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#### Risk assessment of SARS-CoV-2 infection in free-ranging wild animals in Belgium

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## **Summary**

The aim of this review paper is to evaluate the putative susceptibilities of different free-ranging wild animal species in Belgium to SARS-CoV-2 and provide a risk assessment of SARS-CoV-2 infection in those animals. Since the onset of the Covid-19 pandemic, natural SARS-CoV-2 infections have mainly been confirmed in domestic and production animals, and in wild animals kept in captivity, although the numbers remain limited when compared to human cases. Recently, the first SARS-CoV-2 infections in presumably escaped minks found in the wild have been detected, further addressing the much-feared scenario of transmission of the virus to animals living in the wild and its consequences. Considering the most likely origin of the virus being a wild animal and the putative susceptibilities of free-ranging wild animal species to SARS-CoV-2, the risk of infection with possible establishment of the virus in these populations has to be investigated closely. The authors conclude that most attention should be given to surveillance and awareness raising activities for SARS-CoV-2 infection in wild mustelids, bats, wild canids and felids, particularly these collected in wildlife rescue centres. People involved in frequent and close contact with wild animals should take all necessary precautionary measures to protect wild animals against exposure to the virus. One year after the first detection of SARS-CoV-2 in humans, the time has come to increase investments in research and surveillance activities in animals, including in free-ranging wild animals, as part of a One Health control of this pandemic. This study focusing on Belgium could be helpful for other countries with similar animal densities and ecosystems.

#### Keywords

free-ranging wild animals – risk evaluation – SARS-CoV-2 — transmission — wildlife disease management

#### Introduction

Covid-19 is a human disease caused by a betacoronavirus from the *Coronaviridae* family, genus *Betacoronavirus* subgenus *Sarbecovirus*, namely Severe Acute Respiratory Syndrome-

CoronaVirus-2 (SARS-CoV-2). The first human cases of SARS-CoV-2 infection were reported in Wuhan City, China, in December 2019 (Wu, Chen, & Chan, 2020). While, so far, the exact origin of the virus remains unknown, several hypotheses exist (Andersen, Rambaut, Lipkin, Holmes, & Garry, 2020; Latham & Wilson, 2020; Y.-Z. Zhang & Holmes, 2020). The majority considers this to be a wild animal source (OIE, 2020 a) whereby the hypothesis of some species of horseshoe bats (family Rhinolophidae) being original natural hosts for a precursor of SARS-CoV-2 has been put forward (Zhou et al., 2020) with transmission to humans occurring most probably through an intermediate host (Zhao et al., 2020). Within a few weeks following the outbreak in China, SARS-CoV-2 human infections were reported all over the world. In response to the rapid spread of the virus and the increasing number of cases and deaths, WHO officially declared Covid-19 (Coronavirus disease 2019) as a pandemic at the beginning of March 2020 (WHO, 2020). Meanwhile, compared to the human cases, a significantly lower number of infections with SARS-CoV-2 has been diagnosed in pets and production animals, in wild animals kept in captivity and more recently in presumably escaped minks found in the wild (OIE, 2020 b). However, anthropozoonotic events (SARS-CoV-2 transmission from humans to animals) raise new concerns for animal health risk or for the establishment of a new animal reservoir (Munnink et al., 2020). Furthermore, considering particularly the first cases of SARS-CoV-2 infection reported in presumably escaped minks in Utah (Promed, 2020 a) and the possibility of mustelids as intermediate host (Zhao et al., 2020), avoiding the possible establishment of a wild animal reservoir is of great importance. In order to safeguard the health and wellbeing of affected animal species, and of our own, there is need to get a closer look at the risk of SARS-CoV-2 infection in free-ranging wild animal species.

The aim of this review is thus to evaluate the putative susceptibilities of different free-ranging wild animal species in Belgium to SARS-CoV-2 and provide a risk assessment of SARS-CoV-2 infection in those animals.

