia, lung injury, acute respiratory distress, or death. These are often brought on by a cytokine storm caused by the rapid viral replication of SARS-CoV-2; additional severe and uncommon symptoms include stroke, seizures, encephalopathy, or rhabdomyolysis (199,241–249).

Risk factors that increase severity of infection

Risk factors that increase the severity of disease associated with COVID-19 have been well documented, especially increasing age. As of October 22, 2021, there have been 1,683,201 COVID-19 cases, 16,683 ICU admissions, and 28,457 deaths in Canada. Of those,

individuals aged 0 to 29 made up 671,223 (39.9%) cases, 716 (4.3%) ICU admissions, and 93 (0.4%) deaths, while individuals 70 years and older made up 145,305 (8.7%) cases, 5,339 (32%) ICU admissions, and 23,717 (83.3%) deaths (250). For the above results, co-morbidities were not considered. Co-morbidities which impact severity of infection include type I and II diabetes, high blood pressure, coronary heart disease, cerebrovascular disease, immunodeficiencies, and obesity. With an increase in age and the addition of co-morbidities, the chances of a severe outcome when infected with SARS-CoV-2 is further increased (199,251–256).

Other risk factors which contribute to an increased severity of infection include socio-economic-status (SES), race/ethnicity, sex/genetics, the environment, vaccination status, and dose. A lower SES results in increased health disparities like limited access to healthy foods or proper medical care thereby resulting in the increased chance of co-morbidities (257). Studies conducted around the world have demonstrated differences in outcomes of SARS-CoV-2 infection depending on race or ethnicity. For example, in the U.S.A., White Americans were less likely to die from COVID-19 than African Americans, Native Americans, and Latin Americans, and in the United Kingdom, White patients were less likely to be hospitalized than Black patients for SARS-CoV-2 (258). Certain groups being disproportionally impacted by infectious diseases is unfortunately not a new occurrence. Indigenous groups all over the world have suffered higher infection rates and deaths dating back to the 1918 influenza pandemic (259). During the H1N1 pandemic, Australia's Aboriginal people were five times more likely to become infected, and in Canada, First Nations peoples were three times more likely to be hospitalized (259). The reason for these disparities are multifold and include systematic racism which can lead to a lower SES and health disparities (258).

For sex, males are at an increased risk of a severe SARS-CoV-2 infection which is predicted to be due to increased expression of ACE2 and TMPRSS2 in type II alveolar cells (258). Variation in severity of outcome is also associated with differences in blood types, where individuals with blood type A have an increased risk compared to individuals with blood type O, or a genetic variation on chromosome 3, causing change in function in genes important in immune response and SARS-CoV-2 cellular entry (251,260,261). Environmental risk factors such as exposure to air pollutants lead to more severe adverse

health effects (252). Non-vaccinated individuals are also at a higher risk of developing more severe disease associated with COVID-19 infection (262–264). Finally, exposure dose has also shown to affect severity (265). Barring other factors such as co-morbidities, if an individual becomes infected from an asymptomatic or mildly symptomatic case, their symptoms will most likely be mild. Conversely, if an individual becomes infected from a case with severe symptoms, their symptoms are more likely to also be severe. This partially explains why certain healthcare workers without co-morbidities have developed severe symptoms.

Severity of SARS-CoV-2 infection in humans and uncertainty categories

Severity of disease associated with SARS-CoV-2 varies between humans (from totally asymptomatic to ICU admission and/or death). While increasing age and the presence of co-morbidities have been linked to severe outcomes, there are reports of seemingly healthy, young people being hospitalized or succumbing to SARS-CoV-2, which could be due to inoculum dose or genetics. Given the early nature of the literature on clinical disease associated with SARS-CoV-2, for all severity categories, an uncertainty of Medium was assigned. It was also acknowledged that the administration of vaccines will have a protective effect for those who are high-risk but the evolution of the variants raise the uncertainty level (Table 3.10).

Table 3.10. Severity of SARS-CoV-2 infection in a human based on severity of symptoms and care-seeking behaviour.

Severity	Expression of human diseases	Uncertainty	
Very low	No detectable disease	Medium	*Lower risk factors*
Low	Mildly symptomatic, self care	Medium	
Moderate	Moderately symptomatic, seeks care	Medium	
High	Severe symptoms, hospitalization or death	Medium	*Higher risk factors*

Risk estimation

Risk estimation and uncertainty scores

Risk estimation was determined by combining consequence assessment categories (severity) and the overall exposure categories (likelihood of SARS-CoV-2 entry and animal-human exposure) in order to determine the overall risk (likelihood and severity) of exposure of SARS-CoV-2 from a wild animal to a human (Table 3.11; rules for combining as per Table 3.3). The uncertainty scores (in brackets, M or H) were determined by taking the highest level of uncertainty in the consequence assessment and overall estimate of exposure. Cervids presented the greatest risk for humans; however, the estimate of risk varied depending on the severity of a SARS-CoV-2 infection in a human.

Table 3.11. Estimate of risk for a human exposed to SARS-CoV-2 from an infected wild species belonging to one of the four most susceptible animal families.

		Severity of SARS-CoV-2 infection in a human			
Overall likelihood of SARS-CoV-2 exposure		**Very low**	**Low**	**Moderate**	**High**
	Cervidae *Moderate*	Very low (H)	Low (H)	Moderate (H)	High (H)
	Cricetidae *Very low*	Negligible (H)	Negligible (H)	Very low (H)	Low (H)
	Felidae *Low*	Negligible (M)	Very low (M)	Low (M)	Moderate (M)
	Mustelidae *Low*	Negligible (H)	Very low (H)	Low (H)	Moderate (H)

Note. For each of the estimated risks, the uncertainty scores are described in brackets. Estimate of risk was found through the combination of overall exposure and consequence assessment with the rules outlined by (194).

3.4 Discussion

From this qualitative risk assessment, the estimate of risk (probability and consequences) of a person becoming infected with SARS-CoV-2 from exposure to free-ranging North American wildlife ranges from Negligible to High, depending on human risk status and wildlife family. Although the likelihood of SARS-CoV-2 exposure from a wild animal to a human is generally low, the consequences of such events can still be severe for high-risk individuals. Given the high level of uncertainty surrounding SARS-CoV-2 in both humans and animals, discretion should still be undertaken when interacting with wild species from susceptible families (cervids, mustelids, felids and some rodents), regardless of risk level.

