SCIENTIFIC REPORT

Monitoring of SARS-CoV-2 infection in mustelids

European Food Safety Authority and European Centre for Disease Prevention and Control,
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Abstract

American mink and ferret are highly susceptible to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), but no information is available for other mustelid species. SARS-CoV-2 spreads very efficiently within mink farms once introduced, by direct and indirect contact, high within-farm animal density increases the chance for transmission. Between-farm spread is likely to occur once SARS-CoV-2 is introduced, short distance between SARS-CoV-2 positive farms is a risk factor. As of 29 January 2021, SARS-CoV-2 virus has been reported in 400 mink farms in eight countries in the European Union. In most cases, the likely introduction of SARS-CoV-2 infection into farms was infected humans. Human health can be at risk by mink-related variant viruses, which can establish circulation in the community, but so far these have not shown to be more transmissible or causing more severe impact compared with other circulating SARS-CoV-2. Concerning animal health risk posed by SARS-CoV-2 infection the animal species that may be included in monitoring plans are American mink, ferrets, cats, raccoon dogs, white-tailed deer and Rhinolophidae bats. All mink farms should be considered at risk of infection; therefore, the monitoring objective should be early detection. This includes passive monitoring (in place in the whole territory of all countries where animals susceptible to SARS-CoV-2 are bred) but also active monitoring by regular testing. First, frequent testing of farm personnel and all people in contact with the animals is recommended. Furthermore randomly selected animals (dead or sick animals should be included) should be tested using reverse transcriptase-polymerase chain reaction (RT-PCR), ideally at weekly intervals (i.e. design prevalence approximately 5% in each epidemiological unit, to be assessed case by case). Suspected animals (dead or with clinical signs and a minimum five animals) should be tested for confirmation of SARS-CoV-2 infection. Positive samples from each farm should be sequenced to monitor virus evolution and results publicly shared.

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Summary

Since 2020, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a pandemic with millions of human cases and hundreds of thousands of deaths worldwide. Even animal species have been infected by SARS-CoV-2 through contact with infected humans, and instances of backward transmission (from animals back to humans) have been also documented in humans working in mink farms. Therefore SARS-CoV-2 infection in mink and other mustelids could represent a significant health risk for both human and animal health.

Following the indications by the European Centre for Disease Prevention and Control (ECDC), which recommended monitoring and surveillance of mink farms as long as these are exposed to SARS-CoV-2 from humans, the European Commission requested the European Food Safety Authority (EFSA) to recommend options for monitoring strategies, indicating possible objectives, methodologies, in particular as regards sampling and testing methods. Preliminarily EFSA should review relevant scientific literature available globally related to SARS-CoV-2 infection in mink and other Mustelidae, as well as to assess the current situation in the European Union (EU) and elsewhere as regards the risk for human and animal health posed by SARS-CoV-2 infection in mustelids with a view to designing a suitable coordinated monitoring in the Member States (MSs).

Concerning bred mustelids, in the EU, these animals are farmed for fur production and mainly belonging to the species of American mink (Neovison vison). The number of farmed mink has drastically decreased and many countries have already banned fur farming and due to SARS-CoV-2 outbreaks, several countries will ban fur farming in the next years. As from December 2020, following the SARS-CoV-2 epidemics, the number of mink farms in Europe was around 700 farms, mostly located in Finland, Poland, Lithuania and Greece. In the EU, mustelids can be also kept as pets, mainly ferrets. Farmed mink are generally farmed in open housing systems and in contiguous cages. The former situation may allow close contact between caged mink and other animals approaching these facilities, which, if they are susceptible, may acquire SARS-CoV-2 if mink are infected; the latter (contiguous cages) may facilitate efficient animal-to-animal transmission of infectious diseases.

Wild mustelid species (e.g. martens, polecats, badgers, wild mink) are widely distributed in Europe. In principle, they might approach mink farms and eventually acquire SARS-CoV-2 from infected farmed animals or animal products in the farm. Nevertheless, due to their elusive and solitary behaviour, to their low density and to the low number of animal hunted, there is a very low chance of contacts with humans and/or other SARS-CoV-2 susceptible domestic animals. Therefore, the risk of wild mustelids becoming a reservoir for SARS-CoV-2 or the risk of SARS-CoV-2 infection becoming endemic in wild mustelids in Europe is very low.

As of January 2021, SARS-CoV-2 virus has been detected in 400 mink farms for fur production in eight countries in the EU/EEA, namely 290 farms in Denmark, 1 in France, 21 in Greece, 1 in Italy, 2 in Lithuania, 69 in the Netherlands, 3 in Spain and 13 in Sweden. Sporadic SARS-CoV-2 virus cases have been recorded in kept ferrets in Slovenia and in Spain. Different monitoring strategies have led to the detection of the SARS-CoV-2-infected mink farms. Clinical signs were often unspecific (increased mortality, mild respiratory signs and slightly drop in feed intake) and present only in a variable proportion of outbreaks. In most cases, the likely introduction of infection for animals was suspected to be infected humans.

Concerning pathogenesis of SARS-CoV-2 in mustelids, American mink and ferret are highly susceptible to SARS-CoV-2, but no information is available for other mustelid species. Pathogenesis in mink and ferret resembles what has been observed in humans. Virus replication occurs mainly in the respiratory tract with only minor involvement of the digestive tract. The duration of virus excretion seems to be limited to few days.

The infection dynamics of SARS-CoV-2 is characterised by efficient spread from animal to animal once introduced in a mink farm. Transmission by direct contact and indirect contact such as air droplets, dust particles, aerosols and fomites. For this reason, large mink farms with high animal density provide ideal conditions for SARS-CoV-2 replication and transmission. In areas with high density of mink farms, between-farm spread is likely to occur once SARS-CoV-2 is introduced, although mechanisms for between-farm spread are largely unknown to date, it appears that humans have most likely contributed to the observed farm-to-farm spread. The only other risk factor for between-farm transmission identified is short distance to the nearest SARS-CoV-2 positive farm.

The risk for human health posed by SARS-CoV-2 infection in mink and other Mustelidae is represented by the fact that a mink-related variant viruses can establish circulation in the community, but have so far not shown to be more transmissible or causing more severe impact compared with

other circulating SARS-CoV-2; the circulation of mink-related variants in the human population has decreased since the end of 2020. The probability of infection with mink-related variant strains is assessed as low for the general population, moderate for the populations in areas with a high concentration of mink farms and very high for individuals with occupational exposure.

Concerning risk for animal health posed by SARS-CoV-2 infection in Mustelidae, few species of animals present in infected mink farms have been monitored following natural exposure to SARS-CoV-2 from infected mink. Among these, cats and dogs were the only species found infected under field conditions. For other animal species susceptible in experimental infections with SARS-CoV-2 but without evidence of natural infection during outbreaks in mink farms, more investigation is needed to clarify the epidemiological role. Raccoon dogs deserve particular attention as they are susceptible and capable of shedding SARS-CoV-2, and they are bred for fur production as for mink, and are present in the wild with high abundance in Europe. To date, the animal species that may be included in monitoring plans are the ones shown to be susceptible and capable of further transmitting the disease and that can be present in or close to infected mink farms, i.e., American mink (farmed or wild), ferrets, cats, raccoon dogs, white-tailed deer (very locally distributed, in the EU they are present only in Finland and few individuals in Czechia, in the latter country there are no mink farms) and bats belonging to the family of Rhinolophidae. Nevertheless, with new research and new evidence or new mutations of the virus, other species may be involved in the SARS-CoV-2 epidemiology and the monitoring approach could be re-considered.

The monitoring of SARS-CoV-2 in animals can be based either on passive approach (e.g. observation by farmers or veterinarians of indicators, such as reduced feed intake, clinical signs potentially related to SARS-CoV-2 infection and increased mortality rate), or on active approach, i.e. detecting cases of SARS-CoV-2 infection through planned and designed disease monitoring plans and laboratory testing. Passive monitoring should be in place in the whole territory of all countries where animals susceptible to SARS-CoV-2 are bred or kept, or when people infected or suspected to be infected with SARS-CoV-2 are in contact with animals susceptible to SARS-CoV-2 (e.g. workers in mink farms, owners of mustelid pets or hunting animals).

The available diagnostic tests that can be used to monitor SARS-CoV-2 in mustelids include both virological tests (e.g. reverse transcriptase-polymerase chain reaction (RT-PCR) or indirect tests (e.g. enzyme-linked immunosorbent assay (ELISA)).

For SARS-CoV-2 infection in mustelid (mink) farms, at the present time (January 2021), all mink farms that are not yet infected should be considered at risk of infection, as the human disease is widespread, therefore the monitoring objective of choice should be early detection. Early detection aims at containing the disease spread if and once it is introduced, either within the farm and/or to other farms in the region/country. This requires a regular periodic testing of samples to detect the infection at a moment sufficiently close in time to disease introduction into the farm. Early detection cannot be based only on passive surveillance (detection and reporting infected animals showing clinical signs by the farmer) as clinical signs appear only in a proportion of infected animals, therefore an active approach is required. The most important item of the monitoring for early detection of SARS-CoV-2 in mink farms should be the strict and frequent testing (e.g. weekly) of farm personnel and all people in contact with the animals and their household members, as humans are expected to be the most frequent source of introduction of virus into the farm. This is key to prevent the virus introduction into the farm.

In animals, given the fast spread of SARS-CoV-2 in mink farms once introduced, monitoring for early detection could be based on testing using RT-PCR for a sample of animals randomly selected at a sampling frequency ideally not longer than one week, which corresponds to a design prevalence of $\sim$ 5% in each epidemiological unit. Selection of dead or sick animals for testing would increase the probability of detection and pooling samples could be considered. Nevertheless, the monitoring for early detection and the expected prevalence values should be assessed case by case on the basis of epidemiological situation, prevention measures, the level of risk acceptance, etc.

In farms where suspected animals are detected (dead or with clinical signs related to SARS-CoV-2 infection), monitoring for confirmation of SARS-CoV-2 infection should target all of them every time a suspicion is raised, with a minimum of five animals to be tested (50% design prevalence in suspected animals).

Monitoring virus evolution by genetic sequencing analysis should be carried out to identify the origin and the source of the virus (e.g. spread between different populations) and to detect possible virus mutations, including those of public health concerns. The samples should be selected among positive cases to represent each positive epidemiological unit. Testing should be carried out in

accordance with validated protocols and all genome sequences shared with the scientific community, uploaded in open source genetic databases, and viruses sequenced from animals should be compared with those from humans through phylogenetic analysis.

Wild mustelids can be sampled and tested for SARS-CoV-2 infection, to monitor possible spill over from infected mink farms. In this case wild animals trapped around infected mink farms should be tested, mainly *Mustela* spp., *Neovison* spp., *Martes* spp. or badger, but also wild canids such as raccoon dogs or foxes, or wild rabbits or hares.

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1. Introduction

1.1. Background

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a pandemic with millions of human cases and hundreds of thousands of deaths worldwide. Natural SARS-CoV-2 infections in animal species after being in contact with infected humans have been observed and two instances of backward transmission (from animals back to humans) have been documented in humans working in mink farms. Further species are shown to be susceptible although the extent of natural infections of animals is still largely unknown.

SARS-CoV-2 infection in mink (and other animals of the family Mustelidae, especially those farmed for fur production) could represent a significant risk for human health that may pose a considerable challenge to risk managers.

A rapid risk assessment published on 12 November by European Centre for Disease Prevention and Control (ECDC) working with the European Food Safety Authority (EFSA) and European Medicines Agency (EMA), provided a first updated scientific understanding of the risks posed by SARS-CoV-2 in mink to human and animal health, with emphasis on the risks associated with a new virus variant found in mink and humans in Denmark.

The European Union (EU) measures put in place by EU Member States in response to findings of SARS-CoV-2 infection in mink are guided by the ECDC risk assessment and the recommendations given by international organisations like the World Health Organization (WHO) and the World Organisation for Animal Health (OIE).

The ECDC recommends monitoring and surveillance of mink farms (repetitive surveys and/or testing of dead mink on a weekly basis) for as long as SARS-CoV-2 exposure from humans to mink cannot be excluded. It also recommends systematic genotyping of the detected SARS-CoV-2 strains according to validated protocols and that the genome sequences from all infected animals are shared. The WHO also highlights the critical role of strong surveillance, sampling and sequencing of SARS-CoV-2, especially around areas where potential animal reservoirs are identified. In this regard, the OIE recommends active monitoring, as early infections in these animals might be difficult to detect, especially in mink.

1.2. Terms of Reference

There is a need to revise and update the measures put in place by Member States to counteract the challenges posed by the epidemiological situation of SARS-CoV-2 in mink.

An adequate and coordinated monitoring may need to be implemented to provide suitable data to better assess the risks posed to human and animal health and design suitable prevention and, if needed, control measures in response to the different epidemiological scenarios and possible evolution of the disease agent.

In view of the above, and in accordance with Article 31 of Regulation (EC) No 178/2002, the Commission asks EFSA for scientific and technical assistance and specifically to:

1) Review relevant scientific literature available globally related to SARS-CoV-2 infection in mink and other animals of the family Mustelidae.
2) Assess the current situation in the EU and elsewhere with regards to the risk for human and animal health posed by SARS-CoV-2 infection in mink and other animals of the family Mustelidae with a view to designing a suitable coordinated monitoring in the Member States.
3) Recommend options for monitoring strategies indicating possible objectives and suitable methodologies, in particular for sampling, frequencies and testing methods.

1.3. Interpretation of Terms of Reference

Regarding Term of Reference (ToR) 1, i.e. SARS-CoV-2 infection in animals of the family Mustelidae, up to 29 January 2021, SARS CoV-2 infection in mustelids has been reported only in ferrets (*Mustela putorius furo*) and in American mink (*Neovison vison*), both belonging to the subfamily Mustelinae (composed of the genera *Mustela and Neovison*). Therefore, the present assessment focuses on mustelids with a particular attention to the species belonging to the Mustelinae subfamily.

To answer ToR 1, a description of the distribution of wild and farmed mustelids species in Europe is provided in Sections 3.1. In Section 3.2, an overview of findings of SARS-CoV-2 in mustelids in Europe and outside Europe, the available information on genetic analysis of the viruses, on susceptibility of mustelids to SARS-CoV-2 infection, the pathogenesis and dynamic of infection and transmission, is reported.

Concerning ToR 2, the risk for human health posed by SARS-CoV-2 infection in mustelids is assessed by the ECDC in Section 3.3.1. The risk for animal health posed by SARS-CoV-2 infection in mink and other animals of the family Mustelidae is assessed in Section 3.3.2 taking into account farmed mustelids and other domestic animals in the farm, or wild animals in the farm environment. The report does not address animals kept in laboratories, e.g. ferrets for laboratory experiments.

Concerning ToR 3, i.e. options for monitoring strategies, is addressed in Section 3.4, where suggestions for the components of a monitoring plan indicating possible objectives, sampling plan, frequencies and tests to be used in different scenarios, i.e. in farmed mink (including domestic animals in and wild mustelids close to the farm), in kept mustelids such as pets, and wild mustelids are reported.

2.   Data and methodologies

For the present assessment, the following sources of data and information have been considered and reviewed:

- Scientific literature from peer-reviewed journals (see literature search protocol at Annex 2);
- Data reported in Section 3.2.1 about fur animal population in Member States are obtained from EU FUR association[1] and from Member States (MSs) affected by SARS-CoV-2 in mustelids[2];
- Data reported in Sections 3.2.1 and 3.2.2 on SARS-CoV-2 detections in mustelids originated from information either submitted to OIE from affected countries (in the EU and worldwide, up to 29 January 2021) (see OIE portal on SARS-CoV-2 in animals[3]) and/or from the case reports submitted from the affected member state to the European Commission. The information reported in Section 3.2.1 has been checked by the national authorities. A different level of detail of information is therefore available and here reported per affected countries. In this section slightly different figures on the number of mink farms present at country level might be reported by the affected country when compared with the statistics obtained from the EU FUR association reported in Section 3.1.2.

In December 2020, an ad hoc EFSA working group was set up to answer the ToRs of this mandate. For Section 3.4.2 about monitoring scenarios, expert knowledge from the working group and epidemiological analysis have been used as assessment methodologies.

The monitoring approach for SARS-CoV-2 in animals taken by MSs have been reported in Sections 3.4.3 and 3.4.4, based on information collected by national authorities and through a survey conducted by the WHO EURO in December 2020 (WHO EURO, 2020).

3.   Assessment

3.1.   Mustelidae in Europe

Mustelids are a family of mammals belonging to the order of carnivores comprising around 60 species worldwide. Mustelids are distributed both in semi-aquatic (marine and freshwater) and terrestrial habitats (Manes et al., 2020).

The family Mustelidae is composed of eight subfamilies of which five are present in Europe, i.e. Guloninae (martens, wolverine), Ictonychinae (grisons, African polecats), Lutrinae (otters), Melinae (European badger) and Mustelinae (weasels, polecats, stoats, ferrets, mink, sables). The subfamilies Helictidinae (ferret-badgers), Mellivorinae (honey badger) and Taxidiinae (American badger) are present only in America, Asia or Africa.

Among mustelids, the American mink is the only species that is farmed in significant numbers (several millions for fur production). Free-living American mink are normally solitary animals that have contact with each other only in the breeding season and live in family groups only when there are kits. Other mustelid species can be kept as pets (e.g. ferrets (*Mustela putorius furo*)) or as hunting animals, such as ferrets used for e.g. rabbit hunting. Some wild mustelid species, such as otters (*Lutra lutra*)

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[1] https://www.sustainablefur.com/

[2] Slight discrepancies in numbers from different data sources may be due to different moment of data extraction, in the second half of 2020 many fur farms closed and databases may have not been regularly updated every week.

[3] https://www.oie.int/en/scientific-expertise/specific-information-and-recommendations/questions-and-answers-on-2019novel-coronavirus/events-in-animals/

and badgers (*Meles meles*), can approach and live in or close to urban areas, although without direct contact with humans, as they are normally very elusive animals.

3.1.1. Geographical distribution of wild mustelids in Europe

The wild mustelid species occurring in Europe and their area of distribution are shown in Figure 1.

Source: IUCN[4]

Figure 1: Distribution of mustelid species in Europe

American mink originated from North America and became an invasive species in Europe (Genovesi et al., 2011). The species was first introduced in Denmark (after 1900) and Sweden (before 1920), and then in several others European countries (personal communication by Shyama Pagad, IUCN SSC Invasive Species Specialist Group, University of Auckland, New Zealand).

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[4] https://www.iucnredlist.org/resources/spatial-data-download

Few wild mustelid species are hunted in some European countries. Badgers, European polecats (*Mustela putorius*), and American mink represent probably 90% of hunted mustelids in Europe (ENETwild, oral communication). Other species are protected (e.g. otter) and hunting is forbidden.

In Nordic countries, such as Sweden, badgers, American mink, pine martens and European polecats are hunted regularly (around 3,000 American mink were hunted in 2019—2020, according to data collected by ENETwild[5]). Wolverine are hunted as well but there is no season – authorities can sanction a hunt if wolverines are deemed to cause too much damage to the reindeer herding industry. That also applies to otters in fish farms. Weasels (*Mustela nivalis*) and stoats (*M. erminea*) can be hunted only to prevent damage in semi-commercial establishments such as a farm or a garden without any special permit from the authorities.[6]

Wild mustelids are elusive, shy and mostly solitary animals, maintaining low population densities. Most of them are nocturnal, therefore difficult to detect and to monitor (Calenge et al., 2015). For these reasons only, limited information on their abundance and population trends over time is available, which does not allow inferring robust conclusions.

In terms of population trends, some species seem to be increasing in different geographical regions in Europe. This is the case for the badger (Sobrino et al., 2009; Reimoser and Reimoser, 2016) and possibly for the invasive American mink (Brzeziński et al., 2020). In Spain, a 14-year survey on carnivore abundances revealed that badger abundance increased significantly from 1992 to 2006, and that the abundances of polecat, stone marten and weasel remained stable throughout the study period (Sobrino et al., 2009). In the UK, populations of the European badger, European otter, European pine marten and European polecat are growing (Sainsbury et al., 2019). However, in the UK, it is suggested that otter recovery is accompanied by a progressive decline in formerly expanding American mink, showing that wildlife population trends are dynamic and subject to changes throughout time (McDonald et al., 2007). For the polecat, Croose et al. (2018) reviewed its status suggesting a general declining trend in Europe. However, this is not the case in the UK (Sainsbury et al., 2019), and possibly neither in parts of continental Europe such as the Netherlands (Hofmeester et al., 2019).

In conclusion, due to their ecology and behaviour, the low density and the low numbers hunted, the probability of close contact among wild mustelids and between wild mustelids and humans and/or other domestic animals is very low, as also concluded in the qualitative assessment by FAO (El Masry et al., 2020). Therefore, even if wild mustelids may become sporadically infected by SARS-CoV-2, by e.g. contact with farmed mink, the risk of SARS-CoV-2 infection becoming endemic in wild mustelids (wild mustelids becoming reservoir) in Europe is very low.

3.1.2. Farmed and kept mustelids in the EU

Farmed mustelids

Farmed mustelids are bred for fur production. The large majority of mustelid species farmed for fur production are American mink and, to a much lesser extent, sables (*Martes zibellina*) in one farm in Finland. According to the European fur association, up to 2019, 2,900 fur animal farms were registered in the EU, producing around 27 million mink pelts per year (Annex 1). The vast majority of pelts produced in the EU were mink pelts (94%), with minor proportions of foxes (*Vulpes vulpes*, fam. Canidae), chinchilla *(Chinchilla* spp., fam. Chinchillidae) and raccoon dogs (synonym finnraccoon, fam. Canidae) (Figure 2). Farming fur animals of more than one species also occurs.

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[5] www.enetwild.com
[6] https://jagareforbundet.se/jakt/jakttider/daggdjur/
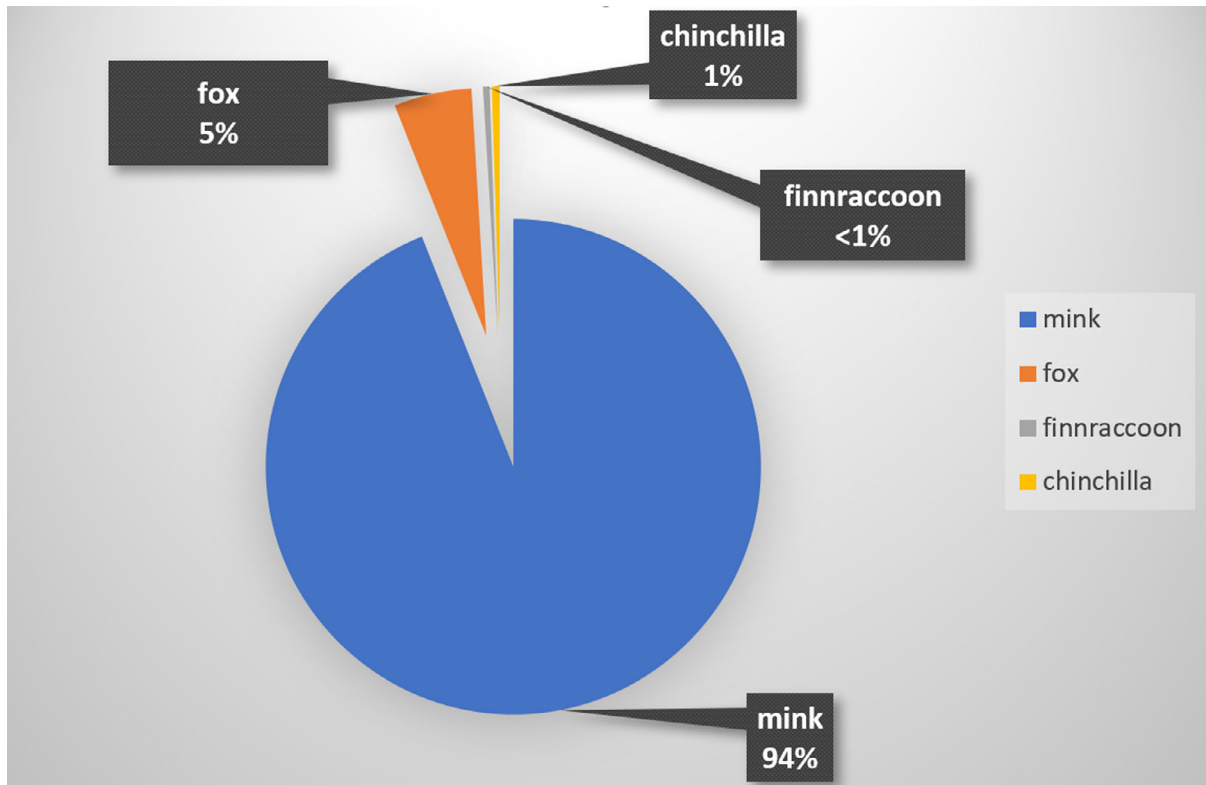
Figure 2: Proportion of pelts produced per species (fox, chinchilla, raccoon dogs (syn. finnraccoon) and mink) in Europe (2019)

According to the WelFur programme,[7] since December 2020 the number of fur animal farms in Europe has decreased to 1,294 farms, out of which 755 mink farms are still active (Figure 3), mostly located in Finland,[8] Poland, Lithuania and Greece. This decrease is due to massive culling of mink and/or closing of farms in some SARS-CoV-2 affected MSs (e.g. in Denmark, the number of mink farms was reduced from 1,100 to one (not yet closed at the time of writing); in the Netherlands all 127 mink farms had been closed by the end of 2020).