# Putative susceptibility of free-ranging wild animal species in Belgium to SARS-CoV-2 infection

The susceptibility of different animal species to SARS-CoV-2 infection has been studied by means of experimental infections, functional and *in silico* analysis. Angiotensin Converting Enzyme 2 (ACE2) is a functional receptor for SARS-CoV-2 (Wan et al., 2020). Depending on sequence similarity of their respective ACE2 orthologues, several animal species should be susceptible to SARS-CoV-2 infection. Functional analysis on various animal cell lines also

revealed a relatively broad host species for SARS-CoV-2 *in vitro* (Tang et al., 2020); but with several species experimentally proven to be not susceptible *in vivo*, indicating that the receptor is necessary but not sufficient to mediate infection. Endogenous proteases, such as TMPRSS2, with proteolytic activity on S have indeed been shown to participate in membrane fusion activity (Hoffmann et al., 2020).

At the day of submission (27 January 2021), a limited number of animal species have been shown to be susceptible at variable degree in experimental infection, including non-human primate, ferret, cat, dog, deer mice, raccoon dog, rabbit, hamster, bank voles, white-tailed deer and Egyptian fruit bat (OIE, 2020 c) (Fagre et al., 2020; Ulrich, Michelitsch, et al., 2020; Palmer et al., 2021). Natural infections have been only diagnosed in pet cats and dogs, captive big cats and great apes, in several minks kept in holdings for fur production in different countries and recently in presumably escaped minks (OIE, 2020 b).

In order to be able to perform a risk assessment of SARS-CoV-2 infection in free-ranging wild animals, we first provide an overview of the several families present in Belgium and their putative susceptibility for infection with SARS-CoV-2. Considering the very limited natural and experimental cases of SARS-CoV-2 infection in wild animals, this information has been deduced from the knowledge from their domestic, production and laboratory counterparts.

## a) Mustelids (*Mustelidae*):

The wild animal species of the family mustelids (*Mustelidae*) present in Belgium are the European pine marten (*Martes martes*), the stone marten (*Martes foina*), the ermine (*Mustela erminea*), the badger (*Meles meles*), the weasel (*Mustela nivalis*), the polecat (*Mustela putorius*) and the otter (*Lutra lutra*) (Libois, 1982; Vercayie et al., 2017; Verkem et al., 2003). Among these, the stone marten and the badger are the species which are the most brought to rescue centres (source: surveillance network wild fauna University of Liège, Agency Nature and Forest Brussels Region and Brussels Environment).

The susceptibility of the ferret (*Mustela putorius furo*) to SARS-CoV-2 infection has been confirmed by several experimental studies (Kim et al., 2020; Shi et al., 2020). Their results showed viral replication at the level of the upper respiratory tract with possibility of isolation of the infectious virus, detection of SARS-CoV-2-genome in the faeces and the

lung (without confirmation of infectious virus) and a rise of neutralizing antibodies (titre of 1/128). Beside the transmission of the virus occurring through direct contact between the animals, Kim et al. (2020) suggest the possibility of an airborne transmission.

The susceptibility of the farmed mink (*Neovison vison*) for the virus was confirmed by the first natural infections of minks on a holding in the Netherlands on 23 April 2020 (Promed, 2020 b). Since, SARS-CoV-2 infections in mink holdings have been reported in Denmark, Spain, the United States, Sweden, Italy, Greece, France, Lithuania, Canada and Poland (OIE, 2020 b – latest update on 23 February 2021). Independent human-to-mink transmission has been identified as the most probable route of SARS-CoV-2 infection in mink instead of farm to farm transmission. For the infected minks in the Netherlands, besides developing respiratory disease symptoms, a slightly increased mortality was also detected on the farms (Oreshkova, Molenaar, Vreman, Harders, Munnink, Hakze-van der Honing, et al., 2020). At the onset of the outbreak in mink holdings in the Netherlands, the Belgian authorities applied a snapshot serological monitoring and a weekly virological monitoring in the 8 remaining mink farms, all tests results being negative. In December 2020, the mink industry in Belgium decided to stop all its activities (source: Belgian Federal Agency for Safety of the Food Chain).