Even though the likelihood of exposure was the same for people regardless of their risk factors for developing severe disease, as the potential consequences of a higher risk individual becoming infected were more severe, this led to a higher overall estimate of risk. Also, higher risk individuals could become infected with SARS-CoV-2 even if exposed to a lower concentration of the virus. For low-risk individuals, who are not likely to develop severe SARS-CoV-2 infection, the overall risk was Negligible for contact with felids, cricetid rodents, and mustelids. People exposed to SARS-CoV-2 from minks in a fur farm did develop respiratory symptoms; however, none succumbed to the virus or were hospitalized (266). Although their risk factors were not known, this demonstrates that exposure does not necessarily equate to a severe SARS-CoV-2 outcome. Similarly, with felids, the risk is Negligible for those who have lower risk factors; this does not come as a surprise as there have been no documented instances of transmission from domestic cats to humans. Further, various governmental agencies have determined that domestic pets like cats do not pose a risk to their owners who have no comorbidities or other individual risk factors for developing severe disease (however, for high-risk individuals there is still some concern) (267,268). With this in mind, it is likely that wild felids would represent even lower risk of exposure based on their infrequent contact with humans. For cricetid rodents, there remains little evidence to support infection in free-ranging populations, although sampling efforts are limited. Cricetid rodents (such as deer mice, *Peromyscus maniculatus)* have the capability of infecting humans with zoonotic diseases like Hantavirus, which can be transmitted through

contaminated feces (269). However, for SARS-CoV-2, the infectious virus shed in the feces would need to survive at a high enough concentration to infect an individual, which would be unlikely; in addition, people in North America already have high levels of concern about contact with rodent feces due to messaging regarding hantavirus.

The overall low estimate of exposure from wildlife was largely determined by the lack of documented animal to human exposure of SARS-CoV-2. Although SARS-CoV-2 has shown the ability to infect animal species from vastly different mammalian families, leading them to be considered susceptible, this does not translate fully into their wild animal counterparts, especially if infection has only been determined through *in vivo* analysis of captive animals. To date, SARS-CoV-2 viral RNA has only been detected in two native wild animal species from the listed families (mink and white-tailed deer), which led them to having higher likelihood scores. There have also been no records of wild animals exposing humans to the virus since the pandemic began, and only one incidence of human exposure of SARS-CoV-2 on a mink farm which likely occurred inside a building. Such conditions are very different from the outdoor environment where a wild mink would be found.

Furthermore, if a species were to be infected, based on the *in vivo* results, viral shedding would need to occur at a high enough intensity for sufficient duration to cause an infection. Based on the *in vivo* results and findings in animals that naturally acquired SARS-CoV-2, the infection appears to be self-limiting even in these highly susceptible species. Interpreting experimental results is challenging as studies used different methods for inoculation and contact (i.e. dose, route, indirect vs. direct contact transmission). There appears to be a short window of opportunity for an individual to come into contact with an infected animal, however, and a low likelihood that the animal is shedding infectious virus at a high enough dose for exposure to occur via the aerosol, droplet, and fomite routes. Furthermore, in an outdoor environment, viral survival and therefore risk of exposure are further reduced by increased airflow, the use of non recycled air, and exposure to UV; less than 10% of transmission occurs outside and odds of superspreading events are decreased (237). This low-risk of exposure may not apply to individuals who interact with wild animals on a frequent basis such as those engaged in subsistence harvest, veterinarians, field researchers, or wild animal rehabilitators, or in captive wildlife, especially those housed indoors.

In hunting, trapping, or harvesting societies, the risk of SARS-CoV-2 exposure from species that belong to these families (mustelids, felids, cricetid rodents, and cervids) is unknown. An infected animal that has a close encounter with an individual while alive has the potential to expose the individuals to infectious virus, albeit in an open-air environment. Based on *in vivo* and environmental studies, infectious virus can be isolated from different tissues, but it is not clear that there is a risk of transmission from carcasses, especially if aerosol generating procedures are avoided and processing occurs outdoors. Humans and animals can become infected with bacterial pathogens such as *Coxiella burnetii* and certain respiratory pathogens like *Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis,* and *Streptococcus suis* during the processing and slaughtering of animals, especially indoors (270–275). However, measures have been developed and employed to reduce the likelihood of a human becoming infected from an animal or animal by-product during commercial slaughtering or harvesting, such as donning PPE (276,277).

This risk assessment also revealed uncertainties surrounding animal infection and exposure to SARS-CoV-2. As SARS-CoV-2 is still novel, new information constantly emerges. Exposure to SARS-CoV-2 infectious virus is more likely to occur inside; however, in outdoor conditions, exposure has occurred and led to infection in stray cats, minks, and white-tailed deer. Exposure to infectious virus fecal-orally is possible, as viral RNA has been detected in waterways and infectious virus can be shed through the feces. Another large uncertainty is the capacity of animals, especially sub-clinically infected animals, to not only expose but transmit the virus back to humans; this may well be the case in white-tailed deer. Very few animals show clinical signs (with the exception of dwarf hamster, mink, and large cats in captivity), and those that do are often mild, and therefore, people may be at risk of exposure without knowing it. On the other hand, if the virus is unable to propagate in these mildly symptomatic animals to levels sufficient to infect humans, then they may not pose a risk.

The behaviour of the animals strongly influences the likelihood of interacting with humans and therefore risk of SARS-CoV-2 exposure. Felids are often solitary except during mating season or when a female is caring for her young; cervids are often found in herds (with the exception of moose); cricetid rodents can be either solitary or found in groups depending on the species and the season; mustelids, depending on the species, may be

solitary or in groups. Animals more likely to be present at high densities (like deer and deer mice) may support more active transmission within their species leading to higher levels of circulating virus in the air and on surfaces in their immediate surroundings. Farms where biosecurity is insufficient to prevent the mixing of captive and wild animals could also allow for enhanced transmission of the virus. This has been recorded in Utah, where wild mink were infected from mink that escaped a nearby fur farm. This could also occur on cervid farms, where wild and farmed cervids have been shown to interact (278). An additional uncertainty is the environment where the species are found, with higher risk of human exposure from species with a peridomestic or commensal relationship with humans, such as deer mice (cricetids) who can inhabit buildings occupied by humans, or deer fed by humans for tourism or hunting.

Variants are another uncertainty that can impact infection and transmission. For humans, the Delta variant has demonstrated increased transmission as compared to the original strain and spread relatively rapidly throughout the world (279,280). Furthermore, new variants may have broader host tropism compared to the original strain. For example, at the beginning of the pandemic, *in vivo* analysis demonstrated that house mice (*Mus musculus*) were not susceptible to SARS-CoV-2, and therefore transgenic mice or SARS-CoV-2 mice adapted strains had to be created. Now, variants in the lineages B.1.351 and P.1 have been shown to replicate in lab strain mice (185). If SARS-CoV-2 circulates in animal reservoirs, new variants will arise, either through mutation or recombination. These variants could spill back into the human population, such as is the case with MERS and dromedary camels (281), with unknown consequences for vaccine resistance.

Uncertainties also surround the consequences of SARS-CoV-2 transmission from an animal to human. Would the outcome mimic results of human to human transmission and thus be dependent on the individual's level of risk (high vs low)? Would animal-adapted strains result in more or less severe infections? Would these strains be outcompeted by human-specific strains of SARS-CoV-2? What effects would SARS-CoV-2 have on food security or the local economy if the affected animal species was used for subsistence hunting? Further work is needed to understand these different variables and outcomes (282). These and other uncertainties can contribute to misinformation and confusion surrounding SARS-CoV-2. Since the knowledge base continues to evolve, what was once

considered true may need to be updated or changed such as how different strains of mice can now be infected or whether deer will prove to be reservoirs of SARS-CoV-2 infection and mutation. If not kept up to date on evolving knowledge, an individual could become confused or misinformed on SAR-CoV-2 prevention.