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[7] https://www.eesc.europa.eu/en/policies/policy-areas/enterprise/database-self-and-co-regulation-initiatives/146
[8] In Finland, the fur farming counts include also 2 million foxes, 180,000 raccoon dogs and a small number of sables.

Source: EU fur association https://www.sustainablefur.com
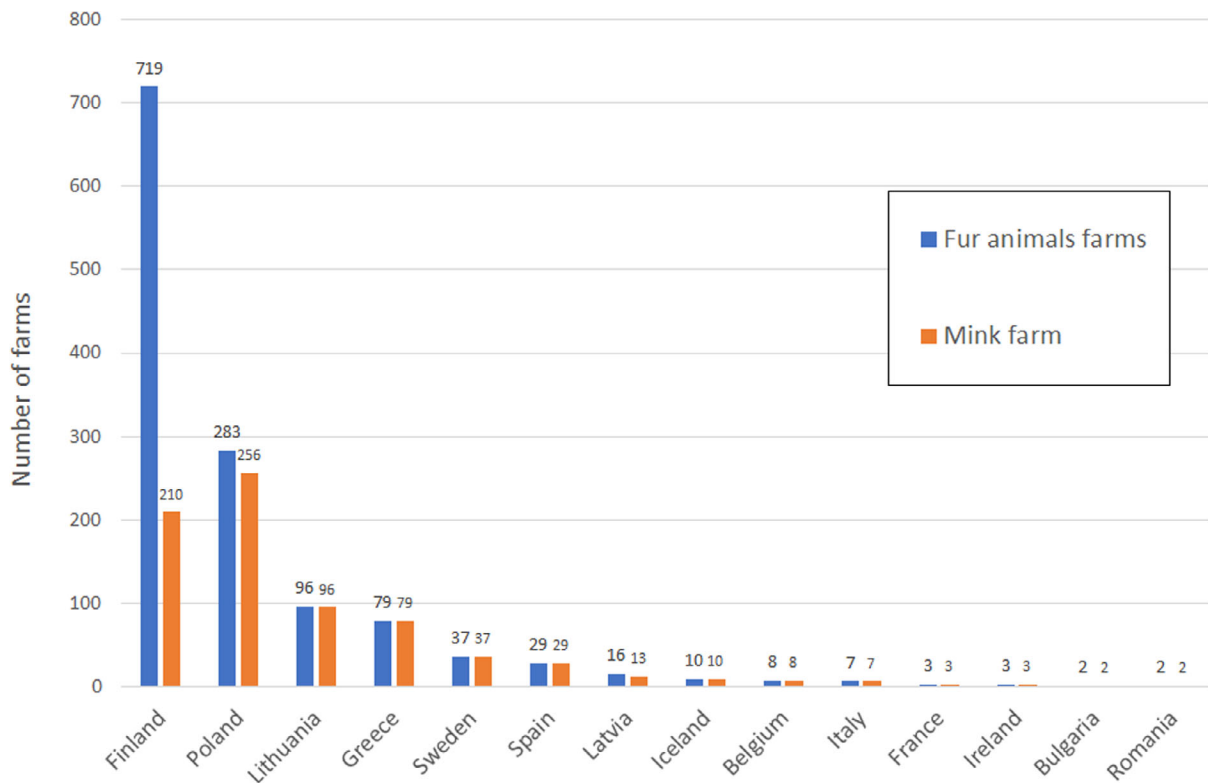
Figure 3: Number of fur animals and mink farms in Europe per country (data as from 17 December 2020)

It is expected, that the number of countries banning fur farming will further increase due to SARS-CoV-2 outbreaks in mink farms, but also due to concerns on animal welfare already raised previously. MSs and neighbouring countries that have already banned fur farming are Austria (2005), Belgium (regions Wallonia and Brussels, 2018), Croatia (2017), Czechia (2019), Luxembourg (2018), North Macedonia (2014), Serbia (2019), Slovenia (2016), Switzerland (2008) and the UK (2000).

Several countries are planning a ban on fur farming (with or without applying a phase-out period). Following SARS-CoV-2 outbreaks on Dutch mink farms in 2020, the Netherlands declared an early shutdown of the industry (originally planned by 2024) by the end of 2020. In Denmark, massive culling of mink farms was applied between September and December 2020 due to SARS-CoV-2 outbreaks and mink farming is now banned until the end of 2021. France announced in 2020 a ban on mink fur farming with a 5-year phase-out period. Ireland committed to a ban and legislation to prohibit fur farming is being prepared. In October 2019, Slovakia introduced a law to prohibit fur farming after a transitional period for existing farms until 2025. In January 2018, Norway decided to prohibit fur farming, with a phase-out period until 2025. In Belgium (region of Flanders), a complete fur farming ban will be implemented by 2023.[9] Bosnia and Herzegovina planned a ban starting in 2028 but, due to the economic decline of the fur industry, the last Bosnian fur farm shut down in 2020. The fur mink industry will be prohibited from 2022 onward in Germany.

The production cycle of farmed mink (including breeding, lactation, weaning, growing and finally killing for pelting) is generally conducted in a closed system on the same farm. Three main production phases can be defined: (i) from pelting to mating (period 1), during this period there may be movements of animals between farms that purchase new breeding stock; (ii) from mating to separation (period 2); and (iii) from separation to pelting time (period 3). In Europe, the mating season starts in March and lasts about 2 weeks. The gestation (from implantation to parturition[10]) varies from between 34 and 38 days. The birth period normally takes place between the end of April

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[9] https://www.furfreealliance.com/fur-bans/
[10] Mink females may give birth to pups from more than one mating. Mating induces the ovulation but the implantation can be delayed. Normally mink are mated more than once at weekly intervals.

and mid-May. The litter size may vary between five (normal litter) to nine (large litters) kits. Once the kits are 8 weeks old, they are weaned from their dam. Around 1 July, the kits are then paired off, female and male, to ensure normal behavioural development (separation phase). By the end of August, the physical development of the mink is more or less complete and continued weight gain consists primarily of fat. The selection of animals for the following year's breeding stock begins at the end of October. This is when the animal's size, behaviour, health and pelt quality are evaluated. Animals that are not selected for breeding are pelted. The pelting season is November/December. Pelting may occur at farms or in pelting centres. After skinning, raw fur pelts are blow-dried for a few hours at room temperature and stored refrigerated or frozen (ECDC, 2020a). After drying, pelts are stored before being transported to auction houses. After pelting, the remaining mink carcasses are rendered for use in the production of biodiesel, fertilisers, among other animal by-products. Further processing (e.g. tanning) of pelts include the use of washing with detergents, antibacterial agents, potassium alum and other salts. The tanning process lasts 4–5 days, during which the leather undergoes several washings, and other mechanical operations to improve its quality.

The most common buildings for farming mink are open housing systems, with cages put outdoors and covered by a roof. In a few cases, halls (closed housing, where cages have no contact with the outside) are used. This open housing system may allow close contact between caged mink and other animals approaching these facilities, such as other wild and/or domestic animals, which, if they are susceptible, may acquire SARS-CoV-2 if mink are infected. Mink are housed in pairs in contiguous wire netting cages with a nest box. This contiguity may facilitate efficient animal-to-animal transmission of infectious diseases. During gestation, animals are kept usually alone, next with the pups until weaning, then one kit stays with the mother and the rest stays in the litter group.

Kept mustelids as pets

Ferrets are the mustelid species usually kept as pets, e.g. in the UK a number between 800,000 and one million ferrets is estimated (personal communication from Dr Madonna Livingstone of Glasgow University) and in Spain the estimated number of ferrets kept as pets or for rabbit hunting and rabbit pest control was 143,000 in 2015.[11] The total number of kept pet ferrets in Europe remains unknown. For comparison, in the US, there are 0.5–0.8 million pet ferrets[12] (Jurek, 1998).

Key messages:

- Wild mustelids are widely distributed in several European countries, with low population densities. In principle, they might approach mink farms, e.g. in search of food or because they are attracted by farmed animals and eventually, if susceptible (they belong to the same species or very closely related), acquire SARS-CoV-2 from infected farmed animals or animal products in the farm. Nevertheless, due to their elusive and solitary behaviour, to their low density and to the low hunting bag, there is a very low chance of contacts with humans and/or other SARS-CoV-2 susceptible domestic animals (such as dogs, cats, ferrets). Therefore, the risk of wild mustelids becoming a reservoir for SARS-CoV-2 and/or the risk of SARS-CoV-2 infection becoming endemic in wild mustelids in Europe is very low.
- In the EU, mustelids farmed for fur production include basically only American mink. Up to 2019, around 2,900 fur animal farms were present in the EU, producing around 27 million mink pelts per year.
- As from December 2020, following the SARS-CoV-2 epidemics in mink farms in the EU, the number of mink farms in Europe has decreased to 759 mink farms, mostly located in Finland, Poland, Lithuania and Greece. Among the biggest mink pelts producers, Denmark and the Netherlands have closed nearly all mink farms.
- Due to SARS-CoV-2 outbreaks, several countries will ban fur farming in the next years.
- Mink are generally farmed in open housing systems, which may allow close contact between caged mink and other animals approaching these facilities, which, if they are susceptible, may acquire SARS-CoV-2 if mink are infected.
- Mink are farmed in contiguous wire netting cages that may facilitate efficient animal-to-animal transmission of infectious diseases and/or to other wild or domestic in-contact animals.
- In the EU, mustelids, mainly ferrets, can be kept as pets but their total number is unknown.

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[11] https://www.mapa.gob.es/es/ganaderia/temas/produccion-y-mercados-ganaderos/20160222_informeestudioparapublicar_tcm30-104720.pdf
[12] https://www.avma.org/resources-tools/reports-statistics/us-pet-ownership-statistics#exotic

3.2. SARS-CoV-2 infection in mustelids

3.2.1. SARS-CoV-2 virus detections in the EU/EEA and UK in mustelids

As of 29 January 2021, SARS-CoV-2 has been reported in 400 mink farms for fur production in eight countries in the EU/EEA, namely 290 farms in Denmark, one in France, 21 in Greece, one in Italy, two in Lithuania, 69 in the Netherlands, three in Spain and 13 in Sweden (Figure 4 and Table 1). Sporadic SARS-CoV-2 cases (virus detected by reverse transcriptase-polymerase chain reaction (RT-PCR) have been recorded in kept ferrets in Slovenia (reported to OIE[13]) and in Spain (Gortázar et al., 2021).

The legal basis for reporting SARS-CoV-2 infection in the EU in mustelids and raccoon dogs is the COMMISSION IMPLEMENTING DECISION (EU) 2020/2183 laying down certain protective measures in relation to reporting infection with SARS-CoV-2 in mink and other animals of the family Mustelidae, as well as in raccoon dogs that entered into force on 21 December 2020[14] and according to which Member States shall submit a report to the Commission within three days after the first confirmation within their territory of the infection of mink, and other animals of the family Mustelidae and of raccoon dogs with SARS-CoV-2.
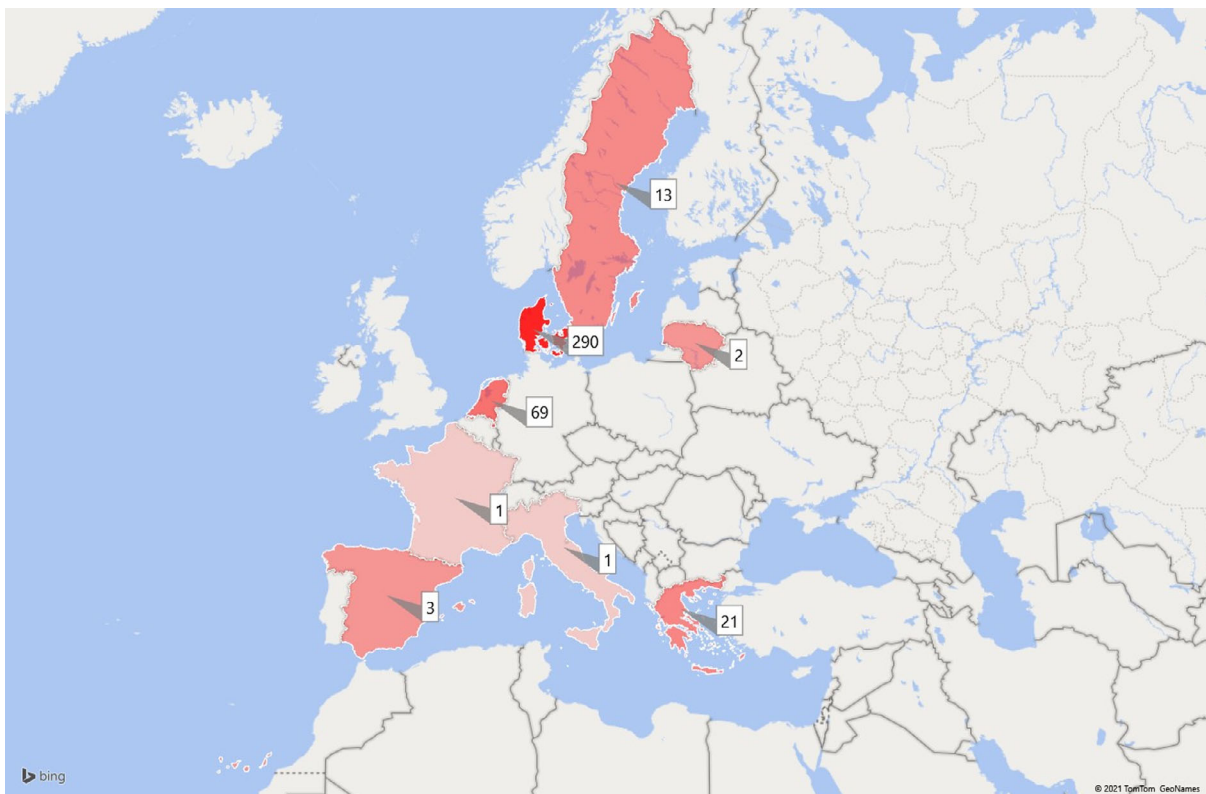
Figure 4: Geographical distribution and number of SARS-CoV-2 outbreaks reported in mink farm in Europe from April 2020 to 29 January 2021. (Source: OIE[3] and case reports submitted from the affected member state to the European Commission.)

Denmark

In total, 290 of approximately 1,140 mink farms have been reported with SARS-CoV-2 in Denmark, since the first case was confirmed on 15 June 2020. In June, SARS-CoV-2 was detected in mink on another two farms. From July to the beginning of August, no further outbreaks in mink were registered. On 14 August, the first outbreak in the second phase of the epidemic was registered, with a slow increase in numbers of affected farms in August, and a steep increase from September onwards. Until October, all the outbreaks were detected in farms located in two municipalities in

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[13] https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=37289
[14] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020D2183&from=EN

Northern Jutland while, from the beginning of October, outbreaks started occurring in neighbouring municipalities (Boklund et al., 2021). Overall, 186 infected farms were detected in Northern Jutland, seven in North-West Jutland, 25 in Mid-West Jutland, 15 in Mid Jutland, 37 in West Jutland, 6 in South-West Jutland, 9 in South-East Jutland, 1 in South Jutland and 4 in an island 40 km from Northern Jutland. Already at the first sampling date, 100% virus prevalence among tested mink was observed in 65% of the infected farms, and in 45% of the farms where no clinical signs of infection were observed (Boklund et al., 2021). At this stage of the production cycle, the autumn, the total of 1,140 Danish mink farms included a total of approximately 15 million mink. On 4 November, it was decided to cull all mink in the country. Most mink were culled and most farms closed, at least until the end of 2021. One or two mink farms remain in the country (12 January 2021). The new Act on the killing of and temporary ban on keeping mink will takes effect on 15 January 2021. The Act imposes a ban on keeping of mink until 31 December 2021. No new SARS-CoV-2 occurrences have been detected since 7 December 2020, when the most recent outbreak was detected. The reason for suspicion were: clinical signs in 33% of the farms, persons tested positive in 24% of the farms, surveillance of dead mink in 32% of the farms, and tracing of contacts to infected farms in 9% of the farms. Mink-associated SARS-CoV-2 strains, characterised by the spike protein change Y453F, were initially observed in mink and humans in June (Hammer et al., 2020). Almost all infected mink farms were infected by mink-associated variant strains and until now 5 clusters of mink-associated variant strains have been identified. These strains have spilled back to the human population and spread in the communities. However, after the culling of mink farms, the incidence of these strains in the human population has decreased significantly (Hammer et al., 2020).

France

On 20 November, one of the four mink farms present in France tested positive to SARS-CoV-2 (OIE[15]). Out of 4,100 animals, 180 were sampled. On those samples, 180 serological tests (ELISA) were performed with 174 positive results, and 110 virological tests (RT-PCR), with 33 positive results. No clinical signs of SARS-CoV-2 infection were reported in the infected farm. Results coming from these other breeder farms were negative (ELISA and/or seroneutralisation and RT-PCR of oropharyngeal swabs).

Greece

As of 29 January 2021, SARS-CoV-2 has been detected on 21 out of the 91 mink farms present in Greece. On 13 November, the first case of SARS-CoV-2 was confirmed on a mink farm in the northern part of the country, in Kozani regional unit (Western Macedonia region). The suspicion was raised on 11 November by the local veterinarian due to the presence of respiratory symptoms, reduced feed intake and increased mortality. As a precautionary measure and based on increased morbidity/mortality, it was decided to cull the animals. A control zone of 10 km radius around the affected farm, where almost 40 mink farms were located, was established. Movement restrictions and strict biosecurity measures were implemented in the control zone. The epidemiological investigation and virus genome sequencing from animals and humans identified two family members working at the farm, who both tested positive for SARS-CoV-2, as the origin of the virus. At the same time, it was decided to test all workers/owners for SARS-CoV-2 in the 91 mink farms present in Greece and to repeat the testing procedure at least every 7 days until vaccination of human population in direct contact with mink (farm staff, owners, veterinarians) is concluded. The first cycle was completed on 13 November 2020 and testing still continues. Between 13 November and 3 December, 11 additional SARS-CoV-2 positive mink farm outbreaks were detected. They are all located in Western Macedonia: five in Kozani, four in Kastoria and two in Grevena regional unit. In 10 out of these 11 farms, suspicion was raised following notification of confirmed human cases as part of the repeated weekly surveillance conducted in all mink farm workers/owners. The 11th infected farm, was detected as the owner observed and reported increased morbidity and mortality; however, also in this case, there was a link with a human case as the owner tested positive to SARS-CoV-2. In total, in 3 out of the 11 farms, increased morbidity and mortality was observed. At this stage, available information indicates a human-to-animal transmission in most of the farms, where the presence of SARS-CoV-2 has been confirmed up to 3 December.

Genetic sequencing analysis was performed on the detected positive samples for the variants in aminoacidic positions found in sequenced genomes in Denmark and UK and discussed in the Rapid

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[15] https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=36748

Risk Assessment by ECDC (ECDC, 2020a,b,c). From these mutations only the Y453F mutation has been detected in the analysed mink genomes from Greek farms indicating that these variants are unrelated to the Danish and Dutch variants.

Following the confirmation of the 11 new outbreaks, it was decided to stop culling animals. Stamping out and official disposal of carcasses were only applied to the first confirmed farm mentioned above. Strict biosecurity measures (e.g. mandatory use of personal protective equipment) and movement restrictions have been implemented in all mink farms (not only in the infected ones) since then. From 3 December 2020 to 8 January 2021, SARS-CoV-2 was confirmed in another nine mink farms in the Western Macedonia. These were located in the regional units of Kozani (5), Kastoria (2) and Grevena (2). In four of those farms, increased morbidity and mortality was observed. Enhanced biosecurity measures and special protocols are also implemented in the establishments where pelting and pelt drying take place. The pelting period concluded in the week 6—12 December. Animals from infected farms were pelted last, under official surveillance, right after pelting for all mink from non-infected farms was completed. All dried raw skins remained stored for at least 4 weeks at the highest possible room temperature. Around 400.000 breeding animals are currently kept on farms. Possible SARS-CoV-2 circulation in the breeding population will continue to be monitored in the following months by repeated clinical and laboratory examinations.

Italy

SARS-CoV-2 was confirmed in a mink farm with 26,600 animals in the Cremona province, Lombardy region on 10 August 2020. After the detection of a human case among workers in the farm on 31 July 2020, 20 dead mink were tested, one gave a weakly positive result to RT-PCR without any anatomopathological findings. All the animals in the farm were in a good health conditions without any suspect signs of infection. After these first positive results, an intensified surveillance was carried on in the whole farm, which encompassed two weekly clinical inspections in each shed, two weekly samplings of 30 oropharyngeal and faecal swabs and virological testing of all dead animals. Overall, 1,124 tests were carried out, leading to the identification of another weak positive RT-PCR result from a faecal swab on 10 October 2020. In both positive samples, the genetic material was insufficient to carry out genetic analysis of the virus. On 6 November 2020, a third weakly positive case from the same farm was confirmed in a faecal swab sampled in a different shed than the one giving the previous positive result. Also, in this occasion, neither clinical signs of infection nor abnormal mortality were observed. On the basis of the diagnostic result, the entire herd was culled in compliance with a Ministerial Order which provides the mandatory notification of SARS-CoV-2 infections in mustelids and the killing and destruction of all the mink present in herds where the presence of SARS-CoV-2 is laboratory confirmed. The same Order also provides, as a precautionary measure, by 23 November, the ban of the breeding activities over the whole territory until the end of February 2021 providing only the maintenance of the breeders without any reproductive activities. The ordinance introduced also the ban on the introduction of new animals in mink farms and the activation of enhanced surveillance in Italian mink farms based on clinical checks, diagnostic tests (serological and virological) on a weekly basis and the application of strict biosecurity measures. In February 2021, an assessment of the surveillance activities carried out on the national territory, also on the basis of the evolution of the epidemiological situation in both humans and animals, will be carried out and it will be decided whether to continue to apply the restrictive measures in the future months or to restart of the breeding activity).