On 11 December 2020, SARS-CoV-2 infection in presumably escaped minks present in the surrounding area of infected mink holdings in Utah was confirmed. Antibodies against SARS-CoV-2 were detected by virus neutralisation in 11 mink escapees, of which 3 had a high cycle threshold detection of SARS-CoV-2 nucleic acid by real time RT-PCR of nasal swabs and a lung tissue specimen. These results were obtained in the frame of wildlife surveillance conducted as part of One Health investigations by the USDA Animal and Health Inspection Services in meso-carnivores and other species around infected minks farms in Utah, Michigan, Wisconsin and Oregon. Although, there is no evidence that SARS-CoV-2 is circulating or has been established in wild populations surrounding the infected mink holdings nor that other wildlife species have been infected, Shriner et al. (2021) stress the concerning possible interaction with susceptible free-ranging wild animal populations. The sequence of the viral genome was identical as those obtained from the infected mink holdings (OIE 2020 b, Promed, 2020 a, and personal communication from USDA Animal and Plant Health Inspection Service).

b) Felids (Felidae):

The wild animal species of the felids family (Felidae) present in Belgium are mostly the stray and feral cat (*Felis Catus*) with a few European wild cats (*Felis sylvestris*), and lynxes (*Felis lynx*) (Libois, 1982; Vercayie et al., 2017; Verkem et al., 2003).

Cats (*Felis catus*) seem susceptible to SARS-CoV-2 according to studies from Shi et al. (2020) and Halfmann et al. (2020). A natural infection of a domestic cat has been confirmed in Belgium (Garigliany et al., 2020) and worldwide (OIE, 2020 b). Transmission from infected humans to domestic cats and wild big cats kept in captivity is the most probable route of SARS-CoV-2 infection.

#### c) Canids (Canidae):

The wild animal species of the canids family (Canidae) present in Belgium are mostly the red fox (*Vulpes Vulpes*), but several raccoon dogs (*Nyctereutes procyonoides*), and a few grey wolves (*Canis lupus*) and stray dogs (*Canis lupus familiaris*) have also been documented (Libois, 1982; Vercayie et al., 2017; Verkem et al., 2003).

Domestic dogs (*Canis lupus familiaris*) have a very low susceptibility for the virus (Shi et al., 2020). In Hong Kong, two dogs (from a cohort of 17) which had been in contact with SARS-CoV-2 positive patients, were confirmed positive but didn't show any signs of the disease nor did they transmit the virus (Promed, 2020 c, d). However, detection of SARS-CoV-2 infection has also been described in dogs showing clinical signs without information on other putative co-infections (Promed, 2020 f). On the other hand, experimental infection of raccoon dogs with SARS-CoV-2 demonstrated their susceptibility to the virus (Freuling et al., 2020). The infected animals showed no clinical signs, a high level of shedding, efficient transmission of the virus and seroconversion.

### d) Bats (Chiroptera):

The wild animal species of the bats family (Chiroptera) present in Belgium are listed in Table 1.

There is currently no evidence of a natural infection with SARS-CoV-2 in bats. An experimental study using Egyptian fruit bats (*Rousettus aegyptiacus*), belonging to a family (Pteropodidae) of chiroptera not present in Europe, showed that the animals became infected but did not show any clinical sign of disease. A transient infection of the respiratory tract has been detected, and infectious virus was confirmed in one of the 9

inoculated bats. Intraspecies transmission of the virus to in-contact animals also occurred. The authors therefore stress the risk of SARS-CoV-2 transmission to some free-living bat populations (Schlottau et al., 2020). As the Egyptian fruit bats are not present in Europe, indigenous bat species to Europe should first be tested for SARS-CoV-2 susceptibility. Furthermore, the Big Brown Bat (*Eptesicus fuscus*), a North American bat species, was experimentally challenged with SARS-CoV-2 but showed no susceptibility for infection (Hall et al., 2020).

Table 1 : bat families and species observed in Belgium (source : Vleermuizenwerkgroep Natuurpunt Studie – update 15 January 2021)

Bat family	Species	Status (r: reproduction, u: present status
		unclear, v: vagrant, m: migratory)
Vespertilionidae	Barbastelle Bat (Barbastella	r
	barbastellus)	
	Northern bat (Eptesicus	u
	nilssonii)	
	Big Brown Bat (Eptesicus	r
	serotinus)	
	Alcathoe's Myotis (Myotis	u
	alcathoe)	
	Bechstein's Bat (Myotis	r
	bechsteinii)	
	Brandt's Bat (Myotis brandtii)	r
	Pond Bat (Myotis dasycneme)	r
	Daubenton's Bat (Myotis	r
	daubentonii)	
	Geoffroy's Bat (Myotis	r
	emarginatus)	
	Greater Mouse-eared Bat	r
	(Myotis myotis)	
	Whiskered Bat (Myotis	r
	mystacinus)	
	Natterer's Bat (Myotis nattereri)	r