3.5 Limitations and conclusions

Qualitative risk assessments are inherently subjective; however, this approach was chosen given the high level of uncertainty in the science and the rapidly emerging nature of the pandemic and literature, coupled with an urgent need for guidance about risks posed by SARS-CoV-2 in wildlife. New information on SARS-CoV-2 is being produced daily; therefore, the information used as the basis of this risk assessment may not accurately reflect the future of the SARS-CoV-2 pandemic and SARS-CoV-2 in wild animals. This includes but is not limited to, additional animals being determined as susceptible and/or capable of transmitting SARS-CoV-2 to humans, or the emergence of new variants of concern with differing zoonotic potential and human and animal pathogenicity. Also, there is the potential for species within the four families selected as being highly susceptible to SARS-CoV-2 to vary in their susceptibility to SARS-CoV-2. Furthermore, the results presented are only a snapshot in time. Also, the selection and definitions of the qualitative categories used and how they were assigned are based on the views of the author and may be interpreted differently by others. Bias may also be present, as the author previously wrote a scoping review investigating which animal species were determined to be susceptible to SARS-CoV-2, which influenced the choice of species/families in this risk assessment. The author also used a western-sciences perspective for classifying and selecting the taxonomic families, which may not accurately reflect what species/families and categories would be investigated by other groups such as those who utilize Traditional Knowledge.

To reduce the likelihood of SARS-CoV-2 exposure, which can lead to transmission to and from wildlife, guidelines outlined by OIE and the International Union for Conservation of Nature's Wildlife Health Specialist Group have been developed (283). In addition to these precautions, further field testing for both exposure and active infection of SARS-CoV-2 in species found within these families should be conducted with special consideration for

community-based research in marginalized and equity-deserving groups to better describe social, cultural, and economic consequences beyond severity of human infection. This will provide a better understanding of which wild animals are being naturally exposed to SARS-CoV-2, how SARS-CoV-2 is being transmitted to and through wildlife populations, and what future steps need to be taken to allow for more in-depth risk assessments to be conducted. Knowing which populations of animals are impacted is useful to inform key stakeholders and risk groups, including Indigenous and subsistence harvesters, rehabilitators, field researchers, hunters, trappers and other wilderness based occupations. At this time, SARS-CoV-2 is still largely transmitted between humans, and based on current evidence, there is a higher likelihood of a human infecting an animal than an animal infecting a human.

CHAPTER 4
KEY FINDINGS, DISCUSSIONS, AND FUTURE DIRECTIONS

In this thesis, I identified animals that are susceptible to SARS-CoV-2 and the risks that specific animal families pose to humans. I now discuss how confusion and misinformation could arise from the various definitions and methods used to determine susceptibility and the uncertainty regarding SARS-CoV-2. Below, the key findings from the two previous chapters are detailed along with an exercise in risk communication.

4.1 Scoping review of animal susceptibility to SARS-CoV-2: key findings and discussion

The most important findings from the scoping review were the identification of the contrasting evaluations and definitions of an animal species susceptibility.

The evaluated susceptibilities of animal species varied by sources using both similar and dissimilar methods, partially due to the capabilities of each method. For instance, *in silico* and *in vitro* can only interpret a small fraction of the infection process, most often viral binding and/or entry. A majority of the sources made claims on a single species susceptibility to SARS-CoV-2, while others ranked or compared the susceptibility among different animal species or taxa. Susceptibility was variably defined due to the different methods used and by different authors; both issues could lead to misinformation and confusion (Figure 4.1). It was concluded that *in silico* and *in vitro* methods are best suited to identify potential species that should be investigated further through *in vivo* or epidemiological analysis.

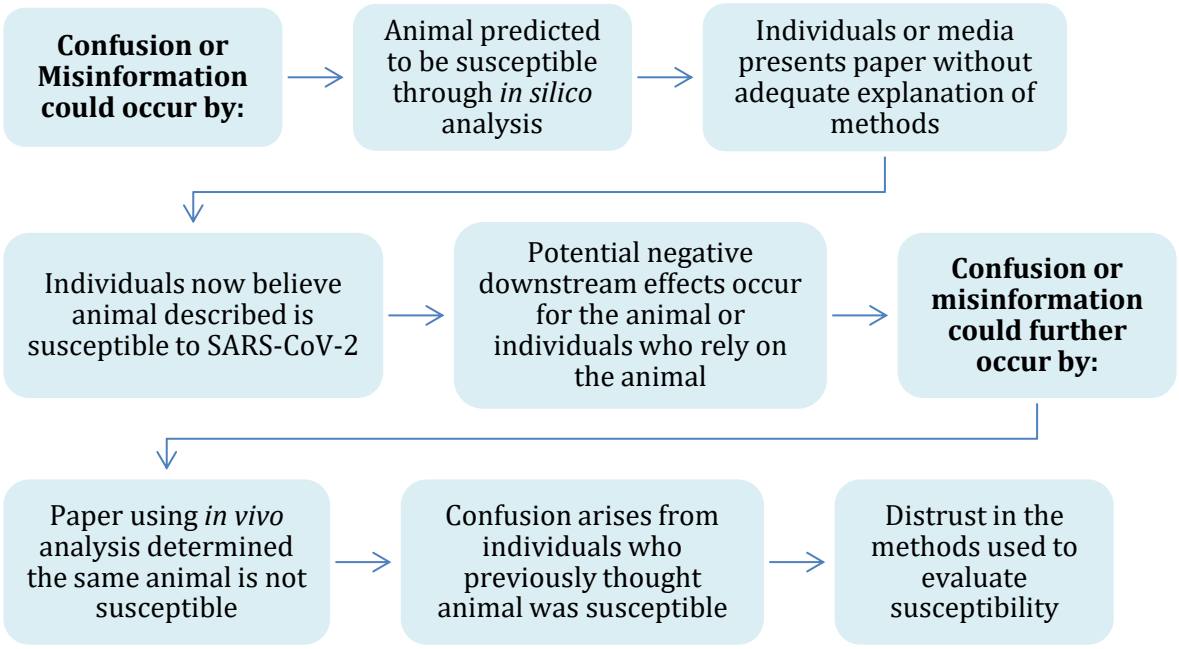
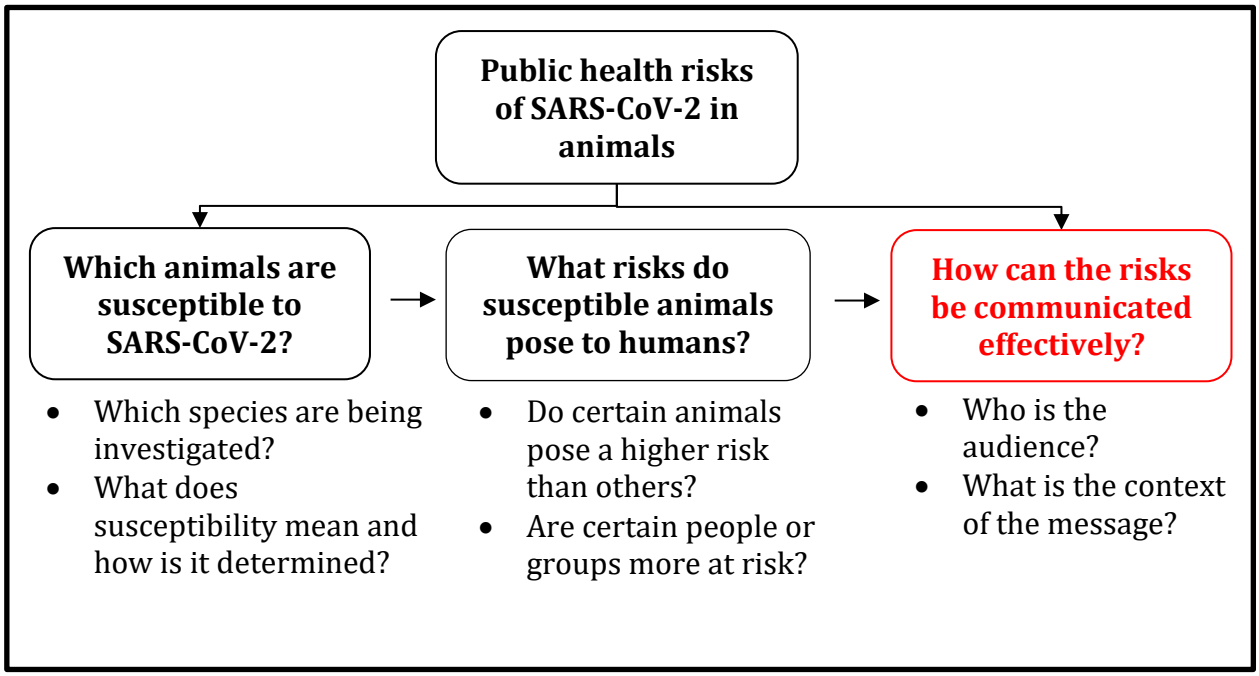