Lithuania

As of 29 January 2021, SARS-CoV-2 has been confirmed in two commercial mink farms in Lithuania. The first outbreak was detected in a farm with 60,000 animals in Varpių village, in the municipalities of Jonava, located in Kaunas County in central Lithuania. On 24 November, the local veterinary authority received a notification about an increase in animal mortality from a mink farm (169 mink from all age groups were found dead, but mainly young animals). Ten samples were randomly taken from dead mink and pooled in groups of five in the National Food and Veterinary Risk Assessment Institute (SFVS). The results were positive for SARS-CoV-2. On 25 November, out of 155 mink found dead, 22 additional samples were taken from different cages with sick mink and were analysed individually by RT-PCR for SARS-CoV-2. On 26 November, all samples tested positive. The SFVS started an epidemiological investigation. According to the preliminary information, it is most likely that the introduction of the virus originated from a farm worker. On 24 November, one worker tested positive for SARS-CoV-2, and additionally five farm workers were later detected positive. Passive surveillance of

COVID-19 is carried out by the SFVS at mink farms. In addition to passive surveillance, up to 26 November, 86 samples were taken from dead mink from all mink farms in Lithuania for SARS-CoV-2 testing (one dead mink from each of 86 mink farms in Lithuania). All samples, apart those taken from the infected farm, gave negative results. On 30 December, a second farm, raising 55,000 animals, was detected to be infected in Pavartyciu village, located in Radviliškis municipality, Šiauliai County. As part of passive surveillance and due to the COVID-19 detection in five workers, five dead mink were sampled and SARS-CoV-2 was detected in four mink (OIE[16]).

The Netherlands

In total, 69 mink farms (out of 126) were reported as SARS-CoV-2 infected in the Netherlands, 44 in the province of Noord Brabant, 23 in the province of Limburg, and two in the province of Gelderland. The first outbreak was diagnosed on 24 April 2020 and the last on 4 November 2020. On 29 farms (42%), the owner noticed clinical signs and reported the suspicion to the competent authority, whereas 39 (57%) infections were detected through the early warning system (EWS) in place (the obligation to test dead animals on a weekly basis). One farm (1%) was detected based on a human link with a previously infected farm. All the infected farms were inspected by an official veterinarian to collect samples for confirmation and only in seven out of the 69 farms (10%) no clinical signs of SARS-CoV-2 infection were seen. All the animals in affected farms were culled. A longitudinal study on the first four outbreaks revealed that the virus was efficiently circulating among mink with variable morbidity and mortality (Molenaar et al., 2020; Oreshkova et al., 2020). At the time of writing this report, the routes of transmission are still unclear, although transmission through infected humans explains most of the infections, and, therefore, epidemiological investigations to give more insight in the sources of virus introduction are still ongoing. Information available indicates at least five different introductions from humans into mink farms (Oude Munnink et al., 2020b). In addition, mink-to-human infection was demonstrated and the overall percentage of SARS-CoV-2-infected humans present on infected mink farms was 66%, where the human virus is always genetically similar to that of the mink (Oude Munnink et al., 2020b). Therefore, information available indicates that most of the outbreaks has been caused by (infected) humans going from farm to farm, but in addition there was a strong spatial clustering of which the transmission route has not yet been elucidated. Around mid-December, all mink have been pelted, implying that currently no farmed mink are bred in the Netherlands and no new infections will occur. Originally, the Netherlands passed a ban on fur farming in 2012 that would phase-out mink fur production entirely by 2024. Following the coronavirus outbreaks on mink farms, the government declared an early shutdown of the industry. This means that from 8 January 2021 onwards, it is no longer allowed to keep farmed mink in the Netherlands.

Poland

Considering the reports of SARS-CoV-2 infections in mink in other European countries and the high incidence of SARS-CoV-2 human infections in Poland, a SARS-CoV-2 private, unofficial sampling in mink was conducted on one farm located in Pomorskie Voivodeship, North Poland. Throat swabs were collected from 91 minks culled for pelting on 17 November 2020. In the laboratory of Gdańsk Medical University SARS-CoV-2 was initially identified in the farmed mink with a prevalence of 16.5% (8.4–28.6%). Following the media reports of COVID-19 infection on the mink farm, the Veterinary Inspection authority, the State Veterinary Institute in Puławy and the State Sanitary Inspectorate (PIS) took immediate action to investigate the farm health status, including daily clinical examinations of the mink on the farm and taking samples for laboratory tests. As a result of the investigation mentioned above, the Veterinary Inspection authorities unequivocally stated that: (i) the results of official laboratory tests showed neither the presence of genetic material of SARS-CoV-2 nor specific antibodies against this virus in samples taken; (ii) the clinically observed flock did not show any symptoms that might indicate SARS-CoV-2 infection in the animals; and (iii) diagnostic tests carried out by the PIS in humans did not confirm SARS-CoV-2 infection in persons professionally linked to the farm mentioned above. The Veterinary Inspection authorities concluded that the media reports about the first confirmation of SARS-CoV-2 infection on a mink farm published by the Medical University of Gdańsk have been verified negatively.

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[16] https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=37389

Slovenia

A ferret kept as pet animal in a COVID-19 positive household was confirmed as SARS-CoV-2 positive by RT-PCR on 1 December 2020. The animal showed clinical gastrointestinal signs and the source of infection was the contact with COVID-19 positive people (OIE[13]).

Spain

One mink farm in the Autonomous Community of Aragon and one in the Autonomous Community of Galicia out of 29 farms in the country were reported as SARS-CoV-2 positive. From the first cases of SARS-CoV-2 reported in mink farms in the Netherlands in April, hygiene and biosecurity measures in mink farms, as well as surveillance on them were reinforced in Spain. During the first month, two reports of clinical suspicions were received on 27 and 28 April, from farms located in the provinces of Ávila and Guipúzcoa, both cases were discarded after analysis of samples in the laboratory. During the second half of May, a human outbreak of COVID-19 was detected in the province of Teruel. Some of those individuals were working on a mink farm with around 19,500 adult and 73,200 young animals. The farm has a feed factory, where food for the animals is produced, as well as its own pelting facility. This is the only mink farm in the Autonomous Community of Aragon that did not have epidemiological links to other mink farms. Since the outbreak of COVID-19 was reported in people related to the farm, on 27 May, samples from lung, spleen, liver and small intestine from four mink from the farm were analysed by RT-PCR and tested negative for SARS-CoV-2. On 8 June, oropharyngeal and rectal swabs and exudates from internal organs from 20 additional animals were sampled. An inconclusive RT-PCR result, in one oropharyngeal swab only, led to a further sample collection at the mink farm. On 22 June, samples of oropharyngeal and rectal swabs from 30 live animals, as well as swab and lung parenchyma samples from six dead animals were collected. One of the oropharyngeal swabs tested positive for SARS-CoV-2 by RT-PCR, and samples from seven other animals gave non-conclusive results (all of them asymptomatic). On 7 July, oropharyngeal and rectal swabs were collected from 90 live animals (30 adults and 60 young animals); 86.67% of animals tested positive for SARS-CoV-2 by RT-PCR. No mortality nor clinical signs compatible with SARS-CoV-2 infection or drop in feed consumption were observed at farm level. A stamping out policy in the farm, including official cleaning and disinfection of the premise after culling was implemented. Genetic analysis from the complete sequence of 34 viruses and the sequence of the gene encoding the spike protein of four more viruses revealed: i) the presence of the D614G mutation, that defines the predominant clade in Spain and Europe, in the spike protein; ii) the absence of the Y453F mutation described in Cluster 5, as well as the rest of the changes that define this cluster that appeared in some of the mink farms in the Netherlands; iii) the presence of the N501T mutation, which is a site in all the sequences analysed; this is a site of host adaptation and antigenic drift that is part of the group of mutations that have been identified in the recent UK viral variant (N501Y); and iv) the F486V and D796H mutations have been always identified together in 22 of the 38 sequences; these changes have not been identified in the GISAID database in either human or mink samples.

On 19 January 2021 a second SARS-CoV-2 outbreak was confirmed in a mink farm in Galicia, in the municipality of A Baña. The farm had a total of 3,100 animals (2,500 females and 600 males) and all were slaughtered few days after the confirmation of the outbreak, in application of the current legal regulations and with the aim of preserving public health. The outbreak had been suspected since the middle of December when, out of 20 oropharyngeal swabs collected on 10 December as part of the active surveillance in place, four gave positive results and three were inconclusive for SARS-CoV-2 by RT-PCR. On 15 and 18 December, an additional 30 and 90 oropharyngeal swabs were taken respectively, and all gave negative results by RT-PCR. On 4 January 2021, 90 oropharyngeal swabs were sampled with four giving an inconclusive result by RT-PCR. On 18 January four samples were collected from the manure of the mink farm and one sample tested positive for SARS-CoV-2 by RT-PCR. Neither clinical signs nor abnormal mortality had been observed among the animals. The origin of the infection remains under investigation, although preliminary information shows that the most probable origin of the introduction of the virus into the farm could has been through two workers from the holding. Both workers, although having no clinical signs of SARS-CoV-2 and being PCR-negative, were tested serologically positive for SARS-CoV-2 antibodies in samples collected on 11 December. The results of the genetic analysis revealed the presence of the pD614G mutation in spike protein (in four samples) and of N501T (in one sample). Neither Y453F nor other mutations and deletions described in the Cluster 5 isolate have been detected. Neither the strain from the United Kingdom VOC 202012/01, nor the South-African 501Y.V2, nor the Brazilian variant included in lineage B.1.1.248 have been detected.

On 22 January, a third SARS-CoV-2 outbreak was confirmed on a farm rearing 1,010 mink (800 females and 210 males) in the Municipality of Navatalgordo, in the region of Castille and León. No signs of SARS-CoV-2 infection were observed at the farm. The origin of the virus remains under investigation, although preliminary information shows that the most probable source could have been the owner of the holding as he had clinical symptoms on 4 January and gave a positive result by antigen test on 8 January 8. Also, the owner and two workers gave positive results for SARS-CoV-2 by RT-PCR in samples taken on 14 January 2021. Genetic analysis of the viruses is ongoing.

Sporadic cases have also been recorded in ferrets in Ciudad Real province, detecting SARS-CoV-2 RNA in six out of 71 ferrets (8.4%) and isolating the virus from one rectal swab (Gortázar et al., 2021). This confirms that natural SARS-CoV-2 infection does sporadically occur in kept ferrets, at least under circumstances of high viral circulation in the human population. However, none of the 20 ferrets resampled 2 months later was PCR positive, including one individual that had given a positive result two months earlier. Therefore, small ferret collections are probably unable to maintain prolonged virus circulation (Gortázar et al., 2021).

Sweden

The Swedish mink fur sector is composed of ∼ 35 farms with in total 600–650,000 animals. Eighteen of these farms are located in the municipality of Sölvesborg, the County of Blekinge, in the south-eastern part of the country. In May to June 2020, a dialogue between the competent authority and representatives from the Swedish mink sector was initiated to raise awareness, to ensure enhanced on-farm biosecurity practices and to raise vigilance regarding increased morbidity and mortality. In early October, given the absence of any reports of morbidity/mortality from the Swedish mink farms, an active surveillance scheme, based on sampling up to five dead mink per farm per week, was initiated. Within the scheme, mink found dead were submitted to the National Veterinary Institute, SVA, in Uppsala, where they were sampled with swabs from the oral cavity and pharynx and analysed by RT-PCR. The surveillance scheme was voluntary and organised in close collaboration with the industry. Between mid-October and mid-November, SVA received 74 submissions of dead mink, representing between 1 and 4 submissions per farm. Thirteen farms gave positive results for SARS-CoV-2. All positive farms were located in Sölvesborg, the County of Blekinge, in the south-eastern part of the country. None of the positive farms had reported increased morbidity or mortality before testing positive but, retrospectively, a slight increase in daily mortalities could be observed in the records from several of the farms.

Movement restrictions and strict biosecurity measures were imposed on all mink farms in Sweden after the confirmation of the first affected farm. None of the affected farms was culled. The Swedish outbreak coincided with annual pelting, when approximately 80% of the mink are killed as part of the production cycle. According to the assessment made, culling of affected farms as part of disease control measures would not speed up the process of reducing the number of susceptible animals compared with the annual pelting and killing, and would therefore not contribute to any significant reduction in the risk of further spread of the disease. Pelting was carried out from mid-November to early December, under strict biosecurity recommendations, to prevent mink-to-human SARS-CoV-2 transmission. After pelting, approximately 90,000 breeding animals remain in Sweden. The situation for SARS-CoV-2 in the mink that remain will be further monitored using serology in live animals, as well as RT-PCR on a sample of animals found dead.

Sequences from humans and mink from the same mink farms cluster closely together, suggesting within-farm human-to-mink and/or mink-to-human transmission.

Table 1: Data on outbreaks of SARS-CoV-2 in mink farms in the EU in 2020

Country	Number of mink farms in the country at the time of SARS-CoV-2 virus first detection	Number of infected mink farms	Date of first and last SARS-CoV-2 virus detection in mink farms[a]	Number of farms where clinical signs of SARS-CoV-2 were observed	Likely source of virus origin[b]
Denmark	1,147	290	15 June to 7 December	145[d]	Human-to-animal transmission suspected or confirmed in some outbreaks. Unclear in most outbreaks
France	4	1	20 November	0	Undetermined but most probably humans on the farm
Greece	91	21	13 November to 8 January	8	Human-to-animal transmission suspected in most outbreaks
Italy	9	1	10 October	0	Human-to-animal transmission
Lithuania	86	2	24 November	2	Human-to-animal transmission
Netherlands	126	69	24 April to 4 November	62	Partly human to mink transmission and part unclear, but with a strong spatial component
Spain	29	3	22 June to 22 January	0	Human-to-animal transmission suspected
Sweden	35	13	23 October to 11 November	No information[c]	Human-to-animal transmission

(a): Suspicion date if available.
(b): Possible source as reported by MSs.
(c): No clinical signs were observed, only retrospectively a slightly increase in the daily mortality was noticed from several of the infected farms.
(d): Boklund et al. (2021) report that, out of 207 outbreaks inspected, in 62 animals showed clinical signs of SARS-CoV-2 infection.

3.2.2. SARS-CoV-2 virus detections outside the EU/EEA and UK in mustelids

From 29 January 2021, outside Europe SARS-CoV-2 virus has been detected in American mink in Canada and the United States.

Canada

From 29 January 2021, SARS-CoV-2 has been detected in two mink farms in Canada. On 8 December, SARS-CoV-2 was confirmed in a mink farm rearing 15,000 animals in British Columbia. On 3 December, workers of the mink farm were diagnosed as COVID-19 positive. When sampling of the mink took place, on 4 December, the animals did not display any signs of infection, but an increased mortality (1%) was observed in the days following sampling. Human-to-animal transmission was suspected as introduction of the infection into mink farms (OIE[17]). On 23 December, a second outbreak was confirmed in a farm rearing 950 mink in British Columbia. Samples were taken on 21 December as the animals had shown clinical signs of diarrhoea and increased mortality (3%). Initial testing has revealed no evidence of human SARS-CoV-2 infection in farm workers. Source of the outbreak is still under investigation. On both farms, animals were euthanised and frozen for pelting (OIE[18]).

United States

From 29 January 2021, the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) confirmed 16 SARS-CoV-2 outbreaks in mink farms in the US (APHIS USDA, 2020a), 11 in Utah, 2 in Wisconsin, 2 in Michigan and 1 in Oregon. The first detection was in Utah and, according to OIE,[19] the outbreak started on 26 July; the last outbreak in a mink farm was

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[17] https://www.oie.int/wahis_2/temp/reports/en_imm_0000036946_20201210_172608.pdf
[18] https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=37348
[19] https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=36731

detected in Oregon and started 22 October (OIE[20]). Overall, 177,357 animals were bred in those farms and 16,130 died due to infection (OIE[20]). Because some workers on these farms had COVID-19, it is likely that infected farm workers were the initial source of the mink infections (APHIS USDA, 2020a).

On 13 December, USDA communicated the first SARS-CoV-2 virus detection in a free-living American mink. The animal was trapped under the monitoring activities carried out around an infected farm in Utah. The animal was confirmed by genetic testing as a wild mink, not an escaped farmed mink.

Key messages:

- Clinical signs of SARS-CoV-2 in mink/mustelids are often non-specific and present only in a variable proportion of outbreaks. They can include increased mortality, mild respiratory signs, slightly drop in feed intake and occasionally mild gastrointestinal signs.
- Different monitoring strategies have led to the detection of the SARS-CoV-2-infected mink farms. A proportion of the infected farms has been detected by passive monitoring, i.e. (i) the farmer noticing and reporting clinical signs or anomalies in production parameters (feed intake, mortality, etc.); (ii) another proportion was detected after a suspicion was raised due to an epidemiological link with SARS-CoV-2 infection in humans (workers/owners); (iii) active monitoring (actively sampling and testing of animals) was needed to detect the rest of the infected farms. According to the epidemiological situation, a different proportion of infected farms was detected by each of these monitoring strategies at country level, e.g. the epidemiological link to human cases led to detection of one out of 69 outbreaks (1.4%) in the Netherlands, and to 57 out 226 outbreaks (25%) in Denmark.
- In most cases, the likely introduction of SARS-CoV-2 infection into farms was suspected to be infected humans. Additional sequence analysis can help in identifying mink farm clusters and mink variants, suggesting or ruling out within-farm human-to-mink, mink-to-mink and/or mink-to-human transmission.

3.2.3. Genetic analysis of SARS-CoV-2 in mustelids

To date, published genetic data on SARS-CoV-2 from mustelids are very scarce and limited to that reported in two studies from the Netherlands and Denmark, respectively, investigating possible transmission between mink and humans (Hammer et al., 2020; Oude Munnink et al., 2020b), and one national report describing the evolution of the SARS-CoV-2 epidemic in mink in Denmark (Boklund et al., 2020). The strains from the Netherlands and Denmark are not closely related and fall into different genetic clades, which excludes a direct link between farms in the two affected countries (ECDC, 2020a). However, in both countries, SARS-CoV-2 strains related to mink farms fall into some separate clusters, suggesting several introductions from humans to mink, followed by transmission chains from farm to farm. The mutation Y453F in the receptor-binding domain (RBD) of the spike protein, suggested as an adaptation of the virus to mink, has been observed in many of the SARS-CoV-2 strains in both countries, independently of the clustering (ECDC, 2020a; Oude Munnink et al., 2020b; Welkers et al., 2020). This mutation has also been observed in SARS-CoV-2 strains associated with mink in, e.g. Greece and Sweden. However the Y453F has also been observed in human cases not related to mink (e.g. South Africa, Belarus, Russia, Switzerland).

In September 2020 in North Jutland, Denmark, a variant referred to as Cluster 5 was detected in five mink farms and in 12 human cases. This Cluster 5 variant contains four mutations (Y453F, I692V, M1229I, S1147L), and one deletion (69–70 del) in the S protein.[20]

The deletion of two amino acids (AAs; 69–70) in the S protein is not present in any of the mink strains from the Netherlands, but has been widely reported in human cases unrelated to mink without Y453F.[21]

Due to its characteristics, and the role of S protein in the virus, i.e. host–cell interaction, it has been hypothesised that the Cluster 5 variant may affect the immune response, probability of reinfection, vaccine efficacy and treatment with convalescent plasma (ECDC, 2020a; Mallapaty, 2020) and may have an increased binding affinity to the human angiotensin-converting enzyme 2 (ACE2) receptor. However, scattered information is only available so far and it is not possible to infer any definitive

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[20] https://files.ssi.dk/Mink-cluster-5-short-report_AFO2
[21] https://www.gisaid.org/about-us/history/

conclusion. Therefore, along with the lineage B.1.1.7, the Cluster 5 variant would merit a mutation surveillance effort (Genomics UK Consortium, 2020).

In conclusion, it is envisaged to monitor the genetic characteristics of virus from all infected mink farms to evaluate the progression of the evolution of the virus, which seems to find in mink a suitable model to evolve and to ascertain that variants of public health concern, such as the Cluster 5, are not circulating.

3.2.4. Susceptibility of mustelids and other animals to SARS-CoV-2

Information on susceptibility to SARS-CoV-2 in animals is reported in Section 3.3.2.

3.2.5. Pathogenesis, clinical signs and immune response

SARS-CoV-2 is an enveloped beta-coronavirus sharing 79% genome sequence identity with SARS-CoV and 96.2% with bat coronavirus RatG13 (Yan et al., 2020). The viral envelope is coated by spike (S) glycoprotein, envelope (E) and membrane (M) proteins. The S protein is composed of two subunits, S_1 and S_2. The S_1 subunit comprises an amino-terminal domain and a RBD (Xiao et al., 2003; Babcock et al., 2004; Wong et al., 2004). The RBD is the ligand for the cellular receptor ACE2. The S_2 subunit is characterised by a fusion peptide (FP) region, which is involved in the release of the viral genome into the host cell cytoplasm (Liu et al., 2004).

The multiple sequence alignments of ACE2 revealed high homology and high conservation in the binding region among different animal species. It has been shown that the 3D structure of human and mink ACE2 is highly conserved and that the binding region of mink ACE2 complements perfectly the SARS-CoV-2 ligand (Hayashi et al., 2020). In addition, ACE2 is most abundantly expressed on type II alveolar and glandular epithelial cells in the trachea and bronchi of ferrets (Van den Brand et al., 2008).

Ferrets (*Mustela putorius furo*) have been shown to be a suitable model for the pathogenicity of SARS-CoV-2 (Johansen et al., 2020; Muñoz-Fontela et al., 2020). Natural infection can occur, presumably after contact with infected humans.[13] In experimental infections, ferrets are highly susceptible to SARS-CoV-2, with efficient virus replication especially occurring in nasal turbinates, soft palate and tonsils. Following mucosal exposure to SARS-CoV-2, clinical signs are generally absent or mild. When clinical signs are observed, these are characterised by lethargy, nasal discharge, wheezing, sneezing and loose stools. In some cases, mild lymphopenia and neutrophilia have also been observed. Virus replication occurs in the upper respiratory tract as soon as 2 days after infection and lasts for few days after infection (Schlottau et al., 2020; Shi et al., 2020). Virus replication in ferrets appears to be restricted to the respiratory and gastrointestinal tracts.

American mink is the other species belonging to the family Mustelidae known to be susceptible to SARS-CoV-2.

Pathogenesis in mink is largely unknown and mostly limited to information from studies evaluating natural infections in farmed mink (Hammer et al., 2020; Molenaar et al., 2020; Oreshkova et al., 2020; Oude Munnink et al., 2020). The available information demonstrates that SARS-CoV-2 can replicate efficiently in the upper and lower respiratory tracts of mink and that host–pathogen interaction resembles that observed in humans and other susceptible animals.

Infection in mink has been associated most commonly to the observation of a reduced feed intake, followed by respiratory signs, nasal discharge and sneezing. Increased mortality in a small percentage of adult mink was reported in different outbreaks in the EU. Post-mortem analyses revealed signs of pneumonia with lung lobes swollen, dark red and without any tendency to collapse. A few animals showed free blood in the upper respiratory tract.

Histological findings of tissues from deceased animals revealed interstitial pneumonia, multifocal areas with thickening and degeneration of alveolar septa with type II pneumocyte proliferation and diffuse alveolar damage. Alveolar lumina were filled with inflammatory leucocytes and desquamated cells. The epithelial cells of bronchioles in affected areas showed severe necrosis and formation of syncytial cells. Trachea showed loss of cilia with swollen epithelial cells. Nasal conchae showed multifocal swelling and degeneration of epithelial cells with diffuse loss of cilia.

The presence of SARS-CoV-2 antigen was found in nasal conchae, trachea, bronchioles and alveolar epithelial cells (Molenaar et al., 2020).

Very recently, results from an experimental infection in mink were reported (Shuai et al., 2020). Mink were inoculated intranasally and killed 4 days afterwards. Viral RNA was detected in the nasal washes and from the ear swabs and rectal swabs of two animals. Infectious virus was detected in the

nasal washes, but not from the conchae swabs or rectal swabs of any animals at any time. Viral RNA was detected in the nasal turbinates, soft palates, tonsils, all lung lobes and submaxillary lymph nodes, in the trachea and in the ileum, but was not detected in heart, kidneys, spleen, liver, pancreas and brain. Virus was detected to a lesser degree in most viral RNA-positive samples. The respiratory tract of the virus-inoculated mink was severely compromised, with mucinous-purulent secretion, containing neutrophil debris and mucus, inflammatory infiltrates, epithelial degeneration and necrosis.