	Greater Noctule Bat (Nyctalus	V
	lasiopterus)	
	Leisler's Bat (Nyctalus leisleri)	r
	Common Noctule (Nyctalus	r
	noctule)	
	Kuhl's Pipistrelle Bat	V
	(Pipistrellus kuhlii)	
	Nathusius' pipistrelle Bat	m
	(Pipistrellus nathusii)	
	Common pipistrelle Bat	r
	(Pipistrellus pipistrellus)	
	Soprano pipistrelle Bat	u
	(Pipistrellus pygmaeus)	
	Brown Long-eared Bat	r
	(Plecotus auritus)	
	Grey Big-eared Bat (Plecotus	r
	austriacus)	
	The parti-coloured Bat	m
	(Vespertilio murinus)	
Rhinolophidae	Greater Horseshoe Bat	r
	(Rhinolophus ferrumequinum)	
	Lesser Horseshoe Bat	r
	(Rhinolophus hipposideros)	
Rhinolophidae	(Pipistrellus nathusii)  Common pipistrelle Bat (Pipistrellus pipistrellus)  Soprano pipistrelle Bat (Pipistrellus pygmaeus)  Brown Long-eared Bat (Plecotus auritus)  Grey Big-eared Bat (Plecotus austriacus)  The parti-coloured Bat (Vespertilio murinus)  Greater Horseshoe Bat (Rhinolophus ferrumequinum)  Lesser Horseshoe Bat	r u r r r

# e) Lagomorphs (Lagomorpha):

The wild animal species of the lagomorphs family (Lagomorpha) present in Belgium are the Brown Hare (*Lepus europaeus*) and the Wild Rabbit (*Oryctolagus cuniculus*) (Libois, 1982; Vercayie et al., 2017; Verkem et al., 2003).

The New-Zealand White rabbit has a low susceptibility for infection with SARS-CoV-2 under experimental conditions (Mykytyn et al., 2020). The rabbits showed limited clinical signs and the virus was detected with PCR at the level of the nose, throat and rectum while the longest infectious virus shedding was taking place in the nose. Neither virus replication nor transmission between animals were evaluated.

## f) Rodents (Rodentia):

The wild animal species of the rodents family (Rodentia) present in Belgium are listed in Table 2.

Syrian or golden hamsters (*Mesocricetus auratus*) (Chan et al., 2020; Sia et al., 2020), deer mice (*Peromyscus maniculatus*), a New World species not present in Europe (Fagre et al., 2020), and transgenic mice for the receptor ACE2 of human origin (Gu et al., 2020) are very susceptible to the virus. The Syrian or golden hamster is, together with the non-human primates and the ferret, an animal which shows clinical signs very similar to those observed in humans. After experimental infection (through the nose with a viral inoculum of 10<sup>5</sup> plaque forming units) shallow breathing and weight loss appear as clinical signs. The experiments showed transmission of the virus between hamsters, but this was not investigated in mice. Furthermore, experimental infection of the bank vole showed a very low susceptibility for SARS-CoV-2 infection and no transmission to contact-animals (Ulrich, Michelitsch, et al., 2020).

Table 2 : rodents families and species present in Belgium (Libois, 1982; Vercayie et al., 2017; Verkem et al., 2003)