Figure 4.1. How misinformation and confusion occur through the variable definition of susceptibility and research method.

4.2 Risk assessment: key findings and discussion

Two key findings were identified in the risk assessment. 1) In North America, free-ranging wildlife species from four taxonomic families were identified as a potential risk of exposure of humans to SARS-CoV-2, especially cervids and 2) the estimate of risk (likelihood of exposure and consequence of infection) of human exposure to SARS-CoV-2 from these four families of wildlife varied from negligible to high. Apart from cervids, there was low likelihood of a SARS-CoV-2 infected animal exposing humans to the virus based on exposure in an outdoor environment, the fact that exposure of SARS-CoV-2 from a wild animal to a human has not been documented, and the solitary and human avoidant behaviour of wild felids and mustelids. Even with the unlikely chance of exposure, individuals identified as high-risk for developing severe SARS-CoV-2 disease should exercise caution when interacting with certain species. Uncertainties were also identified throughout the risk assessment based on the limited evidence and unpredictable nature of SARS-CoV-2; these uncertainties can also contribute to misinformation and confusion.

4.3 Risk communication exercise



The section below is based on my own personal opinions for areas to be cognisant of when communicating risks. This section is based off of my own experiences while completing my thesis and uses an approach similar to Grounded Theory (284). Grounded Theory is an approach to qualitative analysis where the theories are "abstracted from, or grounded in, data generated and collected by the researcher"; i.e. through inductive reasoning, data that is gathered and analysed is used to contrive a theory (283). The risk communication exercise in this thesis partially employs grounded theory for identifying poor and proper uses of risk communication, the "data," and, better practices, the "theory". I use a grounded approach to provide recommendations for areas to be aware of when communicating risks. In my analysis of these experiences, I was careful to abstract meaning from recurring patterns, relate the meaning to core lessons of risk communications, and unite my findings to the context and the potential for growth; that is, I followed a grounded theory process of abstraction (285,286). Once I completed my analysis of the risk communications I was involved in, I identified diversions from the application of those theories – the theoretical

sampling step in grounded theory – and relayed the pitfalls I observed. Although my approach aligns with grounded theory, it is not a fulsome application of the theory and was truncated given the time and resource constraints of my thesis. Grounded Theory is more complex in nature and involves many steps which are cyclical in nature. Areas within Grounded Theory that were not addressed include coding, purposive sampling, memoing, and sensitivity (284).

While participating in the Nunavik ringed seal project, I saw how risk communication can be used effectively for a specific knowledge user community. During the conference call the methods and the results of the monitoring program were shared in a culturally harmonized visual and auditory format by the individuals from the Makivik corporation. This included the use of appropriate imaging, text, and word choice. During the conference call, a translator was present to enhance inclusiveness.

Before I presented the infographics (Appendix A), my supervisor and individuals from the Makivik corporation reviewed the infographics and provided advice on how to best communicate the findings. These findings and recommendations included, using bulleted points and language that could be interpreted, instead of descriptive paragraphs. The bulleted points were also accompanied with related images. These could then be referred to if there was a misunderstanding from the bulleted points and serve as another tool for communicating the risk. For the *Toxoplasma gondii* and *Trichinella* spp infographics, an enlarged photo of the parasites lifecycle was requested. Showing the lifecycle, helped demonstrate how the parasites circulate in the environment and could be referenced while discussing the lifecycle. For the *Trichinella* spp. infographic, it was stressed and reiterated that while *Trichinella* spp have been identified in ringed seals, the parasite has not yet been identified in ringed seals in Nunavik (287). There have been *Trichinella* outbreaks in Nunavik in the past, involving walruses and polar bears, therefore, it would be quite harmful to insinuate or suggest that ringed seals are infected with *Trichinella* spp. By adding the "?" on the photo and writing that the role ringed seals play in the transmission of *Trichinella* is unknown, helped allay fears but also show that there is a degree of concern and uncertainty.

While discussing the infographics, it was important to be cautious of what I was saying, both so it could be translated effectively (avoiding jargon for which there would be no Inuit translation) and would not come across as culturally disrespectful; this included the

methods of prevention. For example, it would have been disrespectful to suggest or recommend that before consuming ringed seal, to always ensure it was fully cooked to a certain temperature. Consuming raw or undercooked seal has a high cultural significance within the Inuit community (287). Lastly, for the infographic that included the various parasites, besides having the taxonomic name listed the location of where the parasite was found in the seal was given. This proved to be helpful as one Inuit hunter described how he recognized some of the other parasites. The round table discussion provided the opportunity for multi-directional discussion of the risks among researchers and harvesters, who held varying points-of-view. For example, elders were more concerned about climate change and orca invasions than about the health of seals, which was the focus of the researchers. The ringed seal conference call demonstrated to me how proper risk communication should occur, and later in my thesis, the experience helped me identify areas where poor risk communication had taken place, ultimately leading to misinformation and confusion. Below is my view of poor risk communication based on the experience of conducting my thesis and how it can lead to misinformation and confusion.