SARS-CoV-2 nucleoprotein antigen was verified in nasal mucinous-purulent secretions, in the epithelium of the nasal mucosa of the vestibular region, respiratory region and olfactory region and that of the tracheal mucosa, suggesting an involvement of nasal secretion in transmission and olfactory function impairment, as also observed in humans (Luers et al., 2020).

The above-mentioned results, along with those described in natural outbreaks, demonstrate that the host-pathogen interaction of SARS-CoV-2 in mink and ferret resembles that observed in humans.

Symptomatic SARS-CoV-2 infections in humans in fact, can progress in the more severe cases in acute respiratory distress syndrome (ARDS) (McGonagle et al., 2020). Viral infection and replication can cause virus linked pyroptosis and vascular leakage. Pyroptosis is a highly inflammatory form of programmed cell death that triggers inflammation with the recruitment of cells and the production of pro-inflammatory cytokines and chemokines IL-1β, IL-6, IFN-γ, MCP1 and IP-10, which in turn prime T and B lymphocytes evoking an adaptive immune response. This response is effective in controlling and resolving the infection, but in some cases, an uncontrolled immune response occurs with the release of a cytokine storm that causes severe tissues damage and multi-organ failure (Tay et al., 2020).

Key messages:

- American mink and ferret are highly susceptible to SARS-CoV-2, but no information is available for other mustelid species. In general, more information is required to have a deeper understanding of the host–pathogen interaction for all mustelid species.
- Pathogenesis in mink and ferret resembles that observed in humans. Virus replication occurs mainly in the respiratory tract with only minor involvement of the digestive tract. The duration of virus excretion seems to be limited to a few days.
- Mustelinae and especially ferrets (due to their suitability as laboratory animal) can play a useful role as preclinical animal model to test diagnostic, therapeutic and prophylactic approaches for SARS-CoV-2 infection, to be further developed for public health purpose.

3.2.6. Infection dynamics and transmission routes

As described in Section 3.3.2, some animal species are susceptible to SARS-CoV-2 and have the capacity to transmit the virus as shown by experimental or natural infections (Freuling et al., 2020; Schlottau et al., 2020). However, to date significant between-animal spread in field settings has only been described for farmed mink.

The high animal density that is present in a typical mink farm (generally 5,000–20,000 animals), provides ideal conditions for viral transmission (Hobbs and Reid, 2020). Once introduced, usually by infected humans as suggested also by outbreak investigations in the affected MSs (Section 3.2.1), SARS-CoV-2 appears to spread efficiently among the animals within the farm, as indicated by high prevalence of antibody and PCR-positive animals (commonly up to 100%) detected in affected farms, in which in-depth investigations have been carried out (Hammer et al., 2020; Oreshkova et al., 2020). In mink, the virus can be found in secretions from the respiratory tract and in faeces (Shuai et al., 2020; Boklund et al., 2021). Extensive virus replication also results in local environmental contamination as shown by detection of virus RNA in air and dust samples collected within (but not outside) the affected premises (Hammer et al., 2020), as observed in the epidemics occurred in mink farms in the Netherlands (Oreshkova et al., 2020). Also, in Denmark, viral RNA has been identified 2–3 metres from mink in two out of eight infected mink farms, while there was no detection beyond 3 metres from the mink in any of the 17 farms where samples were collected in this distance (Boklund et al., 2021). In the Netherlands, viral RNA was observed in the inhalable dust in mink farms and outside, close to the entrance, but not outside the premises of infected mink farms (de Rooij et al., 2021). This indicates that SARS-CoV-2 could be transmitted between animals mainly by direct or close contact via infectious droplets and aerosols, but also via indirect contact via feed or bedding, or in air dust containing faecal matter up to short distance (few metres). Additionally, experimentally transmission was shown in ferrets via air flow between cages (Richard et al., 2020). Further investigation under field conditions is needed to confirm this transmission pathway. Experience from

affected MSs demonstrates that once SARS-CoV-2 has been introduced into an area with high density of mink farms, farm-to-farm transmission is likely to occur, despite enhanced biosecurity implemented to halt the spread (Boklund et al., 2020; Oude Munnink et al., 2020b). The possible transmission chain probably starts with the introduction of the virus into a farm through infected personnel, then it can quickly spread through the animal population, further personnel may acquire the infection from animals and transmit this further to other farms. SARS-CoV-2 has been detected in other susceptible animals, such as cats (Boklund et al., 2021), although evidence for transmission through this pathway is lacking up to date.

Although virus RNA has to date only been found within infected premises, and not outside (de Rooij et al., 2021), aerosol spread can be considered as a possible pathway for between-farm transmission. In the Netherlands, whole genome sequencing indicated that five different virus clusters were circulating, two of which remained limited to a single farm. Markedly, each of these five clusters had been detected by June and no new clusters were seen in mink farms even though SARS-CoV-2 outbreaks were observed until 4 November. In addition, virus from these five clusters has only been observed in mink and persons associated with mink farms, not in any other person, even though efforts have been made to sequence samples from people living in the neighbourhood of mink farms. This suggests between-farm transmission of SARS-CoV-2 virus in the Dutch situation, either through people associated with mink farms or some other unknown mechanisms. A strong spatial clustering of infected farms was observed in Denmark as well as in the Netherlands.

To date, however, the main routes of between farm transmissions remain unknown. From the experience gathered in Denmark and in the Netherlands (Boklund et al., 2021, Fischer et al., in preparation), the only risk factor identified is short distance to the nearest SARS-CoV-2 positive farm and in Denmark also large farm size (Boklund et al., 2021). This pattern of close proximity between affected farms was observed also during the epidemics of porcine epidemic diarrhoea (PED) caused by another coronavirus with a certain ability to survive in the environment, particularly during cold seasons, in the US and Japan in 2013–2014. Despite high biosecurity measures in place the spread could not be halted (Alvarez et al., 2016; Sasaki et al., 2017).

Key messages:

- Once introduced in a mink farm, SARS-CoV-2 spreads efficiently within the farm from animal to animal through transmission by direct contact and indirect contact such as air droplets, dust particles, aerosols and fomites.
- Viral RNA can be found close to infected animals but not outside infected farms.
- Large mink farms with high animal density provide ideal conditions for SARS-CoV-2 replication and transmission, therefore increasing the risk of virus evolution.
- In areas with high density of mink farms, between-farm spread is likely to occur once SARS-CoV-2 is introduced.
- Mechanisms for between-farm spread are largely unknown to date, although transmission through humans probably contributed to the observed farm-to-farm spread. The only other risk factors identified for between-farm transmission are short distance to the nearest SARS-CoV-2 positive farm and in Denmark also large farm size.
- Several other between-farm transmission routes have been investigated including different animal species, domestic or wild animals. These may be involved in transmission of SARS-CoV-2 between farms, although this hypothesis should be confirmed.

3.3. Risk for human and animal health posed by SARS-CoV-2 infection in mink and other animals of the family Mustelidae

It has been demonstrated that SARS -CoV-2 infection can have both anthropozoonotic and zoonotic transmission; therefore, both from human to animals and back from animals to humans (Oude Munnink et al., 2020a). In the following sections, the risk for both human and animal health is assessed, with view of designing monitoring plans.

3.3.1. Risk for human health posed by SARS-CoV-2 infection in mink/mustelids

3.3.1.1. Importance of mink variants and variants for human health

Human cases of SARS-CoV-2 viruses originating from variants related to mink have been identified in Denmark and the Netherlands in the EU/EEA. Moreover, in Denmark community transmission of

such variants has been observed. In total, 10 countries globally (Canada, Denmark, France, Greece, Italy, Lithuania, the Netherlands, Spain, Sweden and the USA; Figure 5) have reported SARS-CoV-2 cases in both mink and humans. In some of these cases, the mink-related Y453F mutation was observed, e.g. Denmark, the Netherlands, Sweden and Greece. However, the Y453F mutation has also been reported to the sequence database from human cases (e.g. South Africa, Belarus, Russia, Switzerland). In GISAID, 1,506 sequences from human cases with the Y453F mutation have been uploaded (South Africa, Belarus, Denmark including Faroe Islands, the Netherlands, Russia, Switzerland, USA), 1,299 sequences related to the B.1.1.298 variant (Denmark and Iceland) and 16 human Cluster 5 sequences (Denmark) are available in the database.
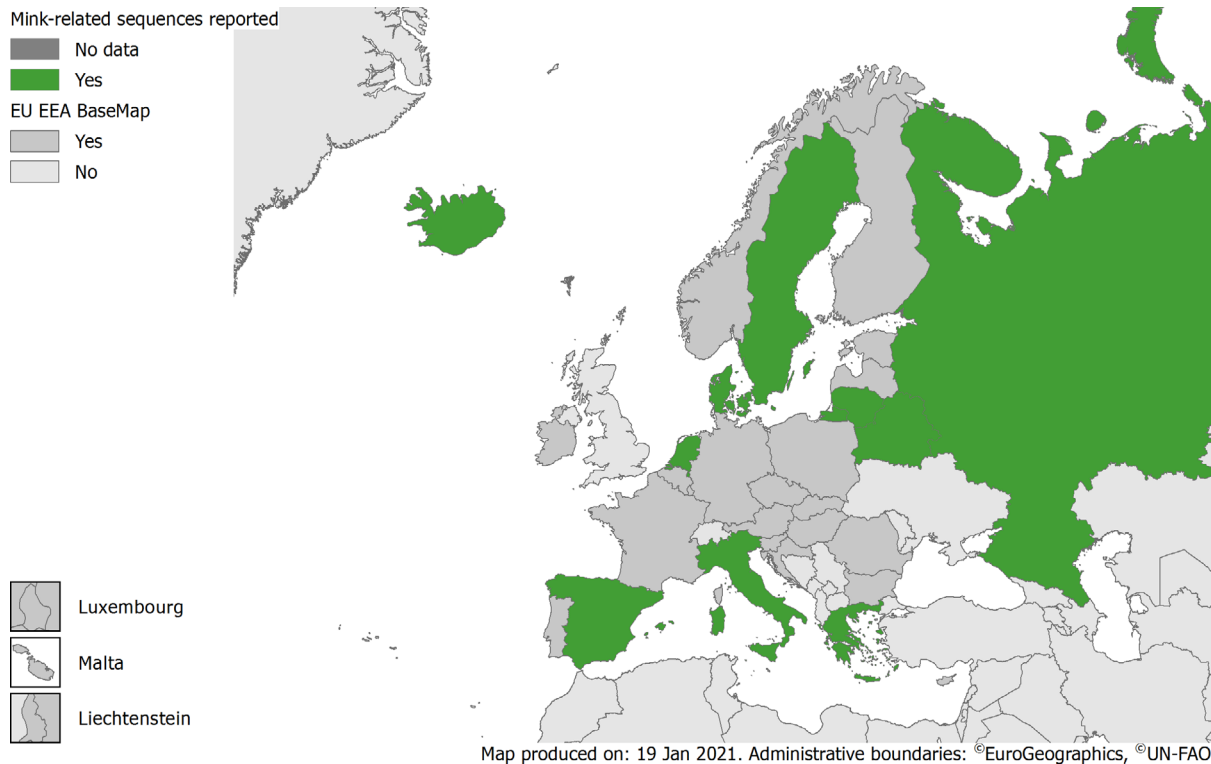
Source: GISAID –Initiative.

Figure 5: Countries reporting SARS-CoV-2 mink-related sequences, European Region as of 19 January 2021

From 8 June 2020 to 2 January 2021, Denmark reported over 1,000 human cases of infection with a mink-related variant virus that were primarily detected in the following regions: North Denmark Region (588), Central Denmark Region (299) and South Denmark Region (90). A further spread across the country was observed and sporadic cases have been reported in other regions with a lower prevalence outside the areas with infected mink herds.[22] Denmark has in the reported period sequenced ~ 16% of all human COVID-19 cases varying depending on sample week and region. In the North Denmark Region, from 8 June to 6 December 2020, in around 30% of sequenced isolates a mink-related variant was identified. Public health measures were implemented in Northern Jutland to reduce the spread of mink variants and it was decided to cull all farmed mink in Denmark. As an effect there was a decrease in the overall number of cases in the region, however, supposedly due to increased handling of mink in that period the proportion of mink variants in sequenced samples increased in all three regions. After the restrictions were lifted, a new increase in the overall number of cases was seen in Northern Jutland whereas the proportion of mink variants gradually declined, after all mink were culled. The proportion of reported mink variants to GISAID is now at a very low level (Figure 6). The Danish Authorities have estimated that more than 4,000 human cases in Denmark

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[22] https://covid19.ssi.dk/-/media/cdn/files/notat-om-den-seneste-udvikling-af-sars-cov2-p-minkfarme-og-blandt-mennesker_uge_50_11122020.pdf?la=da

have been caused by mink variants (personal communication T. Grove Krause, Statens Serum Institute, Denmark) and 1,425 sequences by the mink-related Y453F mutation have been uploaded to GISAID as of 19 January 2021 with the latest sequence uploaded on 28 December 2020. On 18 January 2021, no additional human cases had been reported since the beginning of January from Denmark.
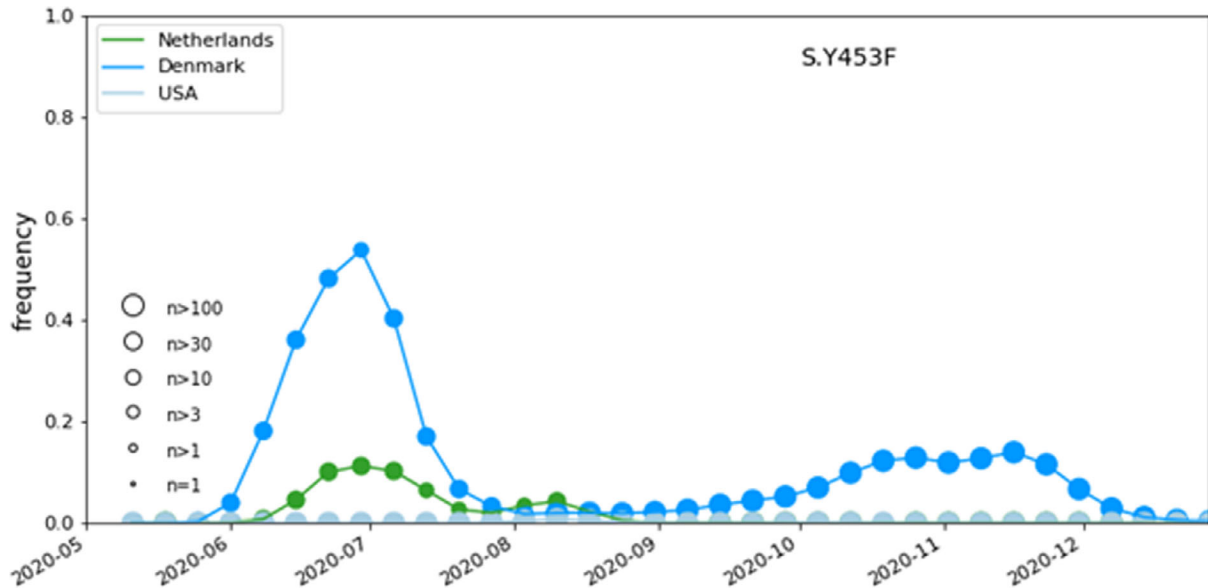
Source: https://github.com/hodcroftlab/covariants/blob/master/table_overview.md#sy453f

Figure 6: Proportion of mink-related viruses of the variant Y453F among all sequences of viruses in Demark, the Netherlands and the USA, up to 19 January 2021

In Sweden, people associated with mink farms are tested for SARS-CoV-2 on a weekly basis using PCR, followed by serology in a second phase. Sweden has previously reported several[23] human cases of mink-related variant viruses in mink farms that differed from the Danish variant.[24] No additional human cases of SARS-CoV-2 infection in mink farms were reported up to 18 January 2021. To date, all sequences from mink and humans associated with mink farms in Sölvesborg belonged to Variant B1.1.39, a variant only seen once in Sweden before the outbreak. Furthermore, sequences from humans and mink from the same mink farms cluster closely together, suggesting within-farm human-to-mink and/or mink-to-human transmission. In sequences from two human samples from one of the negative mink farms the mutation Y453F was observed.

In Lithuania, six human cases of SARS-CoV-2 infection were reported in individuals working in a mink farm, according to the State Food and Veterinary Service report on 31 December 2020.[25]

In Poland, 18 individuals working in mink farms in Pomerania, in the north of the country, tested positive for SARS-CoV-2 infection at the end of November 2020, according to media quoting healthcare agencies.

The French Ministry of Agriculture reported on 9 December 2020, that sequencing analysis of the virus discovered in the Eure-et-Loir farm was not the mink-related variant.[26] No additional human cases have been reported in the country.

Up to 18 January 2021, no SARS-CoV-2 viruses had been detected in 30 mink farms in Finland. No human cases of mink-related SARS-CoV-2 infections have been reported in Finland.[27]

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[23] So far only media reports 14 cases, no official public reports available with number of human cases.
[24] https://www.folkhalsomyndigheten.se/nyheter-och-press/nyhetsarkiv/2020/December/inte-samma-virusvariant-hos-svenska-och-danska-minkar/
[25] https://vmvt.lt/naujienos/audiniu-ukyje-radviliskio-rajone-patvirtintas-naujas-covid-19-zidinys
[26] https://agriculture.gouv.fr/surveillance-du-sars-cov-2-dans-les-elevages-de-visons-bilan-des-investigations
[27] https://www.ruokavirasto.fi/viljelijat/elaintenpito/elainten-terveys-ja-elaintaudit/elaintaudit/turkiselaimet/sars-cov-2-turkiselaimilla/sars-cov-2--seurantatutkimukset-turkistarhoilla/

Italy has not reported further human cases of mink-related SARS-CoV-2 infections as of 18 January 2021. In addition, activities on mink farms were suspended from 21 November 2020 until the end of February 2021 when a new evaluation is expected.[28]

Spain did not report cases of mink-related SARS-CoV-2 infections in humans.

In the Netherlands, mink farming is banned from 8 January 2021. As of 24 December 2020, all mink in 126 farms were culled, of which mink in 69 farms were culled due to SARS-CoV-2 infection. Two human cases with mink-related SARS-CoV-2, possibly infected by mink, were detected in individuals working in two mink farms in May 2020.[29,30] No further human cases of mink-related SARS-CoV-2 have been reported in the Netherlands.

Greece reported on 11 January 2021 that the Y453F mutation in the spike (S) protein has been detected in SARS-CoV-2 genomes from six human cases directly related to mink (farm workers/owners) and on three farms. None of the other mutations described on the Rapid Risk Assessment from 12 November 2020 from EU agencies (ECDC, EFSA, EMA) has been found. Therefore so far, sequenced human samples from the community have not revealed any of the mutations mentioned above.

3.3.1.2. Risk assessment

This assessment is based on information available to ECDC at the time of publication and, unless otherwise stated, the assessment of risk refers to the risk that existed at the time of writing. It follows the ECDC rapid risk assessment methodology, with relevant adaptations (ECDC, 2019). The overall risk was determined by a combination of the probability of an event occurring and its consequences (impact) for individuals or the population (ECDC, 2019).

Limitations have been described in the previous rapid risk assessment on mink.

What is the risk to human health posed by SARS-CoV-2 mink-related variants?

Circulation and transmissibility

Current available evidence indicates that SARS-CoV-2 variants related to mink are able to circulate rapidly in mink farms and also spill over to human communities close to the farms (Koopmans, 2020); however, mink-related variant viruses have so far not shown the ability to be more transmissible compared with other circulating SARS-CoV-2.

The Cluster 5 variant has not been identified since September and Y453F circulation has not been reported since the end of December 2020 in Denmark. In the Netherlands, limited human transmission has been reported outside mink farms since the first mink farm outbreak was identified in April 2020, but no further circulation has been observed in the end of 2020. Implemented prevention and control measures in the community, as well as at the on-farm level, have reduced overall SARS-CoV-2 transmission in general and the circulation of mink-related variants in particular. Nevertheless, undetected circulation cannot be ruled out, as there is a likely underestimate of SARS-CoV-2 cases and a low coverage of viruses sequenced.

Based on the available evidence, the probability of infection with mink-related variant strains is assessed as low for the general population, moderate for the populations in areas with a high concentration of mink farms and very high for individuals with occupational exposure (ECDC, 2020a).

Severity

Patients reported to be infected with these mink-related variants, including the Cluster 5 variant in Denmark, do not appear to have more severe clinical symptoms compared with those infected with non-mink-related variants. Therefore, the current impact of COVID-19 on disease severity in patients infected with any mink-related variant appears to be similar to those infected with non-mink-related variants (ECDC, 2020a). This impact was previously assessed as low for the general population and very high for individuals with risk factors for severe COVID-19 disease, such as the elderly (ECDC, 2020a).

Diagnostics, immunity, reinfection, vaccination and treatment

The emergence of new variant viruses could have an impact on detection assays in use, e.g. through deletions that may cause the failure of RT-PCR tests based on target S alone (Wang et al., 2020).

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[28] http://www.salute.gov.it/portale/news/p3_2_1_1_1.jsp?lingua=italiano&menu=notizie&p=dalministero&id=5183

[29] https://www.rijksoverheid.nl/ministeries/ministerie-van-landbouw-natuur-en-voedselkwaliteit/nieuws/2020/12/24/verbod-pelsd ierhouderij-van-kracht

[30] https://www.rijksoverheid.nl/documenten/brieven/2020/05/19/stand-van-zaken-onderzoek-covid-19-naar-nertsenbedrijven

Multitarget RT-PCR addressing genome regions that code both the N and S proteins does not encounter these problems and, indeed, a divergent result for N and S targets in RT-PCR may be used as an indicator for selecting those samples to be sequenced (ECDC, 2020b).

Diagnostic SARS-CoV-2 RT-PCR assays have not been shown to be affected by the Cluster 5 variant or other Danish mink-related variants. The impact on variant viruses could potentially have an effect on the sensitivity of antigen and antibody detection assays, including rapid antigen tests (RATs), although any effect will probably be small. Of all mink-related variants analysed so far, only the Cluster 5 variant has raised specific concern due to its effect on antigenicity. In preliminary studies, this variant showed reduced sensitivity to neutralising antibodies from COVID-19 patients in 2020.[31]

As not all implications related to SARS-CoV-2 mutations are clearly understood, a great prudence is governing the measures to limit the spread of these variants and several studies are ongoing to better define the immunological and pathogenetic characteristics of these viruses (ECDC, 2020c).

Antibodies generated by natural infection with other variants and the antibodies generated by vaccines may potentially exhibit reduced neutralisation in individuals infected with a mink-related variant virus. Assessment of the T-cell-mediated immune response to mink-related variant viruses has not yet been investigated. Findings are based on limited data available and the impact on (i) the risk of reinfection, (ii) reduced vaccine efficacy or (iii) reduced benefit of treatment with plasma from convalescent patients or with monoclonal antibodies is not fully understood and needs to be further investigated.

The continued likelihood that SARS-CoV-2 is introduced into the mink population in farms and spreads within animals poses the risk that the species adaptation mechanisms of the virus would introduce new mutations with new variants that would emerge to infect workers and also spill over to the rest of the human population. The impact of the emergence of new variant viruses in this population therefore needs to be closely monitored.

Cross-border spread

Cross-border spread of SARS-CoV-2 variants related to mink in the EU/EEA countries and the UK through humans has not been observed so far, but there is no indication that the potential for cross-border spread is different to that for other SARS-CoV-2 variants. Genetic adaptation in mink populations could give rise to a selective advantage in regions with mink farming activity. The mutation Y453F, defining mink-related variants, has also been detected outside of Europe, indicating the potential for circulation of such strains. In Denmark and the Netherlands community transmission has not been observed since end of December 2020.

The mutation Y453F found both in Denmark and the Netherlands and linked to mink, and which is believed to confer a selective advantage in mink-to-mink transmission, has also been detected sporadically in other countries. This could be an indication that this mutation can also arise in humans, or that there is undetected sporadic mink-to-human transmission or low-level circulation of mink-related SARS-CoV-2 in these countries. The geographically uneven sequencing coverage and delay between sampling and upload of sequence data to international databases is delaying the overall availability of information and is limiting the assessment of geographical spread of the new variants beyond affected areas.