Rodent family	Species				
Cricetidae	field vole ( <i>Microtus agrestis</i> )				
	common vole ( <i>Microtus arvalis</i> )				
	bank vole (Clethrionomys glareolus)				
	European pine vole ( <i>Microtus</i>				
	subterraneus)				
	European water vole (Arvicola terrestris)				
	muskrat (Ondatra zibethicus)				
	European hamster ( <i>Cricetus cricetus</i> )*				
Muridae	house mouse (Mus musculus)				
	wood mouse (Apodemus sylvaticus)				
	yellow-necked mouse (Apodemus				
	flavicollis)				
	brown rat (Rattus norvegicus)				

	harvest mouse (Micromys minutus)			
	black rat (Rattus rattus)			
Gliridae	edible dormouse (Glis glis)			
	garden dormouse (Eliomys quercinus)			
	hazel dormouse (Muscardinus			
	avellanarius)			
Sciuridae	red squirrel (Sciurus vulgaris)			
	Eastern grey squirrel (Sciurus			
	carolinensis) fox squirrel (Sciurus niger)			
	Siberian chipmunk (Tamias sibiricus)			
Castoridae	European beaver (Castor fiber)			
Myocastoridae	coypu (Myocastor coypus)			

<sup>\*</sup>The European hamster is near extinction in Belgium.

# g) Hedgehogs (Erinaceidae):

The wild animal species of the Erinaceidae family present in Belgium is the hedgehog (*Erinaceus europaeus*) (Libois, 1982; Vercayie et al., 2017; Verkem et al., 2003).

Currently, the susceptibility of hedgehog (*Erinaceus europaeus*) for SARS-CoV-2 has not yet been studied. This would be useful since this species is highly present in the rescue centres in Belgium and it is known that they are susceptible to another Betacoronavirus, being the Erinaceus coronavirus (EriCov). The latter belongs to the same genus *Betacoronavirus* as SARS-CoV-2 but to a different subgenus, *Merbecovirus* (Corman et al., 2014; Monchatre-Leroy et al., 2017).

h) Bovids, cervids and suids (*Bovinae*, *Cervidae*, *Suidae*) – Reptiles, amphibians and fish - Birds :

Currently, there is no scientific evidence for susceptibility in both natural or experimental infection in these animal families except for bovids and cervids.

A very low susceptibility for cattle for SARS-CoV-2 infection was confirmed based on the information from the experimental study done by Ulrich, Wernike, Hoffmann, Mettenleiter, and Beer (2020). Also, a recent preprint publication has shown that the white-tailed deer (*Odocoileus virginianus*) is susceptible to SARS-CoV-2 via intranasal inoculation and can

efficiently transmit the virus to other white-tailed deer through indirect contact. However, infected animals do not present clinical disease signs. The deer species most related to white-tailed deer in Belgium is roe deer (*Capreolus capreolus*). Their potential status as a reservoir species is unknown and should therefore be investigated (Palmer et al., 2021).

# Assessment of SARS-CoV-2 release, exposure and consequence of the occurrence in freeranging wild animals

Risk assessments for animal health usually consider three main steps (Dufour et al., 2011), namely assessment of the release of the hazard from its source, of exposure to the hazard (while release assessment and exposure assessment are further combined to provide with occurrence probability) and the consequence of the occurrence of the hazard. Considering SARS-CoV-2 as the hazard, occurrence of the hazard should be considered as SARS-CoV-2 infection in free-ranging wild animals. As such, the risk is mainly based on literature survey and experts advice and can be scaled in a qualitative way from 'very low' to 'high', based on a combination of a 'likelihood assessment scale' and a 'consequence assessment scale'.

#### Assessment of SARS-CoV-2 release

The infectious virus is mainly present in respiratory secretions of SARS-CoV-2 infected humans, but also from some pets and some production animal species (minks). Isolation of the virus has also been achieved from urine and stool samples from SARS-CoV-2 infected humans (Cuicchi, Lazzarotto, & Poggioli, 2020; Jeong et al., 2020) and from stool from experimentally infected ferrets (Kim et al., 2020). Therefore, the possibility of presence of SARS-CoV-2 in the natural aquatic environment through waste water treatment plants has also been considered (Franklin & Bevins, 2020). So far, in general, the presence of infectious virus in the natural aquatic environment has not been demonstrated (Patel, Chaubey, Pittman Jr, Mlsna, & Mohan, 2020). There is a lack of scientific information about the quantification, the persistence and the infectivity of the virus in impurified waste water, sewage sludge, surface and sea waters (ANSES advice nr. 2020-SA-0059). Studies performed on the survival of surrogate coronaviruses in surface water, wastewater, sludge and bio-solid waste suggest a very short time of survival of SARS-CoV-2 at temperatures higher than 20°C (Carraturo et al., 2020).