Misinformation and confusion can occur from scientific articles on preprint sites or from previously published and subsequently redacted articles. Similarly, published scientific articles can be misinterpreted or the results misconstrued resulting in misinformation and confusion. Early in the pandemic (2020), two online articles described the potential susceptibilities of walruses and narwhals to SARS-CoV-2 based on their ACE2 receptors (288,289). Both articles referenced the *in silico* study by Damas et al., and as previously described, based on the limitations of the method, *in silico* analysis is unable to fully determine an animals susceptibility (89). Both preprint and *in silico* articles on SARS-CoV-2 suffer the potential that misinformation or confusion will occur as someone reading the articles could believe that either of these species may be infected or capable of transmitting SARS-CoV-2.

One of the news articles was republished in Nunatsiaq News in Iqaluit, the capital city of Nunavut (288). The article was originally published in Arctic Today where it was viewed over 6000 times (290). Nunavut has a population of 35,580 people; of those, 30,135 identify as Inuit (291). Within Inuit culture is the practice of consuming country foods which include harvested animals and plants from the land, water, and ice. Country foods have an

important cultural significance for Inuit (292). Both walruses and narwals are considered country foods and without proper explanation of the methods used by Damas et al. it is possible that an individual unfamiliar with the methods may read the articles and believe walruses and narwals could become infected and/or transmit SARS-CoV-2 (89,292) . While both articles do mention some of the limitations, the uncertainty arising from informed speculation may not be obvious for the public-at-large.

Another experience that I had during my thesis occurred during a webinar attended by researchers and Inuit community members that involved sharing of the same media article. Here the results again were portrayed in a way that made it seem like SARS-CoV-2 infected walruses and narwals were a high likelihood event, and the potential for transmission between humans and wildlife. The misinterpretation caused some individuals attending the webinar to become worried as the foods they consume may cause them to become ill.

Through the experiences of participating in the ringed seal project activities, viewing the two articles, and attending the webinar, I was able to identify key areas that are important for proper risk communication in this context. First, risk communication is not a one-size-fits-all practice; that is, there are different perspectives that need to be accounted for in a knowledge user community. Therefore, knowing about the knowledge user communities' characteristics and preferences is incredibly important. The background of the knowledge user community has equal importance. During the webinar, for example, if the researcher was only presenting to other researchers, then it may have been more appropriate to share the results of Damas et al. as there would be a better understanding of the limitations of *in silico* analysis. Instead, researchers and the general public, including Inuit hunters and harvesters, were on the call together, which made the accurate discussion of an *in silico* study contentious. This leads to the second point; that is, understanding the context within which the knowledge users will learn new information. As walruses and narwhals are country foods, the Inuit on the call may have believed they would need to change their dietary habits or risk becoming infected with SARS-CoV-2 as opposed to someone who did not rely on country foods nor lived near those animals. This is unfortunate; during the pandemic, especially in the North where commercial food shortages are common, and communities have become increasingly reliant on locally harvested wildlife. Although

there are other characteristics of risk communication that are important to be aware of as described in the introduction, these two concepts were made increasingly evident to me during my thesis experience.

Based on my findings from above, if I had the opportunity to communicate the risks of SARS-CoV-2 in cervids to a First Nations or Inuit audience in a single message, assuming this was a requested topic and appropriate form of messaging for the audience it would be, Antlers and hooves not COVID, what you need to watch out for when near deer, caribou, and moose. Through this messaging, my goal would be to demonstrate how at this time, cervids like white-tailed deer and caribou are not a source of human exposure for SARS-CoV-2. Instead, focussing on the known ways cervids could injure a human if they got to close with a humorous undertone.

4.4 Conclusions and future directions

The objectives of this thesis were: 1) to identify which animal species were susceptible to SARS-CoV-2 and 2) to identify the risks of SARS-CoV-2 transmission from wildlife to people in North America, and 3) to explore how to communicate these risks more effectively. The findings from the objectives revealed that animal species varied in their susceptibility to SARS-CoV-2 depending on the method used and the source. These findings led to the second objective, which found that certain taxonomic families, especially cervids, presented a greater risk of SARS-CoV-2 exposure to humans. The combined results also showcased the various uncertainties which can prevent accurate information sharing and conclusions about zoonotic transmission of SARS-CoV-2. As part of my thesis work, I encountered situations where good and poor risk communication occurred and identified areas to be aware of to ensure better risk communication. The SARS-CoV-2 pandemic has exposed various lessons on public and animal health and will serve as a learning opportunity for how human behaviour and the choices of individuals can drive the emergence of infectious diseases in the modern world.

Areas of concern that were identified include how misinformation and confusion can occur when the different methods used to evaluate an animal species susceptibility to SARS-CoV-2 are viewed by individuals or the media. At times, the methodologies or limitations in

scientific reporting are not fully understood by the public-at-large. This can also apply to researchers who may produce studies or give talks without realizing the downstream consequences of what they are reporting based on who may absorb their content. Both of these can lead to knowledge user communities questioning their food safety, which demonstrates the necessity of appropriate and balanced risk communication.

For the methods used in the thesis, the scoping review worked well, showing the range of classes and, the specific animal species that were being investigated. The risk assessment demonstrated knowledge gaps and uncertainties that exist in studying animal transmissibility, and the importance of recognizing and addressing these uncertainties. Both methods helped identify animals that should be further investigated, especially as more natural infection of animals occurs. Together, the scoping review and risk assessment provided a logical path for identifying animals that are susceptible, and then assessing the risks to humans and other animals. Even though numerous papers have been produced addressing SARS-CoV-2, being able to contribute to the literature with the scoping review is valuable. The scoping review identified the pros and cons of the different methods used, and the contrasts apparent in evaluated susceptibilities for certain species. The contribution of the scoping review also includes the illumination of limitations of the snapshot of studies published early in the pandemic.

Within this thesis exist limitations and biases both in my approaches and in the methods themselves. The scoping review and risk assessment are framed in a western sciences point of view which influenced how an animals susceptibility was evaluated, how an animal is identified as susceptible, and the animals that were chosen for the risk assessment. Using a western sciences point of view limited how this information will be taken up by knowledge user communities. An individual who does not follow a western sciences point of view may identify the risks or susceptibility to SARS-CoV-2 differently. Not consulting with other groups is a limitation as the only perspectives portrayed in the scoping review and risk assessment are my own and those of my co-authors, therefore, other researchers' interpretations about animal species or families that could have been included in the risk assessment may differ, as will their interpretation of the risk communication exercise.