Workers employed at mink farms, who are not fully compliant with hygiene measures could also introduce the mink-related variants to the community around the vicinity of the farm, to new areas or to other countries when travelling or returning home while infected. Such events could contribute to the cross-border spread of new variants.

In summary, the probability of cross-border spread of SARS-CoV-2 variants related to mink in the EU/EEA countries and the UK through humans is likely, in the same way it is for other SARS-CoV-2 strains, however, no local circulation in the EU/EEA is currently observed.

3.3.1.3. Options for public health response

Response measures to control the COVID-19 pandemic are also valid for the emergence of mink-related variant viruses (ECDC, 2020a). Increased surveillance, involving collaboration and communication between animal and public health authorities, testing and sequencing of samples from mink farm workers and increasing the representativeness of sequencing viruses from the general population may help to prevent the introduction of SARS-CoV-2 into mink farms, identify cases in workers early and monitor mink-related SARS-CoV-2 variant viruses in the population. Information on

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[31] https://www.ssi.dk/-/media/arkiv/dk/aktuelt/nyheder/2020/mink-cluster-5-kort-rapport.pdf

human testing, sequencing and characterisation of antigenic properties and virus infectivity as well as infection prevention and control measures for mink farm workers and visitors have been previously provided and are still valid. Case-based data from such human cases linked to an animal source like mink should be reported to The European Surveillance System (TESSy) and Early Warning and Response System (EWRS).

More detailed public health measures have been described in the ECDC rapid risk assessment from November 2020 (ECDC, 2020a).

Key messages

- Mink-related variant viruses can establish circulation in the community, but so far have not been shown to be more transmissible or severe compared with other circulating SARS-CoV-2.
- Currently described mink-related variant viruses have not been shown to impact SARS-CoV-2 diagnostic assays.
- The circulation of mink-related variants in the human population has decreased, Cluster 5 variant viruses have not been identified since September and the circulation of the Y453F virus variant has not been reported since the end of December 2020 in Denmark.
- Close monitoring of SARS-CoV-2 infections in mink and mink farm workers is needed to identify SARS-CoV-2 cases early and prevent further transmission to humans and other animals;
- Sequencing of viruses from mink and human cases and sharing this information is needed to identify new variant viruses early so that this may have an impact on diagnostics, transmission, severity or vaccine effectiveness.

The probability of infection with mink-related variant strains is assessed as low for the general population, moderate for the populations in areas with a high concentration of mink farms and very high for individuals with occupational exposure.

3.3.2. Risk for animal health posed by SARS-CoV-2 infection in mink/mustelids

The animal health risk posed by SARS-CoV-2 infection in mustelids is characterised by the probability of acquiring the infection and the adverse outcomes that can result from this infection.

The probability of acquiring the infection depends on host susceptibility (the biological intrinsic characteristics of the host that can lead to infection given a certain level of exposure), and the level of exposure of the host to the pathogen, i.e. the chance of the host to come in contact with the infectious agent (Parkin and Balbus, 2000).

For the risk to animal health following exposure to SARS-CoV-2-infected mustelids, the susceptibility of other animal species is described in Section 3.3.2.1 where both laboratory studies (*in silico*, *in vitro* and experimental infection trials) and observations from data collected under field conditions from animal species exposed to SARS-CoV-2 (observation and investigation of natural infections) are considered.

Because the only reported mustelids known to be susceptible to SARS-CoV-2 are American mink (commonly farmed in the European Union) and ferrets (usually kept in households or breeding centres) as stated in Section 3.2.5, other animals possibly exposed to SARS-CoV-2 from infected mustelids are animals that can be present in infected mink farms and can become infected by close contact with these animals (Section 3.3.2.2). Other animals that could be exposed to infected ferrets (the other mustelid species that can be a source of SARS-CoV-2) in households would be first exposed to infected humans in the same households, as the latter are the first likely source of SARS-CoV-2 in that situation.

3.3.2.1. Susceptibility of mustelids and other animals to SARS-CoV-2

SARS-CoV-2 may have its ancestral origins in bats, in which the closest genetic ancestor is a betacoronavirus (β-CoV) isolated from the horseshoe bat (*Rhinolophus affinis*) (Delahay et al., 2020).

An analysis of the ACE2 protein, the functional receptor for the spike protein of SARS-CoV-2 in a broad range of vertebrates, predicts susceptibility to infection in several mammal species, notably, but not exclusively, carnivores or omnivores (Liu et al., 2020b).

Experimental studies

In silico studies

In addition to observational studies of natural outbreaks and experimental studies using laboratory or farm animals, *in silico* and *in vitro* analyses are alternatives to assess the susceptibility of animals to

SARS-CoV-2. In general, findings from *in silico* or *in vitro* studies are intriguing and they may be predictive of the effects under natural conditions. However, their real significance should always be verified using alternative approaches. *In silico* and *in vitro* approaches can therefore provide valuable preliminary information to drive further experimental studies that aim to better understand the host pathogen interaction.

In silico studies, based on the general consensus that ACE2 binds to the RBD of SARS-CoV-2 spike protein, has been used to compare the key AAs in ACE2, from different species, for the binding ability to the RBD of SARS-CoV-2 spike protein. The simulated structure of the protein complex showed that animals belonging to the families Bovidae and Cricetidae may act as intermediate hosts, while reptiles should not be able to do so (Luan et al., 2020). Another *in silico* study using the multiple sequence alignments of the ACE2 proteins revealed a high degree of conservation among humans, dogs, cats, tigers and mink. Furthermore, the three-dimensional structure of mink and human ACE2 complement perfectly the RDB of SARS-CoV-2 (Hayashi et al., 2020).

In vitro studies

In vitro studies revealed that SK-6 and ST porcine cell lines are permissive to SARS-CoV-2. When inoculated with SARS-CoV-2 those cell lines did not develop a cytopathic effect but showed virus replication. Conversely, PK-15 porcine cell lines and embryonated chicken eggs seemed completely resistant to infection (Schlottau et al., 2020).

Di Teodoro et al. (2021) showed that respiratory *ex vivo* organ cultures of cattle and sheep, but not of pigs, sustain viral replication of SARS-CoV-2 *in vitro* and the virus is associated with ACE2-expressing cells of the respiratory tract of both ruminant species.

Such experimental approaches have also been used to assess the potential biological significance of the different isolates. Analysis of the frequency of the variants over time has suggested a selective advantage of variant D614G over other variants of SARS-CoV-2 (Furuyama et al., 2020; Weissman et al., 2020). *In vitro* studies have also shown that variant D614G expressed increases in infectivity on human lung cells, colon cells and cells rendered permissive by ectopic expression of bat or pangolin ACE2. However, it has been shown that the variant D614G is effectively neutralised by antibodies targeting the RBD. In addition, variant D614G alters the receptor-binding conformation that makes virion membrane fusion with the target cell membrane more probable (Yurkovetskiy et al., 2020).

Other findings showed that although viral loads of the D614G variant is enhanced in the upper respiratory tract of a hamster model of SARS-CoV-2 infection, sera from hamsters infected with classical virus exhibited modestly higher neutralisation titres against the variant D614G virus compared with against classical virus (Plante et al., 2020).

These *in vitro* approaches, cumulatively, support the concept that a mutation may confer a substantial increase in transmissibility compared with other variants and it may have a better fitness to colonise upper respiratory tract.

Experimental infections

Results from studies of experimental infection are available for a small number of animal species (Table 2). Susceptibility to SARS-CoV-2, with varying levels of viral replication and shedding, has been demonstrated in Carnivora: American mink domestic cats (*Felis catus*) dogs (*Canis lupus familiaris*), ferrets, raccoon dogs; Rodentia: Syrian hamsters (*Mesocricetus auratus*); primates: rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*M. fascicularis*), African green monkeys (*Chlorocebus* sp.), common marmosets (*Callithrix jacchus*); Scandentia: Chinese tree shrews (*Tupaia belangeri chinensis*); Chiroptera: Egyptian fruit bats (*Rousettus aegyptiacus*), and Lagomorpha: rabbits (*Oryctolagus cuniculus*); ungulates: white-tailed deer (Delahay et al., 2020; Freuling et al., 2020; Shuai et al., 2020; Palmer et al., 2021).

Dogs appear to have limited susceptibility to SARS-CoV-2; they have low levels of viral replication and infections typically appear to be asymptomatic, while clinical signs of respiratory and/or gastrointestinal disease tend to be mild to moderate in felines (OIE, 2020).

Five studies (Table 2) have reported that it was not possible to experimentally infect pigs (*Sus scrofa domesticus*), despite the capacity of porcine ACE2 to bind SARS-CoV-2 spike proteins (Liu et al., 2020a). However, inconsistent results from virus and antibody tests have been observed in some pigs as these results did not fit with a normal viraemia and humoral immune response following natural infection. It should be considered that experimental studies frequently overestimate susceptibility, as a high titre of infective inoculum is often used to maximise the likelihood of infection. This can explain the contrasting observations of effective transmission to in-contact ferrets following experimental

infection (Schlottau et al., 2020) and the apparent absence of infection in pet ferrets, despite their close contact with infected humans (Sawatzki et al., 2020).

In the experimental study by Ulrich et al. (2020), six inoculated cattle and three contact cattle were tested. Two inoculated cattle experienced viral replication and immune responses without showing clinical signs, but this was not found in contact animals did, this suggests very low susceptibility of cattle to SARS-CoV-2 infection.

For susceptible bat species, SARS-CoV-2, a betacoronavirus, is presumed to originate from horseshoe bats of the Rhinolophidae family (Olival et al., 2020), which is present in central and southern Europe.[32] One study (Ar Gouilh et al., 2018) found SARS-CoV-2 clade member, betacoronavirus EPI1, in Western Europe in *Rhinolophus ferrumequinum,* in addition to a variety of alphacoronavirus, which can also be zoonotic. Moreover, fruit bats species *Rousettus aegyptiacus* (fam. Pteropodidae) have been tested as susceptible and capable of transmission: following experimental studies including nine infected bats and three sentinels, of which seven bats had lesions in the nasal cavity and in other tissues; immune responses were detected even in sentinel bats and one out of three contact bats became infected (Schlottau et al., 2020). Bats belonging to this latter family are not present in Europe, but only in sub-Saharan Africa, India, South-East Asia and Oceania.[33]

White-tailed deer (*Odocoileus virginianus*) was shown to be experimentally infected with SARS-CoV-2 and can transmit the infection to naïve, co-housed deer (Palmer et al., 2021). Four young animals were inoculated intranasally, they showed neither clinical signs nor viraemia, during 21 days follow-up. Nevertheless, viral RNA was detected in nasal secretions and faeces in both infected and in-contact animals (Palmer et al., 2021). White-tailed deer originate from North America, they were introduced last century in Europe in Finland (currently around 100,000 individuals[34]) and in Czechia (estimated 200–400 individuals in total) as free ranging and in some hunting enclosures in Central Bohemia (Anděra et al., 2009).

In the study by Bosco-Lauth et al. (2021), it was shown that deer mice, bushy-tailed woodrats, and striped skunks, are susceptible to infection and can shed the virus in respiratory secretions, while cottontail rabbits, fox squirrels, Wyoming ground squirrels, black-tailed prairie dogs, house mice and racoons were not shown to be susceptible to SARS-CoV-2 infection. Of those species, only house mouse and racoons are present in Europe.

The susceptibility of other animal species to SARS-CoV-2 infection has been investigated, and these appear to be resistant (Table 2).

Table 2: Animal species assessed and deemed not susceptible to SARS-CoV-2 infection based on scientific literature up to 1 December 2020

Species	Type of assessment	Viraemia, clinical and pathological findings	Conclusion	Reference
Cattle (*Bos taurus*)	Experimental study including six inoculated cattle and three contact cattle	Two inoculated cattle experienced viral replication and immune responses. No contact animals responded. No clinical signs.	Low susceptibility to infection	Ulrich et al. (2020)
Chicken (*Gallus gallus domesticus*)	Experimental study including 17 infected chickens and three sentinels	No viral RNA detected. No seroconversion observed. No pathological findings.	Probably resistant to infection	Schlottau et. al. (2020)
Chicken	Experimental study incl. 5 infected chicken and 3 contact chicken	No viral RNA detected. No immune responses No pathological findings	Probably resistant to infection	Shi et al. (2020)
Duck (*Anas platyrhynchos domesticus*)	Experimental study including five infected ducks and three contact ducks	No viral RNA detected No immune responses No pathological findings	Probably resistant to infection	Shi et al. (2020)

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[32] https://www.gbif.org/species/5449
[33] https://www.gbif.org/species/9367
[34] https://yle.fi/uutiset/osasto/news/white-tailed_deer_in_finland_from_5_to_100000_in_80_years/10294577

Species	Type of assessment	Viraemia, clinical and pathological findings	Conclusion	Reference
House mouse and raccoons	Experimental study on six mice and three raccoons, followed up to 28 days post-infection (dpi)	No viral RNA detected No immune responses No pathological findings	Not susceptible	Bosco-Lauth et al. (2021)
Mosquitoes (*Aedes aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*)	Experimental intrathoracic inoculation in 277 adult mosquitoes	No replication	Not susceptible	Huang et al. (2020)
Mouse (*Mus musculus*)	Experimental study in seven transgenic mice with human ACE2 receptor and three wild-type mice	Weight loss, interstitial pneumonia and IgG antibodies in transgenic, but not in wild-type mice	Wild-type mice potentially resistant	Bao et al. (2020)
Pig (*Sus scrofa domesticus*)	Experimental study including nine pigs infected and three sentinels	One suspected viral RNA detection No clinical signs, no immune response. No pathological findings	Probably resistant to infection	Meekins et al. (2020)
Pig	Experimental study including nine infected pigs and three sentinels	No viral RNA detection, seroconversion, clinical and pathological signs observed	Probably resistant to infection	Schlottau et al. (2020)
Pig	Experimental study including five infected pigs and three contact pigs	No viral RNA detected No immune responses No pathological findings	Probably resistant to infection	Shi et al. (2020)
Pig	20 piglets inoculated via different routes	No viral RNA detected Low levels of antibodies were observed in animals inoculated by IV and IM routes No clinical signs No pathological findings	Probably resistant to infection	Vergara-Alert et al. (2020)
Pig	Two contact pigs housed with 16 experimentally infected pigs	Viral RNA detected from nasal swabs in two inoculated pigs three days after inoculation and in tissues of one pig 13 days after inoculation No apparent clinical signs; vague immune responses in two inoculated pigs. None of the contact pigs showed any signs of infection	Probably resistant to infection	Pickering et al. (2021)

Field studies and observations

Natural infections of SARS-CoV-2 have been recorded in pet dogs and cats, in wild felids in zoos including tigers (*Panthera tigris*), lions (*Panthera leo*), puma (*Puma concolor*), snow leopard[35] (*Panthera uncia*) and western lowland gorilla[36] (*Gorilla gorilla gorilla*), in farmed and wild American mink and kept ferrets (Delahay et al., 2020).

Among these susceptible animals, American mink are the only mustelid species farmed on a large scale in many countries (Enserink, 2020; Oreshkova et al., 2020). In this species, disease may vary from inapparent (no clinical signs of infection are shown) to fatal. In Denmark for example, in 30% of 207 mink farms with SARS-CoV-2 infection, no clinical signs were observed at all, whereas 60%

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[35] https://www.aphis.usda.gov/aphis/newsroom/stakeholder-info/sa_by_date/sa-2020/sa-12/ky-snow-leopard-covid
[36] https://www.the-scientist.com/news-opinion/us-confirms-worlds-first-sars-cov-2-cases-in-gorillas-68347

reported clinical signs among kits and 53% among adults. The clinical signs reported were nasal discharge (25%), sneezing (23%), respiratory symptoms (31%), depression (7%), reduced feed intake (54%) and diarrhoea (5%). Out of 212 farms, 134 (63%) observed increased mortality, with a 30-day mortality of 0.45% (median), while the mortality is generally <0.05%. From 30 mink farms, which delivered detailed mortality data, the median daily mortality (on the peak day) observed in mink during the peak of SARS-CoV-2 epidemic was 0.14% (5th–95th percentiles: 0.11–1.9%), compared with a baseline mortality close to zero (e.g. 1–4 animals/month per farm with an average size of 10,000 mink per farm) (Boklund et al., 2021). In the Netherlands, mortality rate ranged from 2.4% to 3.8% compared with a baseline mortality of 0.1% (Molenaar et al., 2020).

In household settings, viral transmission is self-limiting, however, in high-density animal environments, there can be sustained between-animal transmission. To date, animal-to-human transmission has been shown in Denmark and in the Netherlands, in infected mink farms (Oude Munnink et al., 2020b). From many Danish farms, SARS-CoV-2 has been found in samples from mink and persons related to the farm in close correlation timewise. However, it has seldom been possible to determine the direction of the transmission. In the area of Denmark with the highest density of mink farms (before culling) and the most outbreaks of SARS-CoV-2 in mink, 30% of mink farmers and households tested PCR positive. Furthermore, in the period from 10 August to 29 November 2020, approximately 27% of the SARS-CoV-2-strains detected in humans in the community in this area were mink-associated (Larsen et al., 2021).

In the Netherlands, 66% of the tested persons associated with infected mink farms were SARS-CoV-2 positive (either PCR or serology) and the sequence of the virus from humans and mink was always similar. Given the millions of SARS-CoV-2 cases worldwide and ongoing potential for further zoonotic and anthropozoonotic viral transmission, further research and surveillance activities are needed to definitively determine the role of animals in community transmission of SARS-CoV-2 (Hobbs and Reid, 2020).

Natural infection in ferrets was also observed in Slovenia in December 2020[13] and Spain (Gortázar et al., 2021).

Viruses related to the SARS coronavirus were also previously detected by virus isolation and direct RT-PCR from the faecal swab of a raccoon dog and in Himalayan palm civets from Chinese markets, these animals were also seropositive together with a Chinese ferret badger (*Melogale moschata*) (Guan et al., 2003).

Samples (n = 115) from 29 birds (26 gulls (Larinae), one hooded crow (*Corvus cornix*), one jackdaw (*Corvus monedula*), and one common kestrel (*Falco tinnunculus*)) were tested for SARS-CoV-2 using RT-PCR in seven Danish mink farms with high prevalence of SARS-CoV-2-infected mink, and only one gull was positive. The positive sample from this gull was from mud on the foot. This same gull was negative in swabs from the feathers and cloaca (Boklund et al., 2021). This, together with evidence from experiments with chickens and ducks (see above) may suggest that bird species are resistant to SARS-CoV-2.

3.3.2.2. Animal species at risk of infection of SARS-CoV-2 in mustelids

Animal species susceptible to SARS-CoV-2 infection (see Section 3.3.2.1), apart from American mink, that may be present in mink farms, and therefore possibly exposed to SARS-CoV-2 from mink/ mustelids are listed in Table 3. Among these, evidence of natural infection was observed only in cats, dogs, raccoon dogs and ferrets.

Table 3: Overview of susceptible species to SARS-CoV-2 infection that can be present in or close to mink farm and exposed to SARS-CoV-2 based on scientific literature published until 31 January 2020

Species	Type of assessment	Viraemia, clinical and pathological findings	Conclusion	Reference
Cat (*Felis catus*)	Experimental study with two cats	Viral RNA transiently detected in nasal, oropharyngeal and rectal swabs and bronchoalveolar fluid No clinical signs Tracheobronchoadenitis	Susceptible	Gaudreault et al. (2020)

Species	Type of assessment	Viraemia, clinical and pathological findings	Conclusion	Reference
Cat	Experimental study including seven subadult kittens and some kittens	Infectious virus was detected in nasal turbinate, lungs and other tissues Immune responses observed Lesions observed in trachea and lungs of kittens	Susceptible and infectious	Shi et al. (2020)
Dog (*Canis familiaris*)	Experimental study incl. five inoculated and two sentinel dogs	Viral RNA detected in rectal swab in one inoculated dog on day 2, but not in other tissues. Two dogs had immune responses, but no pathological findings	Susceptible, but to a limited extent. Not capable of further transmission	Shi et al. (2020)
Dog	Observational study including 15 dogs	Viral RNA detected repeatedly in 2 dogs. No clinical signs No pathological findings?	Susceptible	Sit et al. (2020)
Ferret	Challenge study (18 ferrets)	Infectious virus shed in nasal washes, saliva, urine and faeces. Elevated body temperature and reduced activity No fatalities	Susceptible and infectious	Kim et al. (2020)
Ferret	Experimental study incl. 9 infected ferrets and 3 sentinels	Viraemia? Immunity? No gross lesions observed, some ferrets with severe rhinitis and lung infiltrates	Susceptible and infectious to other ferrets	Schlottau et al. (2020)
Ferret	Experimental study incl. 6 ferrets for two weeks	Infectious virus detected from nasal, but not rectal, swabs. Fever and loss of appetite observed in 1 ferret. Pneumonia and peribronchitis observed in two ferrets. Immune response in all ferrets. No pathological findings.	Highly susceptible and infectious	Shi et al. (2020)
Golden Syrian Hamster (*Mesocricetus auratus*)	Challenge study Number of animals	Viraemia; Transmission period of 6 days Weight loss	Susceptible	Sia et al. (2020)
Rabbits (*Oryctolagus cuniculus*)	Four groups of 3 animals each inoculated nasally and followed for 4 days. One group was followed for 21 days	No clinical signs observed. Viraemia observed for 6–7 days and all 3 rabbits followed for 21 days seroconverted	Susceptible, not capable of further transmission	Mykytyn et al. (2020)
Raccoon dogs (*Nyctereutes procyonoides*)	Nine raccoon dogs inoculated and three naïve animals exposed to these	Three inoculated animals were lethargic but appeared with no other clinical signs, and no other animals demonstrated clinical signs. Viral shedding occurred in 6/9 inoculated animals. Infection was observed in 2/3 contact animals. Immune responses were observed in 4/7 inoculated animals	Susceptible and capable of further transmission	Freuling et al. (2020)

Species	Type of assessment	Viraemia, clinical and pathological findings	Conclusion	Reference
Bats belonging to the family of Rhinolophidae bats (e.g. *Rhinolophus ferrumequinum*)	SARS-CoV-2 clade member detected in Western Europe		Susceptible, probable reservoir	Ar Gouilh et al. (2018)
White tailed deer	Experimental study with 4–6-week-old fawns	Animals inoculated intranasally showed neither clinical signs nor viraemia, during the 21 days follow-up. RNA detected in nasal secretions and faeces in both infected and in-contact animals	Susceptible without clinical signs but capable of further transmission	Palmer et al. (2021)

Given the transmission dynamics as described in Section 3.2.6, the animals that are susceptible to SARS-CoV-2, and are at risk because of their presence in the vicinity to the mink, may include cats, dogs, other fur animals bred in the same farm such as raccoon dogs and wild animals such as bats belonging to the family of Rhinolophidae.

In Denmark, among 168 SARS-CoV-2-infected mink farms, the following animals were observed to have access to infected farm environments: 4% farms reported escaped mink, 68% reported occurrence of gulls or other birds, 15% had observed stray or feral cats and 19% reported rats or mice on the farm. Furthermore, 34% and 29% were reported to have a dog or their own cat, respectively (Boklund et al., 2021).

Samples from 30 feral cats from 1 October to end November 2020 tested negative in RT-PCR assays, while 21 of the cats tested negative in indirect antibody ELISA (Boklund et al., 2021). In the Netherlands, 11 out of 69 cats tested positive for SARS-CoV-2 (by PCR or serology). Flies caught in one farm were also positive for RNA in the RT-PCR, while flies from another outbreak farm were negative for RNA in the RT-PCR (Boklund et al., 2021). Among the farm animals tested in Denmark were 1 horse (grazing between the mink cages), 8 farm cats, 11 dogs, 14 chickens and 33 rabbits. Among these, only one dog and one farm cat were positive in the RT-PCR, and four dogs were antibody positive by ELISA (Boklund et al., 2021).