Assessment of SARS-CoV-2 exposure

Free-ranging wild animal species are likely to be most exposed to SARS-CoV-2 when in direct or indirect contact with infected humans. Transmission of this respiratory virus mainly occurs through respiratory droplets or airborne aerosols (R. Zhang, Li, Zhang, Wang, & Molina, 2020).

In comparison with pet and production animals, direct contact between humans (including those infected with SARS-CoV-2) and free-ranging wild animals is very limited. The most important risk activities imply catching, keeping, handling, caring and releasing free-ranging wild animals. In this respect, co-operators of wildlife rescue centres (including volunteers), citizens submitting injured animals, and persons involved in activities of research, surveillance and management in wildlife seem to have the highest risk. Besides those, other groups such as nature professionals (foresters, academic researchers, nature guides etc.) and the broad public involved in nature activities have limited contact with free-ranging animals, and thus represent a lower risk, if infected with SARS-CoV-2, of transmitting the virus to free-ranging wild animals. However, it should be noted that certain species such as bats, stone martens and hedgehogs have been observed to make their nesting or resting sites in buildings relatively close to humans.

Uncertainties exist for the frequency and closeness of contact moments between free-ranging wild animals and pets and production animals. Particular attention should be given to transmission that could occur through predator-prey interactions and when using ferrets at hunting activities for rabbits for example. Uncertainties remain for other indirect transmission routes such as the orofecal route or fomites (through inanimate surfaces). Conflicting results on the efficiency of the oral route for SARS-CoV-2 infection have been obtained so far. Rhesus macaques were not infected when inoculated via the transgastric route (Deng et al., 2020) while Syrian hamsters got subclinically infected via the oral route (Lee et al., 2020). Exposure to waste water or material (such as waste) contaminated by humans infected with SARS-CoV-2 should also be considered. For instance, the study of Franklin and Bevins (2020) suggests a hypothetic model for the infection of wild free-ranging animals with SARS-CoV-2 in North America where the virus in the faeces of infected humans would reach the natural aquatic environment through waste water treatment plants. The presence of viral RNA in faeces was also discussed (Bivins et al., 2020).

Consequences of SARS-CoV-2 infection

The occurrence of SARS-CoV-2 infection in free-ranging wild animals may have an impact on the health of both animals and humans.

First, the risk of introduction of SARS-CoV-2 into endangered and susceptible wild animal populations may have negative consequences for their conservation. As observed during the

outbreaks in Dutch minks farms, the infection is very contagious as the virus spreads rapidly throughout the farm and infected minks even if housed separately. The infected minks showed respiratory disease signs, and increased mortality was present in some farms. Also, 90 % of the infected minks developed antibodies against SARS-CoV-2 (Enserink, 2020). Taken into account the high transmissibility within a dense animal population and the high susceptibilities of certain animal species, the epidemic situation in minks farms reflects what could happen in case of infection with SARS-CoV-2 in free-ranging wild animal populations, especially in case of gregarious species.

Then, if a reservoir of SARS-CoV-2 would persistently establish in free-ranging wild animal populations, recurring epidemics in the human population could be even more challenging to control. In the context of the outbreaks in mink farms in the Netherlands, a Dutch research team considered it likely that minks and other mustelids could be a true reservoir of SARS-CoV-2, although further research is needed (Munnink et al., 2020). Given the high susceptibility of the raccoon dog, this animal species could also become a reservoir.

#### Risk assessment of SARS-CoV-2 infection in free-ranging wild animals species

The risk of a SARS-Cov-2 infection is the combined result of the assessment of the likelihood that an infection would occur (4 levels: very low, low, high, very high) and the consequence. Two different consequences were considered, either the severity of clinical consequences for individual human or animal health (4 levels: marginal, minor, medium and major), or the potential that the infection would lead to the installation of a reservoir. The combination of likelihood and consequence led to a final appreciation of the risk for infection going from very low, low, moderate to high (see table 3 below).