Within the methods themselves, the limitations include the large number of papers identified in the first screening stage of the scoping review, which may have resulted in sources being missed during the review process. Also, as most of the *in vivo* analysis involved lab animals, this could have influenced the results of what animals would initially be considered as susceptible. Through completing the risk assessment, the families and animals chosen were influenced by which animals were identified as susceptible according to my view of susceptibility and there is a possibility that I missed other animals. By using families instead of individual species, I believe this helps cover species that may have been missed while discussing animals investigated through *in vivo* and epidemiological analysis. However, using family instead of individual species is also a limitation, as not all species within the family may have the same susceptibility as the example species. This could contribute to misinformation and confusion.

The next steps for this work could include an up-to-date scoping review on animal species that are susceptible to SARS-CoV-2 or the creation of a systematic review to evaluate the different methods and determine their accuracy. Further, the creation of a centralized website or message board that fully tracks animal species susceptible to SARS-CoV-2 could guide species selection for epidemiological analysis. A targeted risk assessment or analysis for wild animals or specific animal utilized by Indigenous harvesters (if invited to do so) or a different audience or group (such as wildlife rehabilitators, hunters, or those who interact with Canadian wildlife on a regular basis) could be conducted. With a targeted risk assessment or analysis, an understanding of where the risks of SARS-CoV-2 are more apparent and how these issues can be mitigated, allowing safer resumption of important activities including wildlife viewing, tourism, and subsistence harvesting. Also, increased epidemiological testing for animals identified as higher risk, such as cervids, would document progression of SARS-CoV-2 transmission and identify areas of potential concern for the general public or specific groups.

The experiences and knowledge I have gained throughout this thesis are multifold and are something I am grateful for. Although there have been various changes and updates made throughout, I am proud of this thesis. For other students who wish to undergo a public health thesis program, I strongly recommend it. I have learned to consider different viewpoints, overcome inexperience and challenges, and synthesize findings, three skills that

will serve me in the future. While completing the thesis, I learned that my committee and supervisor want me to succeed and are there to assist me – the thesis experience thereby facilitated my professional growth, networking, and interdisciplinarity skills. Also, I learned that theses are works in progress; that is, they undergo change, they adapt as the world does, and new ideas emerge; and need to, to face the challenges of this and future pandemics.

To other researchers and scientists working in this field, my thesis experience taught me that it is always important to remember that other views exist besides your own and everyone views the world differently. Therefore, when presenting your research, understand that it may be understood in a way that you are unaware of, more so, if it is being presented to a knowledge user community unfamiliar in that area of research. To help audiences avoid becoming misinformed or confused, it is important to express your limitations, and to be responsive and open to feedback.

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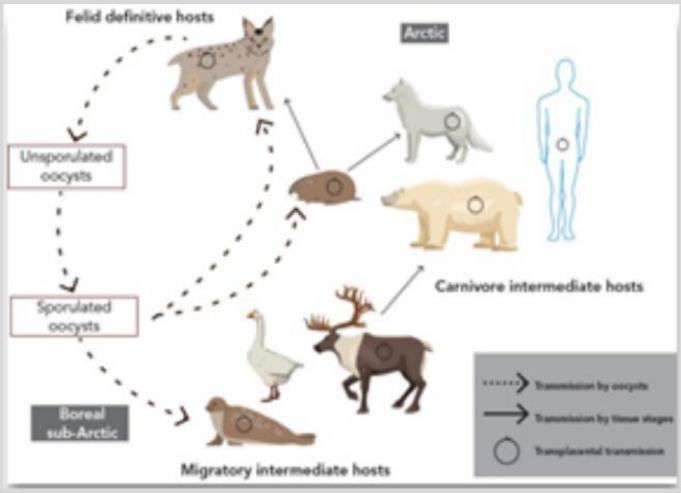
APPENDIX A: INFOGRAPHICS PRESENTED DURING THE RINGED SEAL SURVEILLANCE CONFERENCE CALL

Figures

Microbiological Hazards in Ringed Seals

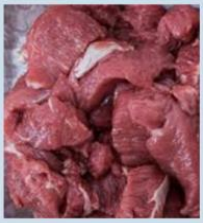
Toxoplasma gondii

Life cycle and Environment



- Felids are the definitive host
- Terrestrial spread of parasite occurs from migratory animals
- Marine spread of parasite occurs from spring runoff into the rivers and oceans

Transmission and Infection in Humans



Human infection can occur by:

- Consumption of raw or undercooked meat
- Improper cleaning of tools used in skinning or cleaning animals
- Transmission from a pregnant mother to her unborn child
- Very few or non specific symptoms

Prevention



Infection can be prevented by:

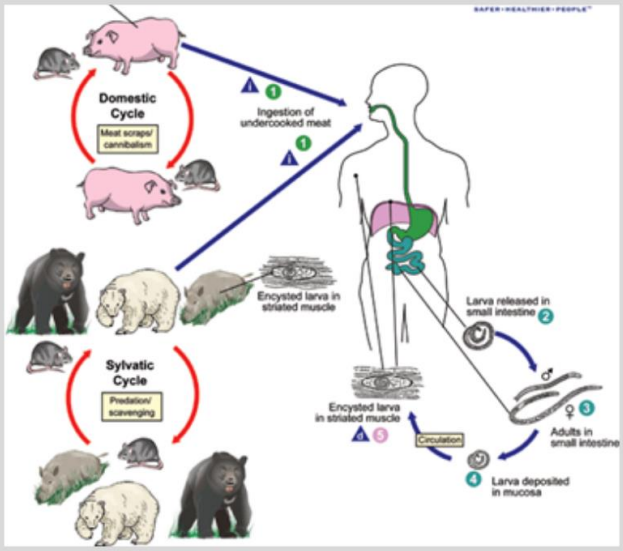
- Washing hands and tools with soap and water
- Freezing meat solid for at least 3 days
- Cooking meat untill it is well done

Figure A. 1. *Toxoplasma gondii* infographic presented during the ringed seal conference call.

Microbiological Hazards in Ringed Seals

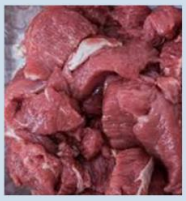
Trichinella

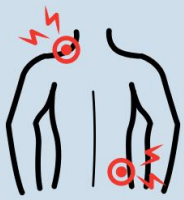
Life cycle and Environment



- Terrestrial and marine cycles
- Only found in animals that consume flesh
- Marine cycle between polar bears and walruses
- How ringed seals play a role in transmission and infection is still unknown
- The NRC tests for *Trichinella* in walruses

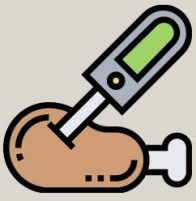
Transmission and Infection in Humans





- Humans become infected through the consumption of raw or undercooked meat
- Symptoms are non-specific
- Two phases of infection - intestinal and muscular

Prevention



- Cooking meat thoroughly is the only way of killing the parasite
- Feeding dogs cooked meat
- Destroying any meat that is contaminated
- Freezing meat is an ineffective method for killing the parasite

Figure A.1. *Trichinella* spp. infographic presented during the ringed seal conference call.