For bats, in Denmark, where continued monitoring of bats for coronavirus is in place, different alphacoronaviruses have been identified in 5 of the 17 bat species present in the country. Nevertheless, bats of the Rhinolophidae family, from which SARS-CoV-2 (betacoronavirus) is thought to have originated, have never been described in Denmark. This family is, however, present in central and southern Europe, therefore monitoring of these species may be considered in this region.

On 15 December 2020, the USDA reported that a wild mink had been found positive; it was trapped around an infected mink farm in Utah.[37] The virus strain is the same as the one circulating in farmed mink. SARS-CoV-2 outbreaks have been reported not only in 16 mink farms in the USA, mostly in Utah, but also in Wisconsin, Oregon and Michigan. No other wild animals (i.e. rodents, skunks, raccoons) have tested positive so far.

Raccoon dogs, which are also bred for fur production and sometimes even on the same farms as mink, are not mustelids but they have also been shown to be susceptible and capable of transmission (Table 3). They are of particular interest, because there is also a large wild population widespread in Europe (Kauhala and Kowalczyk, 2012) that might contribute to the between-farm transmission, given the limited on-farm biosecurity of many mink farms.

Key messages:

1) American mink and ferret are the only mustelid species for which evidence both from experimental and field studies for susceptibility to SARS-CoV-2 is available. There are no other experimental studies available for other wild mustelid species.
2) Few species of animals present in infected mink farms have been monitored following natural exposure to SARS-CoV-2 from infected mink. Among these, cats and dogs were the only species found infected under field conditions. For other animal species susceptible in

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[37] https://www.nationalgeographic.com/animals/2020/12/wild-mink-tests-positive-coronavirus-utah/

experimental infections with SARS-CoV-2 but without evidence of natural infection during outbreaks in mink farms, more investigation is needed to clarify their epidemiological role.

3) Raccoon dogs deserve particular attention as they are susceptible and capable of shedding SARS-CoV-2, and they are both bred intensively for fur production as mink, and present in the wild with high abundance in Europe.

4) To date, the animal species that may be included in monitoring plans are the ones shown to be susceptible and capable of further transmitting the disease and that can be present in or close to infected mink farms, i.e. American mink (farmed or wild), ferrets, cats, raccoon dogs, white-tailed deer (very locally distributed, in the EU they are present only in Finland and few individuals in Czechia[38]) and bats belonging to the family Rhinolophidae. The monitoring approach can be similar to the ones described in Section 3.4.

5) With new research and new evidence or new mutations of the virus, other species, in particular widespread species such as rodents (about which there is still uncertainty about their epidemiological role), may be involved in the SARS- CoV-2 epidemiology and the monitoring approach could be reconsidered.

3.4. Monitoring SARS-CoV-2 infection in mustelids

In this Section, different SARS-CoV-2 monitoring scenarios in mustelids are presented. The different components to be considered in monitoring plans include the objectives, target animals, sampling frequency and sample size for each possible scenario and are described and assessed for the categories of mustelid animals that can be monitored, i.e. farmed animals, animals kept as pets or as hunting animals, and for wild mustelids.

3.4.1. Monitoring components

3.4.1.1. Diagnostic tests

Accurate diagnostic tools for direct, as well as indirect, detection of SARS-CoV-2 infection in animals including mustelids are available; the main ones that can be used for planning monitoring schemes and their characteristic are described below.

Detection of virus nucleic acids

Published protocols for real-time RT-PCR initially designed for detection (screening or confirmation) of SARS-CoV-2 nucleic acids in human samples (e.g. Corman et al. (2020)) are currently in use in veterinary laboratories set up also for testing of mustelids (Hammer et al., 2020; Oreshkova et al., 2020). Real-time RT-PCR kits specifically for veterinary use are also commercially available, e.g. IDEXX SARS-CoV-2 (COVID-19) RealPCR Test,[39] with 100% sensitivity, according to the manufacturer (CDC, 2020).

Mutations of PCR targets may decrease the sensitivity of the RT-PCR tests based on the single target S protein (Wang et al., 2020). Multitarget RT-PCR addressing genomic target for both N and S protein can overcome this issue and indeed divergent results for N and S targets in RT-PCR may be used for selecting those samples to be sequenced (ECDC, 2020b).

Samples for the real-time PCR test are usually pharyngeal swabs from dead or live mink, this is the sample matrix of choice. Expiration air directly collected from animals by using electronic air collector tools (e.g. AeroCollect® System, Force Technology) can also be used as a sample matrix, which avoids the manipulation of animals. Conjunctival and saliva swabs have also been suggested as valid sample matrices, as well as environmental sampling (Fernández-de-Mera et al., 2020), by collecting swabs in particular from cages and bedding and, to a lesser extent, from faecal material, drinkers or feed leftovers (de Rooij et al., 2021). Also according to Oreshkova et al. (2020), a higher viral load is detected in the upper respiratory tract compared with gastrointestinal tract, and virus RNA is not always detected in the faeces from pharyngeal-positive animals (see Section 3.2.5).

Nevertheless, more information is needed about test sensitivity and validation by using sample matrices different from pharyngeal swabs, especially during testing of newly infected animals, as is the case when monitoring for early detection. This might be solved by increasing the sample size, but, since sensitivity is unknown, the sample size cannot be quantified.

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[38] It should be reminded that in Czech mink farming and in general fur animal farming is prohibited.
[39] https://www.idexx.de/de/veterinary/reference-laboratories/overview-idexx-sars-cov-2-covid-19-realpcr-test/

Detection of SARS-CoV-2-specific antibodies

For indirect serological diagnosis, a multi-species ELISA for detection of antibodies against SARS-CoV-2 in animals with diagnostic specificity and sensitivity of 100.0% and 98.3%, respectively, has been described (Wernike et al., 2020). Two commercial multi-species ELISAs, based on double antigens (ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA, IDVet[40]; WANTAI SARS-CoV-2 Ab ELISA) are also available and in use in veterinary laboratories for serological testing of mustelids (Hammer et al., 2020).

As mink could be infected with other coronaviruses related to transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhoea virus (PEDV) (Have et al., 1992), this may impact the specificity of ELISA for SARS-CoV-2. With this in mind, and given the absence of appropriate validation studies using mink sera for the available ELISAs, some extent of cross-reactivity leading to false positive results cannot be excluded. To confirm the positive serological results obtained from ELISAs, virus neutralisation tests (VNT) can be used.

The WANTAI SARS-CoV-2 Ab ELISA is a two-step incubation antigen 'sandwich' ELISA[41] developed for human plasma/serum samples but also used in mustelids for veterinary use. From the limited validation studies in humans the following validation parameters could be retrieved: the sensitivity and specificity (and their 95% CI) were estimated at 93.1% and 100%, respectively. However, it should be mentioned that, for the validation of this method, the samples were not randomly selected, and sensitivity and specificity estimates may not be indicative of the performance of the ELISA in the field. The number of samples in the tested panel was a minimally viable sample size that still provides reasonable estimates and confidence intervals for test performance. However, the samples used may not be representative of the antibody profile observed in patient populations.

The matrix sample for the detection of SARS-CoV-2-specific antibodies is blood serum. Blood samples from mink and other mustelids can be achieved from a variety of veins including the cephalic, jugular, saphenous, femoral, and if under general anaesthetic, the cranial vena cava.

Currently there are no reports nor publications available of appropriate validation studies using serological assays such as antibody ELISAs – developed for human use – in mink. Only a limited amount of sera derived from infected mink farm and/or experimental studies were used to validate results obtained from serological testing, but this does not allow a validation for a particular use and to estimate the performance characteristics, such as repeatability, analytical sensitivity (Se) and specificity (Sp), accuracy, diagnostic Se and Sp (according to OIE chapter 1.1.2 'Principles and methods of validation of diagnostic assays for infectious diseases'[42]). For mink and mustelids, there is a need for reference serum samples (reference material) as well as the need to monitor the assay performance after initial validation. The latter requires quality control programmes (batch validation and release) and proficiency testing, normally organised by European Reference laboratories. Currently, there is lack of a harmonised validation programme and quality control programmes for serological testing in mink.

3.4.1.1.1. Diagnostic time window

For monitoring purposes, it is important to know the time period from and until when SARS-CoV-2 infection can be detected. At the animal level, in experimental settings in ferrets, RNA can be detected between 2 and 14 days post-infection (dpi), and antibodies can be detected from 8 dpi at least until 36 dpi (Ryan et al., 2020). In mink, antibodies are detected from 14 dpi onwards (Shuai et al., 2020). A 2-week lag time for seroconverting after infection can be considered as a suitable time period to be taken into account in monitoring plans in mustelids.

3.4.1.2. Monitoring approaches for SARS-CoV-2

Different approaches for monitoring SARS-CoV-2 in animals can be based either on observations by farmers or veterinarians of indicators, such as reduced feed intake or lower productivity, clinical signs potentially related to SARS- CoV-2 infection and increased mortality rate (passive monitoring), or on detecting cases of SARS-CoV-2 infection through planned and designed disease-monitoring plans (active monitoring). The latter can be carried out in the animal population (either at animal or at farm level), by defining the epidemiological unit, at random or targeted (risk-based), and they can be based on virological detection of infection in both (diseased) live or dead animals or serological testing to detect inapparent infections. Considering that SARS-CoV-2 infection is notifiable to OIE as emerging

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[40] https://www.id-vet.com/produit/id-screen-sars-cov-2-double-antigen-multi-species/
[41] https://www.fda.gov/media/140929/download
[42] https://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/chapitre_validation_diagnostics_assays.pdf

disease, passive monitoring should be in place across the whole territory of all countries where animals susceptible to SARS-CoV-2 are bred or kept, or where people infected or suspected to be infected with SARS-CoV-2 are in contact with animals susceptible to SARS-CoV-2 (e.g. workers in mink farms, owners of mustelid pets or hunting animals). Information campaigns and other initiatives could be considered to increase the awareness of farmers, veterinarians, ferret owners and the public for this disease.

3.4.1.3. Time to detection and possible design prevalence values for different monitoring objectives

The evidence collected from affected MSs, mainly Denmark and Netherlands, can provide the basis to estimate the design prevalence and the sampling frequency for monitoring of SARS-CoV-2 in mustelids, mainly on mink farms, according to different monitoring objectives. An overview of the possible monitoring objectives applicable to the different categories of mustelids is shown in Table 4 and in the paragraphs below.

Table 4: Objectives for monitoring in the different categories of mustelids[(a)]

Animal categories	Monitoring objectives			
	Early detection of SARS-CoV-2	Measuring exposure to SARS-CoV-2	Confirmation of SARS-CoV-2 infection in suspected animals	Monitoring virus evolution
Farmed mustelids	X	X	X	X
Mustelids kept as pets or work animals[(b)]	X		X	X
Free-living mustelids			X[(c)]	X

(a): A cross indicate whether the monitor objective can to be considered for each animal category. An empty cell indicates a non-relevant combination for monitoring purposes.
(b): This refer also to ferrets in breeding centres, see Section 3.4.2.2.
(c): Suspicion for wild mustelids would refer to closeness to infected mink farm.

From the experience gathered in Denmark, in most infected farms (69%), at farm level the RT-PCR positivity rate and seroprevalence in mink were close to 100% by the time the outbreak was detected (ECDC, 2020a). The sampling frequency for both RT-PCR and ELISA on dead mink (in pool of five) in counties without detection of SARS-CoV-2 in mink was at 3 weeks intervals until 9 November 2020, while samples were submitted every week from farms in affected counties. The prevalence at the time of detection was still found to be very high, close to 100%. From 9 November to the beginning of December, all Danish mink farms submitted one pool of five samples every week.

Design prevalence for early detection

For SARS-CoV-2 infection in mustelid (mink) farms, at the present time (January 2021), all mink farms should be considered at risk of infection, as the human disease is widespread; therefore, the monitoring objective should be early detection.

A monitoring plan for early detection aims at containing the disease spread, either within the farm where it is introduced and/or to other farms in the region/country. Monitoring with the aim of early detection surveillance is used to detect first occurrence of a disease in a previously free animal population or the unusual increases of a disease frequency if the disease is present. The former purpose is particularly important for SARS-CoV-2 infection in farmed mink due to the serious animal health and public health consequences and the cost of delayed detection of a disease potentially moving to new domestic and/or wild population. How fast should the disease spread be contained and the related speed of detection needed by the monitoring is different case by case as this is influenced by several factors such as the epidemiological situation of the farm (closeness to other infected mink farm, infection rate in the human population that may influence the risk of introduction, etc.), by factors of risk management remit, such as the prevention and control measures applied (level of biosecurity, culling policy that may be adopted, level of monitoring of the farm personnel, etc.) and the level of risk acceptance, etc. For SARS-CoV-2 in mink farms, the disease should be ideally contained before it largely spread within the farm so to avoid (i) infection of high number of animals and high

chance of virus mutation, (ii) transmission to farm workers, and (iii) that the virus could spread outside the infected farm, putting at risk other farms in the region.

Early detection cannot be based only on passive surveillance (detection and reporting infected animals showing clinical signs by the farmer, see above) as clinical signs appear only in a proportion of infected animals, therefore an active approach is required.

However, the performance of this passive surveillance may vary due to underreporting, delays in detecting clinical signs and/or lack of knowledge and awareness. Therefore, an active sampling (monitoring) should be considered for early detection. Each monitoring for early detection normally requires a regular periodic testing of samples to detect the infection at a moment sufficiently close in time to disease introduction into the farm (or other epidemiological unit that should be defined). In general, the higher the transmission rate of the disease, the more frequent should be the testing to detect the introduced disease early. From the evidence available from Denmark, in a report by Boklund et al. (2020),[43] the cumulative incidence of SARS-CoV-2 in a mink farm was simulated based on observations conducted in one infected farm and according to 1, 5 or 10 infected animals introduced (Table 5 and Figure 7).

In this Danish study, the investigated farm was tested three times with throat swabs from mink: (i) round 1:12.5% positive by PCR; (ii) round 2 (4 days later): 92% positive in PCR, and iii) round 3 (8 days later): 95% positive by PCR and 97% seropositive. This information was used to produce a standard infection curve that was used to estimate time of introduction. First, a simple susceptible/ infected/recovered (SIR) model was developed that would simulate spread of SARS-CoV-2 among mink on a farm. In this model it was assumed that the herd had a size of 4,200, which was equivalent to the investigated farm, and that there was homogenous mixing of animals. The infectious period was modelled with a probable value of 5 days (Kissler et al., 2020), with a variation from 4 to 6 days. The transmission rate, was used as a calibration factor for reaching an increase from 12.5% to 92% in 4 days, as observed in the farm. By starting with 12.5% of the animals infected and varying the transmission rate, a transmission rate of 1.45 was found to be suitable to obtain the observed increase.

From the values reported in Table 5 and in Figure 7, it is evident how fast the infection can spread within the farm and how after two weeks the disease reached an incidence close to 100%.

Table 5: Cumulative incidence (%) of SARS-CoV-2 in a mink farm at different time from virus introduction simulated according to 1, 5 or 10 infected animals introduced

Time after introduction (sampling frequency)	Median cumulative incidence (5th–95th percentiles)		
	1 animal infected introduced	5 animals infected introduced	10 animals infected introduced
7 days	1.1 (0.4–2.4)	5.5 (3.6–8.0)	6.1 (4.5–8.0)
10 days	6.7 (2.3–14.1)	28.2 (18.8–38.8)	46.7 (37.8–56.6)
15 days	48.6 (21.4–74)	90 (81–95)	97 (94.5–98.4)
20 days	99.6 (97.5–99.8)	99.9 (99.8–100.0)	99.9 (99.8–100.0)

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[43] https://dkvet.dk/raadgivning/raadgivningssvar/covid-19-i-mink/Epidemiologisk_udredning_i_SARS-COV-2_smittede_minkfarme_13-11-2020.pdf
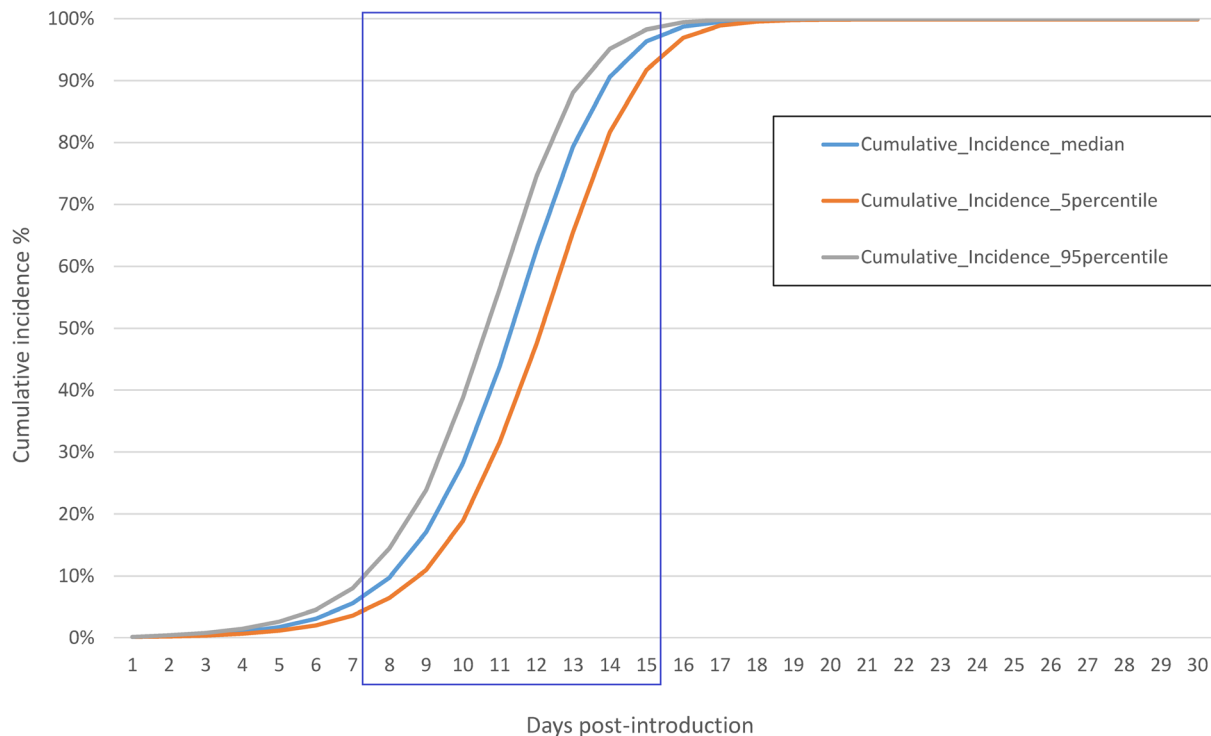
Epidemic started with 5 minks, as from Boklund et al. (2020). The 1-week time window when the incidence increase up to 100% is highlighted in blue.

Figure 7: Simulated curves of cumulative incidence of PCR-positive animals in a farm

Based on Table 5, monitoring for early detection could be based on testing by RT-PCR a sample of animals randomly selected in the epidemiological unit (farm, building, to be defined by the competent authority, etc.) at a sampling frequency ideally not longer than one week (see Table 5, corresponding to introduction of infection in 1–5 animals), which would correspond to a design prevalence of approximately 5% in the epidemiological unit. Selection of dead or sick animals for testing will increase the probability of detection.

If this frequency of testing was not feasible, a risk-based approach could be followed, to be evaluated case by case (see considerations at the beginning of the Section), so to reduce the sample size and/or decreasing the sampling frequency (testing less often), although the sampling frequency, due to the high infection rate of SARS-CoV-2, should be considered as more important than the number of samples. However, it is important to be aware that with longer delays between sampling and/or smaller sample sizes the infection is expected to be detected later after introduction, and therefore a larger proportion of animals is expected to be infected at the time of detection.

In any case, the most important item of the monitoring for early detection for SARS-CoV-2 in mink farm should be the strict and frequent monitoring of farm personnel and all people in contact with the animals and their household members, as humans are expected to be the most likely introduction of virus into the farm.

Confirmation of suspected animals

In absence of suspected infected animals, either dead or those with clinical signs, the suspicion could be raised based on contact tracing or infected people on the farm, then the monitoring scheme described for early detection above would be applicable.

By contrast, in farms where suspected animals are detected, sampling should target all of them, with a minimum of five animals, enabling the detection of 50% prevalence. In fact, based on evidence from monitoring mink in the Netherlands, in suspected animals tested by RT-PCR, the RT-PCR positivity rate (based on a sample of 20 suspected animals per farm) varied between 5% and 100% and in only 16 out of 69 farms (23%) the RT-PCR positivity rate was below 50%. Therefore the target prevalence for confirmation test of suspected animals could be 50%. This was also suggested by USDA (APHIS USDA, 2020b).

Nevertheless, considering the few observational studies available to date and the consequently high uncertainty on this aspect, the expected prevalence values should be assessed case by case on the basis of the results of the epidemiological investigation above all with respect to the possible period of exposure and on the distribution of the infection on the farm. This latter is a fundamental aspect to understand whether to randomly sample animals or to perform a risk-based sampling, i.e. to focus on some areas, categories or parts of the farm.

Measuring exposure level of animals to SARS-CoV-2

Although at the present time (January 2021) the monitoring objective of choice should be early detection in all mink farms in the EU (see above), it can be useful to know if the animal population has ever been exposed to the virus, either in regions without any experience of outbreaks reported or at farm level to prove that the production cycle is virus-free. This can be achieved by cross sectional serological survey, e.g. at the beginning and at the end of the production cycle of one year. In any case the serological test would not be suitable for early detection, as antibodies develop only after two weeks (see Section 3.4.1.1.1), when the disease, if introduced, would have already spread throughout the epidemiological unit (Table 5). For the design prevalence for this type of monitoring it is assumed that, when the virus is introduced into a mink farm, the seroprevalence will reach a high level very quickly. Indeed, it has been observed that a 80–100% incidence is reached in the animals in each sampling unit (to be assessed on a farm-to-farm basis) within two to three weeks (Table 5). Therefore, the design prevalence to be considered for this monitoring could be 80%.

Serological testing can be useful also when the epidemics has waned and the source of virus will be no longer present (in the case of SARS-CoV-2 this is mainly the infection in the human population) to prove freedom from virus, by testing non-immune animals (e.g. newborn animals).

The sample matrix for a serological test is blood serum, mink at farms should ideally be sampled by capillary sampling, while few animals (ferret pets) could also be sample by vein puncture (see Section 3.4.2.2). In the Netherlands, in addition to the first farms, where the seroprevalence was close to 100%, seroprevalences observed in infected farms was generally low, or even zero. In addition to the stage of infection, this difference may be due to the use of a different ELISA and different matrix used, i.e. blood drop from toe clipping on filter paper, by this sample matrix the test sensitivity could be decreased.

Monitor virus evolution

In case of virus detection, genetic analysis should be carried out to characterise the virus, to detect possible virus mutations and to identify the origin and the source of the virus (e.g. spread between different populations). This can be carried out by sequencing of specific targets in the RNA that show a certain degree of variability in the virus population or by whole genome sequencing. The samples should be selected from among the positive cases, as soon as positive animals are detected. If many positive animals are detected and the capacity to sequence all of them is limited, a subset should be selected, at least to represent each positive farm. Sample metadata (e.g. epidemiological link, spatial data, temporal information) should be collected as well to select the samples to be sequenced and allow comparative analysis.

3.4.2. Monitoring scenarios

In this Section different monitoring scenarios are presented by animal categories, i.e. farmed mustelids (e.g. mink), mustelids kept as pets (e.g. ferrets) and free-living mustelids. The related characteristics of the monitoring (objective, target animals, sampling frequency, sample size) are described and assessed in the below Sections.

3.4.2.1. Scenario 1: farmed mustelids

American mink is the only farmed mustelids breed in the EU for fur production, apart for sables bred in just one farm in Finland (see Section 3.1.2).