		Consequence assessment			
		Marginal	Minor	Medium	Major
Likelihood assessment	Very High	Low	Moderate	High	High
	High	Low	Low	Moderate	High
	Low	Very low	Low	Moderate	High

	Very low	Very low	Very low	Low	Moderate

Tab le 3

: Risk matrix used to classify the level of risk: each assessment comes from expert opinion based on the results of experimental infection in the species itself or closely related and/or on reports of individual infections from the field. These results and data allow the qualitative assessment of the 'likelihood of occurrence' of the SARS-CoV-2 infection (infectivity for the species as well as transmission potential to congeners) and the 'consequences' of this infection (sensitivity to the infection at the clinical level as well as putative reservoir establishment). The method is described in the Guidelines for the opinions of the Scientific Committee established by the FASFC (http://www.favv-

afsca.fgov.be/scientificcommittee/publications/brochures/guidelinesopinions/\_documents/2017-04-19\_GuidelinesfortheopinionsoftheScientificCommittee\_en.pdf, adapted) which is based on classical Zepeda matrix (Zepeda, 1998 and 2002) and OIE's method for risk at import in animal Health (OIE, 2016).

The risk assessment was performed for the following five possible transmission routes:

a) Risk of transmission of SARS-CoV-2 from an infected person to a free-ranging wild animal (see figure 1 – transmission route A):

Currently, infection of free-ranging wild animals by humans is the most probable route of infection. The wildlife rescue centres form the most probable location where such transmission could occur.

The risk for individual animal health due to the infection of free-ranging wild animals by infected humans is qualified as "moderate" for wild mustelids (by analogy with the minks and ferrets); "low" for wild felids, wild canids and rodents; and "very low" for all other free-ranging wild animal species.

Taken into account the risk infected raccoon dogs represent for a SARS-CoV-2 reservoir, the authors are in favour of setting up an appropriate surveillance and risk mitigation measures strategies for kept and free-ranging wild raccoon dogs. Also we have to take into account that raccoon dogs have been suggested as intermediate hosts for severe acute respiratory syndrome coronavirus (SARS-CoV) (Xu et al., 2009). Attention should also be given to the possible

transmission of SARS-CoV-2 from human to foxes as the taxonomic proximity of this species is close to raccoon dogs.

b) Risk of SARS-CoV-2 transmission from an infected domestic animal (production or pet animal) to a free-ranging wild animal (see figure 1 – transmission route B):

Currently, the risk linked to the infection of a free-ranging wild animal through transmission from a domestic animal infected with SARS-CoV-2 is qualified as "very low" to "low" (for feral cats and wild mustelids through domestic animals other than production minks) and "moderate" (for wild mustelids through transmission from escaped production minks).

Mink industry usually relies on closed buildings. However infected mink escaping from a contaminated holding is a continuing risk for susceptible animals in the wild. This risk should be taken into account in every country where minks are used for the fur industry.

Attention should also be paid to domestic cats infected with SARS-CoV-2 by their owner as they could possibly transmit the virus to stray or feral cats, which in turn could infect surviving prey animals like rodents or bats if susceptible for the virus (Ancillotto, Serangeli, & Russo, 2013) (De Bruyn et al. 2020), or production animals such as minks (Oreshkova, Molenaar, Vreman, Harders, Munnink, Honing, et al., 2020). Anecdotic infection of stray cats have already been reported during the outbreaks in the mink farms in the Netherlands even if the infection route for these infections have not been clarified (final report Dutch government, 2020).

c) Risk of SARS-CoV-2 Transmission from free-ranging wild animals to humans (see figure 1 – transmission route C):

In the context of the current human pandemic, the risk for humans getting infected with SARS-CoV-2 via wild animals is qualified as "very low" compared to the risk of infection from person to person or to the risk of infection from a contaminated environment.

For persons working in wildlife rescue centres or in research centres, the risk is qualified as "low to moderate". This risk is "higher" for the more vulnerable groups of the human population ( the elderly, people with comorbidities, etc.).

d) Risk of SARS-CoV-2 transmission between free-ranging wild animals (see figure 1 – transmission route D) :

The risk of transmission of SARS-CoV-2 between free-ranging wild animals has been qualified as "very low" for all susceptible animal species except for mustelids. For mustelids, the risk of transmission between individuals and of persistent establishment is qualified as "moderate". Indeed, SARS-CoV-2 infection spread quickly in minks farms in the Netherlands. Besides that, in the past, other viruses, such as the canine distemper virus (caused by a paramyxovirus) or the Aleutian mink disease (caused by a parvovirus), have been introduced and spread within the population of mustelids in the wild indicating effective transmission and dissemination possibilities for an emerging virus in the wild if some animals are susceptible (Akdesir et al., 2018; Knuuttila et al., 2015). The risk of persistent establishment of SARS-CoV-2 in the wild mustelid population is thus qualified as "moderate".