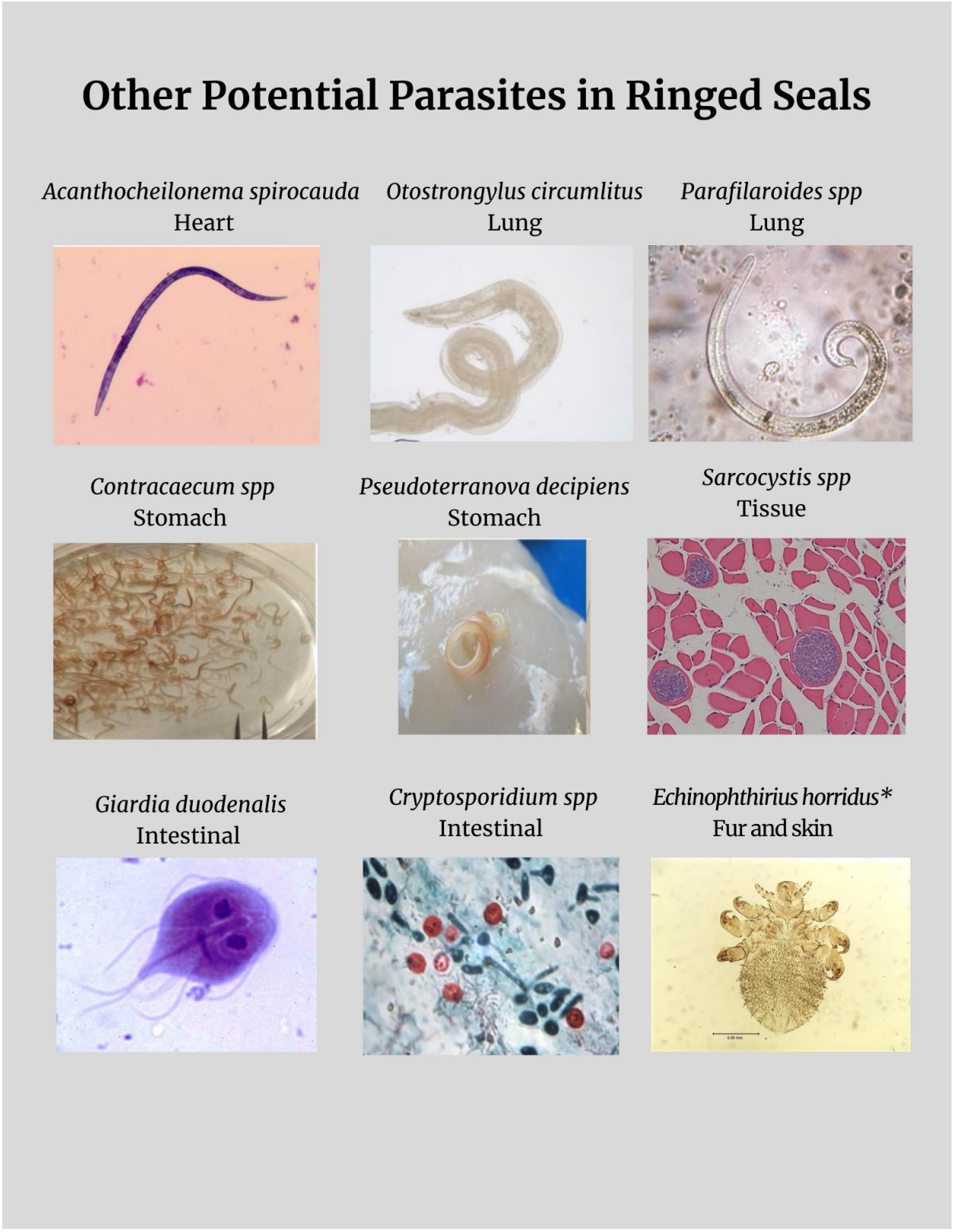


Figure A.2. Infographic of additional parasites potentially found in ringed seals presented during the ringed seal conference call.

APPENDIX B: SUPPLEMENTAL INFORMATION FOR SCOPING REVIEW

Electronic Supplements

In addition to the thesis are two Excel spread sheets.

ES B.1. Contains the individual characteristics of the sources selected for the scoping review. The characteristics of each source include: the first three authors, source title, date uploaded / published, document type, country of first author, susceptibility evaluating method, an overview of the methods used, number of animal species investigated, and an overview of the findings.

ES B.2. Contains the animal species investigated by each source. For each animal species, their class, scientific, and common name are listed. For each source, the first three authors are listed.

Figure

(COVID19 OR COVID-19 OR COVID2019 OR COVID-2019 OR SARSCoV2 OR SARS-CoV-2 OR SARS-CoV2 OR "SARS Coronavirus 2" OR 2019-nCoV OR 2019nCoV OR nCoV2019 OR nCoV-2019)
AND
(Animal* OR Wildlife OR Mammal* OR Bird* OR Reptil* OR Fish*)
AND
((Transmi* AND Infect*) OR (ACE2 OR ACE-2 OR "ACE 2"))

Figure B.1. Search terms for identifying literature sources in the Public Health Database searched from July 9th - 13th 2020 and December 30th - January 2nd, 2021.

Tables

Table B.1. The number of sources that investigated each taxonomic class sorted by susceptibility evaluating method (see Figure 2.3).

Class	*In silico*	*In vitro*	*In vivo*	Epidemiological
Mammalia	46	20	33	11
Aves	21	5	4	1
Insecta	0	1	1	1
Reptilia	13	2	0	0
Actinopterygii	6	1	0	0
Amphibia	8	0	0	0
Chondrichthyes	5	0	0	0
Coelacanthimorpha	4	0	0	0

Table B.2. Self-reported limitations from the sources selected for the scoping review, sorted by analysis method.

Limitations described by sources	References
In silico	
Susceptibility and risk were generalized to an entire species however intraspecies variation may exist in the ACE2 sequence which could alter a subspecies susceptibility	(72,77,84,86,104)
ACE2 isoforms may be present which could alter an animals predicted susceptibility	(87)
Only mammal's species used which had phylogenetic, ecological, and geospatial data available	(110)
More species should have been evaluated, however limited due to missing information, ACE2 receptors not found in databases, or phylogenetic, ecological, and geospatial data was unavailable	(79,86,90,104,110)
Majority of animal species only selected which were known to be infected with CoVs	(110)
Only RNA expression of ACE2 investigated did not consider protein expression	(87)
More to an animal's susceptibility than ACE2 expression	(87)
ACE2 expression levels were unknown	(73)
The crystal structure, the protein sequence, and what effects expression of TMPRSS2 is unknown which could impact susceptibility	(73)
Possible that other receptors besides ACE2 or regions on the ACE2 receptor are used by SARS-CoV-2 therefore susceptibility predictions may not be accurate	(80,84,89)