As long as SARS-CoV-2 is circulating in humans or animals, passive monitoring should always be implemented. Farmers should immediately report any suspicion of SARS-CoV-2 presence in the farm to the local human and veterinary health authorities. SARS-CoV-2 should be suspected, if signs of possible infection are observed in the animals, such as increased mortality, respiratory or gastrointestinal signs or reduction in feed intake. Furthermore, if human cases are confirmed among workers/owners of the farms or their household members, investigations to confirm or rule out suspicion of presence of SARS-CoV-2 in the animals should be carried out.

As clinical signs have not been observed in a substantial proportion of infected mink farms, passive monitoring should be complemented by active monitoring, aiming at either i) early detection of the infection if introduced in the farm, ii) confirming a suspicion of infection, iii) monitoring virus evolution and if needed 4) measuring level of exposure to the virus. These different monitoring scenarios are presented based on the possible epidemiological situations. The related characteristics (objective, target animals, sampling frequency, sample size) are described and assessed. The objectives of a monitoring plan in the farmed mink population may depend on the population structure, distance and possible contact ways to other farms, e.g. same farm personnel working in different farms.

3.4.2.1.1. Objective 1: early detection of SARS-CoV-2

Early detection aims at detecting the virus infection as soon as possible after it enters the farm (or other epidemiological unit, to be defined by the competent authority). This is to safeguard animal health, public health and safeguard farmers' working conditions. It is crucial to design the sample size according to the expected prevalence. As reported in Section 3.4.1.3, in the first week after the virus entered a mink farm, the prevalence of the infection among the animals in the farm may vary between 5 and 10%. The diagnostic test used should target the detection of the virus and not the antibodies. Below an example of monitoring scheme for early detection is presented:

- **Diagnostic test:** RT-PCR (diagnostic sensitivity (Se) 100%).
- **Sample matrix:** oropharingeal swabs from alive or dead animals (see Section 3.4.1.1).
- **Target population:** dead and sick animals from each epidemiological unit should be preferred as they might be infected; however, since the baseline mortality can be sometimes very low (e.g. in Denmark 1–4 animals/month per farm with an average size of 10,000 mink per farm(ECDC, 2020a)), there might not be enough dead mink to be weekly tested, therefore samples should also be taken from live animals to reach the expected sample size.
- **Sampling frequency:** weekly.
- **Duration of active monitoring plan:** as long as SARS-CoV-2 circulates in humans/animals in the country.
- **Design prevalence:** 5% (see Section 3.4.1.3).
- **Sample size:** considering a farm of, e.g. 10,000 animals and the above parameters, with a design prevalence of 5%, 59 animals should be sampled weekly from each epidemiological unit, to achieve a confidence of 95%. If a confidence of 99% is desired, then 90 animals should be tested weekly. Pooling samples could also be carried out in order to reduce the number of laboratory diagnostics assays needed, the pool size should be adequately considered.

If this frequency of sampling or amount of samples to be tested would not be feasible, an alternative approach could be followed (see considerations expressed at Section 3.4.1.1 and 3.4.1.3). An example could be testing all dead or sick animals as soon as they are detected in the farm. If no dead or sick animals are available to be tested in at least 2-week period, the laboratory examination of at least 14 randomly selected animals (corresponding to a design prevalence of 20%, 95% of confidence level) should be made. This alternative monitoring approach would nevertheless lead to a later warning, i.e. the infection is expected to be detected later after introduction, and therefore a larger proportion of animals is expected to be infected at the time of detection.

3.4.2.1.2. Objective 2: confirmation of SARS-CoV-2 infection in suspected animals or to confirm active virus circulation in the farm

In case of suspicion of SARS-CoV-2 infection in a farm, due to the presence of signs of SARS-CoV-2 infection (e.g. increased mortality, respiratory symptoms, decrease in feed intake) or the detection of cases of human infections among workers/owners/household members, active finding of virus should be performed in the farms. Also, where there is a positive result from serological screenings, further test to detect the virus should be performed to exclude active circulation of the virus. Below an example of monitoring scheme is presented:

- **Diagnostic test:** RT-PCR (diagnostic Se 100%).
- **Sample matrix:** oropharyngeal swabs.
- **Target population:** all suspected animals, i.e. increased mortality compared with the baseline mortality rate of that specific production period and/or clinical signs in animals or personnel.

- **Sampling frequency:** every time a suspected animal is detected, or a positive serological result is obtained in the farm.
- **Duration of active monitoring plan:** as long as SARS-CoV-2 circulate in humans/animals.
- **Design prevalence:** 50%, see Section 3.4.1.3, with a minimum of five animals to be tested.
- **Sample size:** considering a farm of 10,000 animals and the above parameters and a 50% design prevalence, testing five suspected animals would provide a confidence of 95% (seven animals for 99% confidence).

3.4.2.1.3. Objective 3: monitoring virus evolution

In case of virus detection, genetic analysis should be carried out to characterise the virus, to detect possible virus mutations and to identify the origin and the source of the virus (e.g. spread between different populations). Genome sequences generated should be shared with the scientific community and uploaded in open source genetic databases. Viruses sequenced from animals should be compared with those from humans through phylogenetic analysis. Below an example of monitoring scheme is presented:

- **Diagnostic test:** sequencing, WGS.
- **Sample matrix:** RNA from positive cases.
- **Target population:** confirmed positive animals.
- **Sampling frequency:** as soon as positive animals are detected.
- **Duration of active monitoring plan:** as long as positive animals are detected.
- **Design prevalence and sample size:** all animals confirmed as positive. In the case of many positive animals detected, there may be limited capacity to sequence all isolates, therefore a subset should be selected, at least to represent each farm. If PCR assays addressing multiple variable targets are used, the divergent results in case of mutations may be used for selecting those samples to be included in the pool to be sequenced (see Section 3.4.1.1).

3.4.2.1.4. Objective 4: measuring exposure to SARS-CoV-2

Knowing the exposure to SARS-CoV-2 in mink farms may be needed to check if animals have come into in contact with the virus. Below an example of monitoring scheme for proving freedom from SARS-CoV-2 infection in a mink farm is presented:

- **Diagnostic test:** serological test (ELISA). Se: 98.3%.
- **Sample matrix:** blood sample at pelting time, in dead animals or collected ad hoc in vivo.
- **Target population:** all animals in the farm.
- **Duration of active monitoring plan:** 1 year (one productive cycle).
- **Design prevalence:** value chosen for this scenario: 80% (see Section 3.4.1.3).
- **Sampling frequency:** this testing can be carried out e.g. at the beginning and at the end of the production cycle of 1 year.
- **Sample size:** considering a farm of 10,000 animals and the above parameters, testing 3 animals per sampling unit would provide a confidence of 95% (4 animals for 99% confidence)**.**

3.4.2.2. Scenario 2: Mustelid kept as pets or work animals

Mustelids, usually ferrets, may be kept as pets or as work animals for rabbit hunting and rabbit control. Generally, each owner or household has contact with only one or very few ferrets. Breeders represent an exception and may own between 10 and 100 ferrets per facility and contact even larger numbers through their networks. Therefore, surveillance schemes for kept mustelids should first target registered ferret breeding facilities. Below an example of monitoring scheme is presented:

- **Objective:** Confirmation of SARS-CoV-2 infection in suspected animals.
- **Diagnostic test:** virological test (RT-PCR).
- **Sample matrix:** oropharyngeal or rectal swabs.
- **Target population:** ferrets aged older than 3 months in breeding facilities.
- **Sampling frequency:** every time a suspected animal is detected or a SARS-CoV-2 cases in persons in contact with the animals.
- **Duration of active monitoring plan:** as long as SARS-CoV-2 circulates in humans/animals.
- **Design prevalence:** the limited available information indicates an expected prevalence of 10%.
- **Sample size:** in facilities with less than 30 ferrets, all animals over 3 months should be samples. In facilities with up to 100 ferrets or more, 30 animals should be sampled and tested, to achieve 95% confidence.

For the objective of confirmation of SARS-CoV-2 infection in suspected animals, see Section 3.4.2.1.3.

For the objective of testing exposure to virus, serological test may be carried out in all animals. Blood serum sampling is needed for this purpose. Blood sampling in ferrets can be achieved from a variety of veins including the cephalic, jugular, saphenous, femoral and if under general anaesthetic, the cranial vena cava.[44]

Pet and hunting ferrets are attended by clinical veterinarians. Veterinarians can be involved in disease surveillance and take swabs and blood samples in case of suspicion, for instance, in the presence of respiratory signs or diarrhoea, mortality or suspected or confirmed COVID-19 in the owner or household. If someone in the household is infected, all owned ferrets should be tested.

3.4.2.3. Scenario 3: free-ranging mustelids

- **Objectives**: Wild mustelids, mainly the species belonging to genera *Mustela* or *Neovison* can be sampled for SARS-CoV-2 monitoring, either to monitor possible spill over from infected mink farms (testing wild animals trapped around infected mink farms) or for generating information on SARS-CoV-2 circulation in wildlife hosts. In the latter case samples can originate from individuals found dead (mainly road kills) or hunted (in countries where selected mustelids are game species).
- **Diagnostic test:** RT-PCR on fresh carcasses or live animals, in the case of recent infections, and ELISA to test exposure to the virus that may have occurred earlier.
- **Sample matrix:** oropharyngeal and rectal swabs, or samples from lung or upper airway tissues in dead animals, or serum obtained from blood samples taken from the heart, the thoracic cavity, from muscle juice or from the cavernous sinus in larger species. Serum (or serum-like liquids such as muscle juice) obtained from dead animals will generally be suitable for ELISA but not for serum neutralisation. Alive wild mustelids can be sampled as farmed ones, usually under anaesthesia, taking oral and rectal swabs for PCR and drawing blood for serum from the vena cava. Individuals over 800 g can be blood-sampled, taking 0.5–1 mL of blood.
- **Target population:** individuals found dead (e.g. road kills) belonging to genera *Mustela* or *Neovison*, hunted individuals (in countries where selected mustelids are game species), or trapped individuals for research purpose or around known-infected mink farms. In the latter case, if other animal species that are known or suspected to be susceptible to SARS-CoV-2 are trapped not only around infected mink farms, e.g. wild mustelids mainly from *Mustela* spp., *Neovison* spp., *Martes* spp. or badger, but also wild canids such as raccoon dogs or foxes, or wild rabbits or hares, it would be advisable to test them as well.
- **Duration of monitoring plan:** as long as SARS-CoV-2 circulates in humans/animals.
- **Sample size and frequency:** sampling is opportunistic (as it depends on road kills) and should be carried out depending on the source (all individuals trapped or collected close to risk sites such as mink farms, but only a proportion of common road killed species) and type of samples (all fresh carcasses from road-killed or hunted mustelids of the genera *Mustela* and *Neovison*, a proportion of those belonging to other genera).

3.4.3. Monitoring of SARS-CoV-2 in farmed mink in place in EU MSs

The monitoring approach for SARS-CoV-2 in animals taken by MSs is described below, based on information collected by national authorities and through a survey conducted by the WHO EURO in December 2020 (WHO EURO, 2020). In this survey, answers were collected from 30 countries (Armenia, Austria, Azerbaijan, Belgium, Cyprus, Czechia, Finland, Georgia, Germany, Greece, Holy See, Hungary, Iceland, Israel, Italy, Kosovo,[45] Kyrgyzstan, Latvia, Liechtenstein, Lithuania, Netherlands, Norway, Poland, Portugal, Republic of Moldova, San Marino, Slovakia, Slovenia, Spain and Sweden). All countries with mink farms present up to December 2020 (Belgium, Finland, Greece, Iceland, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Slovakia, Spain and Sweden) stated that a SARS-CoV-2 monitoring system is implemented to detect this virus in mink on fur farms. In this Section, the monitoring system is described for the MSs listed above, which have mink farming, plus Denmark and France.

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[44] https://www.vettimes.co.uk/app/uploads/wp-post-to-pdf-enhanced-cache/1/overview-of-ferrets-part-one-conditions-and-behaviour.pdf

[45] All references to Kosovo in this document should be understood to be in the context of United Nations Security Council resolution 1244 (1999).

Belgium

A serological screening was conducted in September 2020 on 60 mink from each farm (8 farms in total). Furthermore, from September until December 2020, compulsory notification of increased mortality, SARS-CoV-2-related symptoms in more than 5% of the mink, and weekly testing of dead animals (five animals per farm) have been implemented. In case of suspicion, the official veterinarian should visit the farm within 2 days and perform investigation with anamnesis, clinical research and sampling. In this period of three and a half months, a total of 423 cadavers were analysed, all were negative in PCR for SARS-CoV-2. Since December 2020, all mink farms have stopped their activities and with the official operating stop in 2023, there is a great probability that Belgium has definitively stopped this activity.

Denmark

After detection of the first three outbreaks in Denmark, a survey was conducted in June including 125 randomly selected mink farms. From each of these farms, 30 mink were sampled (blood samples and throat swabs) for analyses; none was found to be positive. Since late June, four main mechanisms for surveillance have been used, including testing of: i) suspicions based on clinical signs in mink, ii) dead mink submitted for laboratory testing every week or third week depending on the geographical area and time period during the outbreak, iii) mink following SARS-CoV-2-positive test in humans associated with the farm, and iv) contact tracing to infected farms. Overall, most infected farms had all animals testing positive in RT-PCR and antibody ELISA on the first sampling, suggesting rapid transmission within the farm or that detection occurred relatively late in the course of infection (Boklund et al., 2021). The monitoring in case of clinical suspicion consisted of swab sampling of 10 live mink with clinical signs of respiratory disease, but with some variation (1–30 samples), as well as samples from 5 to 10 dead mink. If no respiratory disease was noticed, mink from all geographical areas of the farm were selected for sampling. In the situation where mink from a farm were undergoing culling or already culled 10 pharyngeal swab samples were taken from culled animals. If possible, the samples were taken from culled animals that had symptoms.

Finland

Preliminary surveillance was carried out on 30 mink farms (out of 200 mink farms/a total of 700 fur animal farms) in November 2020, targeting areas with highest number of mink fur production. Each farm was asked to send five dead mink for analysis, and all tested negative for SARS-CoV-2.

In December 2020, surveillance was launched on all fur farms that raise minks or other animals of the family Mustelidae. Each farm was asked to send three dead mink every 3 weeks for SARS-CoV-2 testing. If the number of dead animals is insufficient, live animals will be sampled. Before moving or selling breeding animals, 30 animals from the holding must be tested for SARS-CoV-2. In addition, samples will be collected from holding if clinical symptoms in animals are present or an increase in mortality is observed or if a person working on a farm has been diagnosed with a COVID-19 infection. Samples are throat swabs and they are analysed by RT-PCR. Surveillance is scheduled to continue until at least the summer of 2021, and monitoring is planned to be extended also to raccoon dog farms.

France

France has planned to set up active surveillance based on serological surveys to be carried out on a sample of the mink population, coupled with clinical suspicion-based surveillance. This strategy, applied to all mink farms (four farms in France), is to characterise the health status of these farms in the current context of the pandemic. The number of mink per farm in France can vary from approximately 10,000 to 30,000 individuals. A design prevalence of 5% (with a risk of error of 5%) was recommended and was adjusted per farm, and each farm is expected to be considered as an epidemiological unit. This would lead to a sample size of 59 animals, for an epidemiological unit with 10,000 animals. The authorities can refine this sampling plan, by defining the epidemiological units (per building in farm or per farm) and their size (1,500–10,000), depending on the configuration and organisation of work within each farm.

If the serology is negative in all buildings of the farm, this can exclude an infection with exception of very recent one, which can be detected by suspicion-based surveillance (see below). If instead a positive sample is detected at serological test, this result suggests a past or recent infection in the farm, and the RT-PCR undertaken at the same time is established to confirm an infection in progress. The design prevalence of these RT-PCR analysis would, in this case, be much more variable over time

compared with that defined for serology, because the RNA of the virus is detected during the first days of infection (up to 10–14 days post-inoculation in ferrets) (Kim et al., 2020). A serological test on domestic carnivores located in the environment of farms that were detected positive by PCR for SARS-CoV-2 should also be conducted to detect the first animal contaminated (mink or other domestic animals).

Clinical surveillance induces a suspicion-based surveillance consisting of analysing sick or dead animals, to confirm an infection. Increased mortality constitutes an alert, when it significantly exceeds the current mortality rate in the farm. A mortality rate greater than 1% is considered as an alert, a value to be adjusted, if the current mortality rates in the farm were high. Necroscopy is performed on dead animals to check for lesions and to collect lung and tracheal tissue for RT-PCR analysis. In cases in which there is no mortality, but existence of clinical signs (lethargy, runny nose or eyes, cough, sneezing, breathing difficulties or shortness of breath, vomiting, diarrhoea) in more than 5% of the animals, RT-PCR tests have to be implemented on samples of oral (or pharyngeal) swabs and serological tests to be carried out in parallel on several sick animals (ANSES, 2020).

Greece

Following the announcement of the first outbreak in the Netherlands in April, clinical surveillance, including control of morbidity and mortality rate, was put in place in mink farms.

On November 2020, it was decided to adopt a different strategy and to implement an integrated surveillance system at the human–animal interface (One Health). Screening for SARS-CoV-2 of all workers in mink farms is conducted weekly and results are directly notified to veterinary authorities. In animals, passive surveillance and a risk-based approach are combined. Veterinary authorities always carry out clinical investigation and laboratory testing in mink farms upon notification of increased morbidity/mortality in animals or of human cases directly related to an establishment. In the context of ongoing surveillance, 32 farms were sampled and tested until 20 January. At least 20 mink are tested from farms without symptoms or increased mortality/morbidity, while 10 mink are tested from farms with symptoms or increased mortality/morbidity, compared with baseline mortality. Oropharyngeal swabs are collected from the animals and tested using real-time PCR for the presence of SARS-CoV-2. Sequencing of virus genomes from human and mink samples is conducted.

Italy

Controls are carried out in case of notification of clinical signs related to SARS-CoV-2 or increased mortality or if positive cases are detected in farm workers.

Latvia

Nine clinical inspections were conducted between May and June 2020, and 14 visits for checking the level of animal welfare[46] were conducted between July and December, as well as wastewater samples (all negative). Testing of dead mink is planned.

Lithuania

Passive surveillance is combined with active surveillance; dead mink are collected at farms and tested for SARS-CoV-2. From the beginning of December, one sampling campaign has been applied.

Netherlands

Surveillance in the Netherlands is based on testing dead mink on weekly basis for the presence of SARS-CoV-2 by PCR (the EWS) and passive surveillance by notifying a clinical suspicion and increase in mortality. Moreover, in June 2020 a serosurvey has been performed on all mink farms, followed by a serosurvey of the farms in the region where most outbreaks occurred (Limburg and Noord Brabant) in September 2020. Initially, five dead mink per farm were tested by PCR for the EWS. Later, from September onwards all dead mink with a maximum of 50 per farm were included in the sample. It is difficult to estimate the performance of the surveillance using dead animals since assumptions need to be made for the transmission on a farm. Nevertheless, the surveillance system in the Netherlands combined with the EWS and the notification obligation, seemed an appropriate programme to detect active infections on farms, as both systems focus on clinically affected animals. In total 68 farms were detected using this combination, 39 were detected on the EWS and 29 on the notification obligation. Only one farm was detected using the serosurvey performed in September 2020.

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[46] https://www.sustainablefur.com/animal-welfare/

Poland

In accordance with the Ordinance of the Minister of Agriculture and Rural Development, SARS-CoV-2 in mink is a notifiable disease subject to official monitoring in accordance with the following scheme.

1) Pharyngeal or nasopharyngeal swabs taken from at least 10 diseased mink, if there are clinical signs in the mink herd, especially from the respiratory system, and if there are also mink deaths in this mink herd, lungs taken from a) 10 dead mink, when the number of dead mink is at least 10, or b) all dead mink, if the number of dead mink is less than 10.
2) Pharyngeal or nasopharyngeal swabs taken from 20 live mink, if there are no clinical signs in the mink herd, at least in 15% of the mink farms in the district, every 8 weeks, so that in a given calendar year the tests are carried out in 100% of the mink farms in the district.

Following confirmation of infection with SARS-CoV-2 virus in mink: the movement of animals, by-products, products and equipment to and from the holding shall be suspended; an epidemiological inquiry shall be carried out; the killing and disposal under official supervision of all animals and raw skins obtained from the farm are ordered; in addition, appropriate procedures are taken for cleaning and disinfecting the holding; a 10-km surveillance zone is established around the outbreak for 30 days following the initial cleansing and disinfection; re-population of the holding is possible 30 days after final cleansing and disinfection.

Due to the potential zoonotic nature of the disease, activities related to the control of SARS-CoV-2 in mink are also coordinated with the State Sanitary Inspectorate (PIS) according to the 'One Health' principle.

Spain

Passive surveillance in all mink farms was performed during 2020, suspicion is raised in case of detection of clinical symptoms, epidemiological link with other positive mink farm to SARS-CoV-2, detection of an active infection in personal in close contact with the animals. Oropharyngeal swabs are taken from suspected animals for PCR analysis. Additionally, active surveillance in mink farms was carried out during the pelting period from November to December 2020, taking 15 to 20 oropharyngeal swabs from each mink farm for PCR analysis. Finally, active surveillance has been included in the monitoring programme of SARS-CoV-2 in mink farms from 2021, including the analysis by PCR every two weeks of oropharyngeal swabs taken from dead mink detected in the farms. Surveillance on kept ferrets occurs in the context of an ongoing research grant.

Sweden

Surveillance in Sweden is based on testing mink found dead, aiming at five mink per farm and weekly and passive surveillance with increased mortality or morbidity to be notified by mink farm owners. A serosurvey has also been carried out in breeding animals in all mink farms during December 2020 to January 2021. Targeted surveillance on mink workers is also carried out.

3.4.4. Monitoring of SARS-CoV-2 in farmed mink in place in Canada and United States

Canada

The Canadian surveillance guidelines for SARS-CoV-2 in farmed mink aim to provide guidance to the provincial and territorial animal and public health authorities on the surveillance approaches to be applied for SARS-CoV-2 in Canada's farmed mink population. The guidelines were developed by the Canadian Food Inspection Agency (CFIA) in collaboration with the Federal/Provincial/Territorial COVID-19 One Health Working Group. The guidelines address surveillance design and sampling plans for: (i) early detection of the first case (in an unaffected area), (ii) outbreak surveillance, and iii) post-outbreak surveillance. As provincial/territorial authorities are the lead on the SARS-CoV-2 response in farmed mink in Canada, they use the surveillance guidelines to develop situation specific surveillance plans, with any required additional guidance from federal partners. In addition, the Public Health Agency of Canada is collaborating with the CFIA to produce ongoing risk maps overlaying mink farm location with human case incidence. These are being shared with each province to help to inform their risk-based approach to surveillance.

United States

The Centers for Disease Control and Prevention (CDC), the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS), and other partners jointly developed several guidance documents to apply a One Health approach to address SARS-CoV-2 in people, mink, and other animals on affected mink farms. First, the 'Interim SARS-CoV-2 Guidance and Recommendations for Farmed Mink and Other Mustelids' (USDA and CDC, 2020) is intended to provide the most up-to-date information related to SARS-CoV-2 and farmed mink and other mustelids and guidance related to SARS-CoV-2 prevention and control strategies for public and animal health officials and partners in the mink industry. Second, 'Response and Containment Guidelines: Interim Guidance for Animal Health and Public Health Officials Managing Farmed Mink and other Farmed Mustelids with SARS-CoV-2' (APHIS USDA, 2020b) is available which outlines multiple strategies to contain a SARS-CoV-2 outbreak involving farmed mink or other mustelids and addresses topics including animal health, biosecurity, surveillance and monitoring, quarantine measures, worker safety, and more. As state animal health authorities are the lead to address farmed mink, state animal health officials use these guidance and recommendations in their jurisdictions. Lastly, guidelines on 'Surveillance and Release Testing Options for State Animal Health Officials (SAHO) Managing SARS-CoV-2 Positive Mink Premises' are also available from USDA-APHIS for SAHOs to manage SARS-CoV-2 positive mink premises, create farm plans, and implement testing requirements for quarantine release. This document also outlines active surveillance protocols for non-infected farms. USDA-APHIS maintains a list of SARS-CoV-2 cases in mink and other animals in the United States (APHIS USDA, 2020a) that have been confirmed by USDA's National Veterinary Services Laboratories. CDC has worked with the mink industry to conduct webinars for mink farm workers on preventing SARS-CoV-2 introduction onto mink farms including appropriate usage of personal protective equipment, worker safety, and the importance of timely reporting of human illnesses and occupation to state officials.