Attention should also be paid to raccoon dogs as they could represent a similar chain of transmission although at very lower level in Europe. Indeed, they represent a massive industry in China, where they are bred on farms and caught in the wild for their fur and they were found susceptible to SARS-CoV-1 (Cherry & Krogstad, 2004). Such industry does not exist in Western Europe and the risk can thus be considered as very low.

# e) Risk of SARS-CoV-2 transmission from a free-ranging wild animal to a domestic animal:

The risk of a domestic animal getting infected with SARS-CoV-2 by a free-ranging infected animal has been qualified as "very low" for all domestic species. Many risk factors have an influence on the probability of domestic animal exposure to a free-ranging wild animal, such as habitat, outdoors access, hunting behaviour of the pet, the level of biosecurity of the farm in case of production animals (open or closed type), etc.

#### Conclusions and perspectives

Based on the current state of knowledge, most attention should be paid to wild mustelids, bats, wild felids and canids. Following the reported risk of SARS-CoV-2 infection in free-ranging wild animals, the following recommendations can be made concerning surveillance and awareness raising:

- For all activities at risk involving human-to-animal contacts, biosecurity measures (including guidelines and procedures) should be applied in order to prevent the

transmission of the virus to free-ranging wild animals such as personal protective equipment in all circumstances, and, if SARS-CoV-2 positive, staying away from free-ranging wild animals during a period of at least 15 days since the diagnosis. Guidelines have been published about the preventive measures to take while handling free-ranging wild animals in times of the Covid-19 pandemic (IUCN SSC WHSG and OIE, 2020);

- Testing of all bats, wild mustelids, wild felids and canids in rescue centres (regardless of the presence of clinical signs) by qPCR before releasing them into the wild. If possible, oropharyngeal samples should be taken, or, if not possible, rectal swabs or faeces;
- Awareness raising of the different concerned stakeholders such as the wildlife rescue centres;
- Providing guidelines for the broad public on how to handle dead or injured free-ranging wild animals, and information about which organizations need to be contacted;

Interplay between humans, animals and environment in the epidemiology of SARS-CoV-2 infection cannot be ignored. The proven susceptibilities of certain animal species, the reported outbreaks in many minks holdings in the world, the documented reverse zoonotic transmission in mink holdings in the Netherlands and Denmark, and the recent detection of SARS-CoV-2 infection in presumably escaped minks found nearby infected production animal holdings in the United States indicate the urge to invest the necessary means in further research and surveillance activities in animals, more specifically in free-ranging wild animals. A multidisciplinary One Health approach is needed to tackle the Covid-19 pandemic, and to prevent the next recurring epidemics if a reservoir would establish among free-ranging wildlife populations.

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(vii) ethical approval;

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

(viii) conflict of interest statement;

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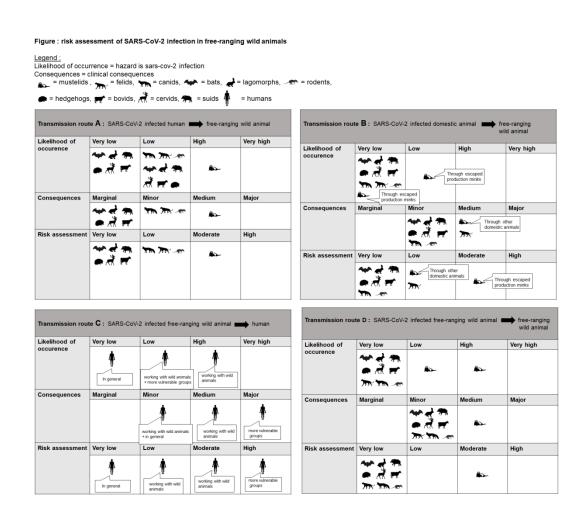
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