Table B.2. Cont'd	
Limitations described by sources	**References**
In silico	
There is more to an animal being susceptible besides the interaction between the SARS-CoV-2 RBD and the ACE2 receptor other factors that need to be considered, host immune response, factors which would allow virus to prosper, underlying health conditions, host behavior and number of contacts, age, atmospheric temperature, population density, airflow, ventilation, and humidity	(18,77,84,86,89,91,92,94,101,102,106)
Predictions based on the homology to the hACE2 receptor may under or overestimate the impact of single mutations, glycosylated residues, or other factors which could increase or reduce binding	(72,77,79,86,92)
That predictions should be further verified using additional information or methods like experimental infection, epidemiological investigations, biochemical or biophysical approaches	(18,19,70,72,79,83,86,89,98,101,102,107,109,111)
More data on how the mutations on the ACE2 receptors will impact binding	(89)
Different crystal structures of the hACE2 receptor binding to the SARS-CoV-2 RBD have been used to model an animal species ACE2 receptor binding to the SARS-CoV-2 RBD which can give different binding results	(100)
Only used CoVs with full length genomes used	(110)
How only one SARS-CoV-2 strain was used, and other strains or variants may have a different outcome	(84,102)
In vitro	
Species specific cell lines were not used	(95,96)
Possibility that other receptors besides ACE2 can be used	(95)
Binding and or entry does not equate to susceptibility additional factors that can impact an animal's susceptibility include entry after binding, other cellular factors, transmission of the virus	(92,95,96,112)
ACE2 polymorphisms present in an animal which can give conflicting results	(103)
Experimental infections or epidemiology analysis needed to further determine intermediate hosts	(69,114)
A pseudotyped virus system is limited in scope and does not allow for further experimentation	(17)
Only assessed ACE2 functionalities for determining host range other factors might also contribute	(92)
ACE2 sequences obtained from a database no experimental evidence that these genes can code a functional protein	(92)
Accurate predictions are difficult due to the lack of animal infection data and biochemical interactions between the SARS-CoV-2 RBD and ACE2 receptor of different animals	(83)
In vivo	
Small sample size	(118,126,138,151)
Experimental animals were young, healthy, and/or immunocompetent, animals naturally infected may respond differently to infection possibly having increased susceptibility, severity, transmission, or shedding	(115,116,118,129,130,132,136,146,150)

Table B.2. Cont'd	
Limitations described by sources	**References**
In vivo	
Differences in age, breed, and colony of animals between experimental studies can affect outcomes between studies	(151)
Comparison between rechallenged and primary challenged not conducted therefore unable to determine if the results from the rechallenged animals are from the primary or rechallenged infection	(145)
Inoculum doses differed or other doses needed to fully describe and understand patterns in pathogenesis	(99,126,127,151)
Variants or other strains can alter pathogenesis and susceptibility	(115,127,151)
Limited as an animal model as infection not fully replicated, no severe disease present, infectious virus not found, reinfection studies not conducted, or treatment group was not included	(117,118,125,129,135, 146,149)
Physiological body temperatures were not known prior to the study	(136,146)
Chemokines or cytokines expression were investigated partially or not at all	(99,146,148)
No positive control samples	(123)
Only SARS-CoV-2 spike gene sequences instead of whole genome	(99)
Could not determine transmission pathway aerosol, droplet, or both	(139)
Usure how transmission studies will relate to the real world including both animal to human and animal to animal	(130)
Pathology between animals could not be compared as animals sacrificed at different times and were outbred	(146)
Unsure how infection was established through oral inoculation	(135)
Unable to determine what areas of the immune system provided protection from reinfection	(125)
Epidemiological	
Small sample size	(154,156,157,159)
Experimental infections needed to further determine susceptibility	(119,153)
Neuter status unknown prevents better comparison with humans	(156)
Detection of viral RNA from oropharyngeal swab may have been due to cat coming into contact with viral RNA instead of viral infection	(157)
Cannot determine if virus actively replicated as no culture assays conducted	(157)
A focus should be on different ages and different degrees of viral load	(159)
Unable to estimate time of infection in the animals	(156)
Could only determine exposure could not determine naturally if dogs and cats can transmit or become infected with SARS-CoV-2	(152)
Missing SARS-CoV-2 infection status of pet owners	(155)

Table B.3. Sources that evaluated and described the susceptibility to SARS-CoV-2 of the six most investigated animal species from the scoping review, by evaluation method.

Species	Source ranking	*In silico*	*In vitro*	*In vivo*	Epidemiological
Cats N=47	**Not Susceptible**	(76)			(153)
	Very low susceptibility				(159)
	Medium / Intermediate susceptibility	(89,104,108)			
	Potentially susceptible	(75,80,82,98)			
	Susceptible	(17,69,70,74,78,81,87,88,90–92,94,96,97,100–102,110,111)	(17,69,81,88,92,96,112)	(129,131)	(10,30,152,154–157,160)
	Highly susceptible	(19,72,73,85,107)		(122,124)	
Dogs N=39	**Not Susceptible**	(74,76,78,104)		(113)	(153)
	Very low susceptibility			(124)	(159)
	Low susceptibility	(19,85,89,108)		(122)	
	Medium / Intermediate susceptibility	(73)			
	Potentially susceptible	(80,82,98)			
	Susceptible	(17,69,70,72,87,88,90,92–94,96,97,100–102,110)	(17,69,88,92,93,96,112)		(10,30,152,154,156)
Pigs N=31	**Not Susceptible**	(76,78,94,100)	(114,115,118)	(115,118,122)	(153)
	Low susceptibility	(19,89)			
	Medium / Intermediate susceptibility	(73)			
	Potentially susceptible	(80,90)			
	Susceptible	(69,70,72,88,91–93,96,97,101,102,108,110,111)	(69,88,92,93,96,112,121)	(151)	
House Mice N=31	**Not Susceptible**	(17,69,70,73,76,80–82,87,88,90–93,96,98,100–102,110,111)	(17,69,81,88,92,93,96,112,121)		(153)
	Very low susceptibility	(89)			
	Low susceptibility	(19,72,94,107,108)			
	Potentially susceptible	(75)			

Table B.3. Cont'd					
Species	**Source ranking**	***In silico***	***In vitro***	***In vivo***	**Epidemiological**
Ferrets N=24	**Not Susceptible**	(76)			(153)
	Very low susceptibility	(89)			
	Low susceptibility	(108)			
	Medium / Intermediate susceptibility	(73)			
	Potentially susceptible	(80,90)			
	Susceptible	(70,78,87,91,92,94,98,100–102)	(92,112,118)	(118,139,149)	(30)
	Highly susceptible	(19)		(122)	
European rabbits N=24	**Not Susceptible**	(76,98)			(153)
	Very low susceptibility				
	Low susceptibility				
	Medium / Intermediate susceptibility	(89)			
	Potentially susceptible	(90)			
	Susceptible	(17,69,70,74,78,81,88,92,94,97,101,102,106,110,111)	(17,69,81,88,92,112,116)	(116)	
	Highly susceptible	(19,72)			