4.   Conclusions and recommendations

MUSTELIDAE IN EUROPE

- In the EU, mustelids farmed for fur production are mainly American mink. From 2900 fur animal farms present in the EU in 2019, producing around 27 million mink pelts per year, as from December 2020, following the SARS-CoV-2 epidemics in mink farms in the EU, the number of mink farms in Europe has decreased to 759 mink farms, mostly located in Finland, Poland, Lithuania and Greece. Among the biggest mink pelts producers, Denmark and Netherlands have closed nearly all mink farms. Due to SARS-CoV-2 outbreaks, several countries will ban fur farming in the next years.

- Mink are farmed in contiguous wire netting cages that may facilitate efficient animal-to-animal transmission of infectious diseases and/or to other wild or domestic in-contact animals.

- Mink are generally farmed in open housing systems, which may allow close contact between caged mink and other animals approaching these facilities, and which, if they are susceptible, may acquire SARS-CoV-2 if mink are infected.

- Wild mustelids are widely distributed over several European countries, with low population densities. In principle, they might approach mink farms and eventually acquire SARS-CoV-2 from infected farmed animals or animal products in the farm. Nevertheless, due to their elusive and solitary behaviour, to their low density and to the low numbers hunted, there is a very low chance of contacts with humans and/or other SARS-CoV2 susceptible domestic animals (such as dogs, cats, ferrets). Therefore, the risk of wild mustelids becoming a reservoir for SARS-CoV-2 and/or the risk of SARS-CoV-2 infection becoming endemic in wild mustelids in Europe is very low.

- In the EU, mustelids are also kept as pets, mainly ferrets, but their total number is unknown.

SARS-CoV-2 DETECTION IN THE EU

- As of 29 January 2021, SARS-CoV-2 virus has been reported in 400 mink farms for fur production in eight countries in the EU/EEA, namely 290 farms in Denmark, 1 in France, 21 in Greece, 1 in Italy, 2 in Lithuania, 69 in the Netherlands, 3 in Spain and 13 in Sweden. Sporadic SARS-CoV-2 virus cases have been recorded in kept ferrets in Slovenia and in Spain.

- Different monitoring strategies have led to the detection of the SARS-CoV-2-infected mink farms. A proportion of the infected farms has been detected by: (i) passive monitoring, i.e. the

farmer noticing and reporting clinical signs or anomalies in production parameters (feed intake, mortality, etc.); (ii) another part was detected after a suspicion was raised due to an epidemiological link with SARS-CoV-2 infection in humans (workers/owners); (iii) active monitoring (actively sampling and testing of animals) was needed to detect the rest of the infections. According to the epidemiological situation, a different proportion of infected farms was detected by each of these monitoring strategies, e.g. the epidemiological link to human cases led to the detection of one out 69 outbreaks in the Netherlands, and to 57 out 226 outbreaks in Denmark.

- Clinical signs of SARS-CoV-2 in mink/mustelids are often non-specific and present only in a variable proportion of outbreaks. They can include increased mortality, mild respiratory signs and slightly drop in feed intake.
- In most cases, the likely introduction of SARS-CoV-2 infection into farms was suspected to be by infected humans.
- **Recommendation**: Additional sequence analysis can help to identify mink farm clusters and mink variants, suggesting or ruling out within-farm human-to-mink and/or mink-to-human transmission.

GENETICS, SUSCEPTIBILITY AND PATHOGENESIS

- American mink and ferret are highly susceptible to SARS-CoV-2, and no information is available for other mustelid species. **Recommendation**: More information is required to have a deeper understanding of the host-pathogen interaction for all mustelid species.
- Pathogenesis in mink and ferret resembles that observed in humans. Virus replication occurs mainly in the respiratory tract with only minor involvement of the digestive tract. The duration of virus excretion seems to be limited to a few days.
- Animals belonging to the subfamily of Mustelinae and especially ferrets (due to their suitability as laboratory animal) can play a useful role as preclinical animal models to test diagnostic, therapeutic and prophylactic approaches for SARS-CoV-2 infection, to be further developed for public health purpose.

INFECTION DYNAMICS

- Once introduced in a mink farm, SARS-CoV-2 spreads efficiently within the farm from animal to animal through transmission by direct contact and indirect contact such as air droplets, dust particles, aerosols and fomites.
- Viral RNA can be found close to infected animals but not outside infected farms.
- Large mink farms with high animal density provide ideal conditions for SARS-CoV-2 replication and transmission, therefore increasing the risk of virus evolution.
- In areas with high density of mink farms, between-farm spread is likely to occur once SARS-CoV-2 is introduced.
- Mechanisms for between-farm spread are largely unknown to date, although transmission through humans probably contributed to the observed farm-to-farm spread. The only other risk factors identified for between-farm transmission identified is short distance to the nearest SARS-CoV-2 positive farm and in Denmark also large farm size.
- **Recommendation**: Several other transmission routes of SARS-CoV-2 have been investigated including different animal species, domestic or wild. These may be involved in transmission of SARS-CoV-2 between farms, although this hypothesis should be confirmed by more ad hoc investigation.

RISK FOR HUMAN HEALTH posed by SARS-CoV-2 infection in mink and other animals of the family Mustelidae with a view to designing a suitable coordinated monitoring in the Member States

- Mink-related variant viruses can establish circulation in the community, but have so far not been shown to be more transmissible or cause a more severe effect compared with other circulating SARS-CoV-2.
- Currently described mink-related variant viruses have not been shown to affect SARS-CoV-2 diagnostic assays.
- The circulation of mink-related variants in the human population has decreased, Cluster 5 variant viruses have not been identified since September and circulation of the Y453F virus variant has not been reported since the end of December 2020 in Denmark.

- Close monitoring of SARS-CoV-2 infections in mink and mink farm workers is needed to identify SARS-CoV-2 cases early and prevent further transmission to humans and other animals.
- Sequencing of viruses from mink and human cases and sharing this information is needed to identify new variant viruses early that may have an impact on diagnostics, transmission, severity or vaccine effectiveness.
- The probability of infection with mink-related variant strains is assessed as low for the general population, moderate for the populations in areas with a high concentration of mink farms and very high for individuals with occupational exposure.

RISK FOR ANIMAL HEALTH posed by SARS-CoV-2 infection in mink and other animals of the family Mustelidae with a view to designing a suitable coordinated monitoring in the Member States

- American mink and ferret are the only mustelid species for which evidence both from experimental and field studies for susceptibility to SARS-CoV-2 is available. There are no other experimental studies available in other wild mustelid species.
- Few species of animals present in infected mink farms have been monitored following natural exposure to SARS-CoV-2 from infected mink. Among these, cats and dogs were the only species found infected under field conditions. For other animal species susceptible in experimental infections with SARS-CoV-2 but without evidence of natural infection during outbreaks in mink farms, more investigation is needed to clarify the epidemiological role.
- Raccoon dogs deserve particular attention as they are susceptible and capable of shedding SARS-CoV-2, and they are bred for fur production as with mink, and are present in the wild with high abundance in Europe.
- To date, the animal species that may be included in monitoring plans are the ones shown to be susceptible and capable of further transmission of the disease and that can be present in or close to infected mink farms, i.e. American mink (farmed or wild), ferrets, cats, raccoon dogs, white-tailed deer (very locally distributed, in the EU they are present only in Finland and few individuals in Czechia, in the latter country there are no mink farms) and bats belonging to the family of Rhinolophidae.
- With new research and new evidence or new mutations of the virus, other species, in particular widespread species such as rodents (about which there is still uncertainty about their epidemiological role), may be involved in the SARS-CoV-2 epidemiology and the monitoring approach could be reconsidered.

MONITORING of SARS-CoV-2

- Monitoring of SARS-CoV-2 in animals can be based either on passive approach (e.g. observation by farmers or veterinarians of indicators, such as reduced feed intake, clinical signs potentially related to SARS-CoV-2 infection and increased mortality rate), or on active approach, i.e. detecting cases of SARS-CoV-2 infection through planned and designed disease-monitoring plans.
- Passive monitoring should be in place in the whole territory of all countries where animals susceptible to SARS-CoV-2 are bred or kept, or where people infected or suspected to be infected with SARS-CoV-2 are in contact with animals susceptible to SARS-CoV-2 (e.g. workers in mink farms, owners of mustelid pets or hunting animals).
- The plan for active monitoring strategies for SARS-CoV-2 in mustelids should cover the following aspects: the objective of the active monitoring, the target population, the diagnostic test, the sampling frequency and the sample size.
- The available diagnostic tests for detection of SARS-CoV-2 in mustelids include both virological tests (e.g. RT-PCR) and indirect tests (e.g. ELISA). Both have high sensitivity and specificity, although there is lack of reference material to enable a harmonised validation programme and quality control programme on diagnostic assays for SARS-CoV-2- in animals.

  - **Recommendation**: after initial validation, quality control programmes and proficiency testing should be organised by expert laboratories.

- Monitoring for SARS-CoV-2 in mustelids can be applied to:

  o farmed mustelids (mink) with the objectives of early detection of infection, measuring exposure to virus (freedom from disease), confirmation of suspected infected animals and monitoring virus evolution

  o animals kept as pets or work animals (ferrets), with the objectives of early detection of infection, confirmation of suspected infected animals and monitoring virus evolution

  o wild mustelids, with the objectives of confirmation of suspected infected animals and monitoring virus evolution

- The objectives of monitoring SARS-CoV-2 in mustelids include early detection, measuring exposure to SARS-CoV-2, confirmation of SARS-CoV-2 infection in suspected animals and monitoring virus evolution.

- For SARS-CoV-2 infection in mustelid (mink) farms, at the present time (January 2021), all mink farms that are not yet infected should be considered at risk of infection, as the human disease is widespread, therefore the monitoring objective of choice should be **early detection**.

  o Early detection aims at containing the disease spread if and once it is introduced, either within the farm and/or to other farms in the region/country. This requires a regular periodic testing of samples to detect the infection at a moment sufficiently close in time to the disease introduction into the farm.

  o Early detection cannot be based only on passive surveillance (detection and reporting by the farmer of infected animals showing clinical signs) as clinical signs appear only in a proportion of infected animals, therefore an active approach is required.

  o **Recommendation**: The most important item of the monitoring for early detection for SARS-CoV-2 in mink farms should be the strict and frequent testing (e.g. weekly) of farm personnel and all people in contact with the animals and their household members, as humans are expected to be the most frequent introduction of virus into the farm. This is key to prevent virus introduction into the farm. Suspicion should be raised and further investigations be conducted at farms with a previous or current history of personnel with SARS-CoV-2.

  o **Recommendation**: Given the fast spread of SARS-CoV-2 in mink farms once introduced and based on the evidence gathered from outbreak investigation in Denmark, monitoring for early detection could be based on testing by RT-PCR a sample of animals randomly selected at a sampling frequency ideally not longer than one week, and which correspond to a design prevalence of approximately 5% in each epidemiological unit. Selection of dead or sick animals for testing would increase the probability of detection. Pooling samples could be considered.

  o **Recommendation**: The monitoring for early detection and the expected prevalence values should be assessed case by case based on the epidemiological situation, prevention measures, the level of risk acceptance, etc.

- **Recommendation:** In farms where suspected animals are detected (dead or with clinical signs related to SARS-CoV-2 infection), monitoring for confirmation of SARS-CoV-2 infection should target all of these every time a suspicion is raised, with a minimum of five animals to be tested (50% design prevalence in suspected animals).

- **Recommendation**: **Monitoring virus evolution** by genetic sequencing analysis should be carried out to identify the origin and the source of the virus (e.g. spread between different populations) and to detect possible virus mutations, including those of public health concerns. The samples should be selected from among positive cases, to represent each positive epidemiological unit. Testing should be carried out in accordance with validated protocols and all genome sequences shared with the scientific community, uploaded into open source genetic databases, and viruses sequenced from animals should be compared with those from humans through phylogenetic analysis.

- If it is needed to **monitor the exposure level** of animals to SARS-CoV-2 (i.e. whether the animal population has ever been exposed to the virus), a cross sectional serological survey can be performed. As it is assumed that, if the virus is introduced into a mink farm (epidemiological unit), the seroprevalence will reach a high level very fast, the design prevalence to be considered for this monitoring could be 80%.

- Wild mustelids can be sampled and tested for SARS-CoV-2 infection, to monitor possible spill over from infected mink farms. In this case wild animals trapped around infected mink farms

should be tested, mainly *Mustela* spp., *Neovison* spp., *Martes* spp. or badger, but also wild canids such as raccoon dogs or foxes, or wild rabbits or hares.

- Wild mustelids can be sampled and tested for SARS-CoV-2 infection and also for generating information on SARS-CoV-2 circulation in wildlife hosts. In this case samples can originate from found dead individuals (mainly road kills) or hunted individuals (in countries where selected mustelids are hunted). The sampling strategy is convenient sampling, as it depends on availability of animals to be tested.

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Abbreviations

AA	amino acid
ACE2	angiotensin-converting enzyme 2
APHIS	Animal and Plant Health Inspection Service
ARDS	acute respiratory distress syndrome
β-CoV	betacoronavirus
CFIA	Canadian Food Inspection Agency
ECDC	European Centre for Disease Prevention and Control
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EWRS	Early Warning and Response System
EWS	early warning system
FP	fusion peptide
MAFFT	multiple alignment using fast Fourier transform
MS	Member State

PED   porcine epidemic diarrhoea
PEDV   porcine epidemic diarrhoea virus
PPE   personal protective equipment
RAT   rapid antigen test
RBD   receptor-binding domain
RT-PCR   reverse transcriptase-polymerase chain reaction
SIR   susceptible/infected/recovered
TESSy   The European Surveillance System
TGEV   transmissible gastroenteritis virus
ToR   Term of Reference
USDA   US Department of Agriculture
VNT   virus neutralisation test
WHO   World Health Organization

Annexes

Annex 1.   Fur farming in the EU

Table 6:   Number of fur animals farms (mainly mink) and mink skins produced in 2019

Country	Number of fur animals farms[a]	Number of mink skins produced
Total for Europe 2019	**2,976**	**27,737,900**
Denmark	1,123	12,150,000
Poland	296	5,750,000
Netherlands	150	4,000,000
Lithuania	104	1,234,000
Greece	83	1,200,000
Finland	923	1,000,000
Spain	31	550,000
Sweden	49	500,000
Latvia	15	455,000
Norway	145	320,000
Bulgaria	2	120,000
Belgium	11	100,000
Romania	2	100,000
Ireland	3	85,000
Italy	11	70,000
Iceland	16	65,000
France	5	32,000
Bosnia and Herzegovina	2	6,400
Estonia	4	500
Austria	0	0
Croatia	0	0
Czechia	1	0
Germany	0	0
Hungary	0	0
Moldova	0	0
Portugal	0	0
Serbia	0	0
Slovakia	0	0
Slovenia	0	0
Switzerland	0	0
United Kingdom	0	0

(a): The number of farms include a minority of fox and finnraccoon farms too.
Source: annual internal Fur Europe survey.

Annex 2.   Literature search protocol

COVID19

    (((2019-nCoV[tiab] OR 2019nCoV[tiab] OR "2019 nCoV"[tiab] OR COVID-19[tiab] OR COVID19 [tiab] OR "COVID 19"[tiab] OR SARS-CoV-2[tiab] OR SARSCov2[tiab] OR "SARS CoV 2"[tiab] OR SARS-COV-19[tiab] OR SARSCOV19[tiab] OR "SARS CoV 19"[tiab] OR ((wuhan[tiab] OR hubei[tiab] OR Huanan[tiab] OR China[tiab]) AND (betacoronavirus*[tiab] OR coronavirus*[tiab] OR CoV[tiab] OR nCoV[tiab] OR pneumonia[tiab])) OR "COVID-19 "[Supplementary Concept] OR "severe acute respiratory syndrome coronavirus 2"[Supplementary Concept]))

ANIMALS

(("animal experimentation"[MeSH Terms] OR "models, animal"[MeSH Terms] OR "invertebrates"[MeSH Terms] OR "Animals"[Mesh:noexp] OR "animal population groups"[MeSH Terms] OR "chordata"[MeSH Terms:noexp] OR "chordata, nonvertebrate"[MeSH Terms] OR "vertebrates"[MeSH Terms:noexp] OR "amphibians"[MeSH Terms] OR "birds"[MeSH Terms] OR "fishes"[MeSH Terms] OR "reptiles"[MeSH Terms] OR "mammals"[MeSH Terms:noexp] OR "primates"[MeSH Terms:noexp] OR "artiodactyla"[MeSH Terms] OR "carnivora"[MeSH Terms] OR "cetacea"[MeSH Terms] OR "chiroptera"[MeSH Terms] OR "elephants"[MeSH Terms] OR "hyraxes"[MeSH Terms] OR "insectivora"[MeSH Terms] OR "lagomorpha"[MeSH Terms] OR "marsupialia"[MeSH Terms] OR "monotremata"[MeSH Terms] OR "perissodactyla"[MeSH Terms] OR "rodentia"[MeSH Terms] OR "scandentia"[MeSH Terms] OR "sirenia"[MeSH Terms] OR "xenarthra"[MeSH Terms] OR "haplorhini"[MeSH Terms:noexp] OR "strepsirhini"[MeSH Terms] OR "platyrrhini"[MeSH Terms] OR "tarsii"[MeSH Terms] OR "catarrhini"[MeSH Terms:noexp] OR "cercopithecidae"[MeSH Terms] OR "hylobatidae"[MeSH Terms] OR "hominidae"[MeSH Terms:noexp] OR "gorilla gorilla"[MeSH Terms] OR "pan paniscus"[MeSH Terms] OR "pan troglodytes"[MeSH Terms] OR "pongo pygmaeus"[MeSH Terms] OR ((animals[tiab] OR animal[tiab] OR mice[Tiab] OR mus[Tiab] OR mouse[Tiab] OR murine[Tiab] OR woodmouse[tiab] OR rats[Tiab] OR rat[Tiab] OR murinae[Tiab] OR muridae[Tiab] OR cottonrat[tiab] OR cottonrats[tiab] OR hamster[tiab] OR hamsters[tiab] OR cricetinae[tiab] OR rodentia[Tiab] OR rodent [Tiab] OR rodents[Tiab] OR pigs[Tiab] OR pig[Tiab] OR swine[tiab] OR swines[tiab] OR piglets[tiab] OR piglet[tiab] OR boar[tiab] OR boars[tiab] OR "sus scrofa"[tiab] OR ferrets[tiab] OR ferret[tiab] OR polecat[tiab] OR polecats[tiab] OR "mustela putorius"[tiab] OR "guinea pigs"[Tiab] OR "guinea pig"[Tiab] OR cavia[Tiab] OR callithrix[Tiab] OR marmoset[Tiab] OR marmosets[Tiab] OR cebuella[Tiab] OR hapale[Tiab] OR octodon[Tiab] OR chinchilla[Tiab] OR chinchillas[Tiab] OR gerbillinae[Tiab] OR gerbil[Tiab] OR gerbils[Tiab] OR jird[Tiab] OR jirds[Tiab] OR merione[Tiab] OR meriones[Tiab] OR rabbits[Tiab] OR rabbit[Tiab] OR hares[Tiab] OR hare[Tiab] OR diptera[Tiab] OR flies[Tiab] OR fly[Tiab] OR dipteral[Tiab] OR drosphila[Tiab] OR drosophilidae[Tiab] OR cats[Tiab] OR cat[Tiab] OR carus[Tiab] OR felis[Tiab] OR nematoda[Tiab] OR nematode[Tiab] OR nematoda[Tiab] OR nematode[Tiab] OR nematodes[Tiab] OR sipunculida[Tiab] OR dogs[Tiab] OR dog[Tiab] OR canine[Tiab] OR canines[Tiab] OR canis[Tiab] OR sheep[Tiab] OR sheeps[Tiab] OR mouflon[Tiab] OR mouflons[Tiab] OR ovis[Tiab] OR goats[Tiab] OR goat[Tiab] OR capra[Tiab] OR capras[Tiab] OR rupicapra[Tiab] OR chamois[Tiab] OR haplorhini[Tiab] OR monkey[Tiab] OR monkeys[Tiab] OR anthropoidea[Tiab] OR anthropoids[Tiab] OR saguinus[Tiab] OR tamarin[Tiab] OR tamarins[Tiab] OR leontopithecus[Tiab] OR hominidae[Tiab] OR ape[Tiab] OR apes[Tiab] OR pan[Tiab] OR paniscus[Tiab] OR "pan paniscus"[Tiab] OR 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seahorse[Tiab] OR seahorses[Tiab] OR mugil curema[Tiab] OR atlantic cod[Tiab] OR shark[Tiab] OR sharks[Tiab] OR catshark[Tiab] OR anguilla[Tiab] OR salmonid[Tiab] OR salmonids [Tiab] OR whitefish[Tiab] OR whitefishes[Tiab] OR salmon[Tiab] OR salmons[Tiab] OR sole[Tiab] OR solea[Tiab] OR "sea lamprey"[Tiab] OR lamprey[Tiab] OR lampreys[Tiab] OR pumpkinseed[Tiab] OR

sunfish[Tiab] OR sunfishes[Tiab] OR tilapia[Tiab] OR tilapias[Tiab] OR turbot[Tiab] OR turbots[Tiab] OR flatfish[Tiab] OR flatfishes[Tiab] OR sciuridae[Tiab] OR squirrel[Tiab] OR squirrels[Tiab] OR chipmunk[Tiab] OR chipmunks[Tiab] OR suslik[Tiab] OR susliks[Tiab] OR vole[Tiab] OR voles[Tiab] OR lemming[Tiab] OR lemmings[Tiab] OR muskrat[Tiab] OR muskrats[Tiab] OR lemmus[Tiab] OR otter[Tiab] OR otters[Tiab] OR marten[Tiab] OR martens[Tiab] OR martes[Tiab] OR weasel[Tiab] OR badger[Tiab] OR badgers[Tiab] OR ermine[Tiab] OR mink[Tiab] OR minks[Tiab] OR sable[Tiab] OR sables[Tiab] OR gulo[Tiab] OR gulos[Tiab] OR wolverine[Tiab] OR wolverines[Tiab] OR minks[Tiab] OR mustela[Tiab] OR llama[Tiab] OR llamas[Tiab] OR alpaca[Tiab] OR alpacas[Tiab] OR camelid[Tiab] OR camelids[Tiab] OR guanaco[Tiab] OR guanacos[Tiab] OR chiroptera[Tiab] OR chiropteras[Tiab] OR bat[Tiab] OR bats[Tiab] OR fox[Tiab] OR foxes[Tiab] OR iguana[Tiab] OR iguanas[Tiab] OR xenopus laevis[Tiab] OR parakeet[Tiab] OR parakeets[Tiab] OR parrot[Tiab] OR parrots[Tiab] OR donkey[Tiab] OR donkeys[Tiab] OR mule[Tiab] OR mules[Tiab] OR zebra[Tiab] OR zebras[Tiab] OR shrew[Tiab] OR shrews[Tiab] OR bison[Tiab] OR bisons[Tiab] OR buffalo[Tiab] OR buffaloes[Tiab] OR deer[Tiab] OR deers[Tiab] OR bear[Tiab] OR bears[Tiab] OR panda[Tiab] OR pandas[Tiab] OR "wild hog"[Tiab] OR "wild boar"[Tiab] OR fitchew[Tiab] OR fitch[Tiab] OR beaver[Tiab] OR beavers[Tiab] OR jerboa[Tiab] OR jerboas[Tiab] OR capybara[Tiab] OR capybaras[Tiab]) NOT medline[subset])))

FOOD

("Diet, Food, and Nutrition"[Mesh] OR "Foodborne Diseases"[Mesh:noexp] OR food[tiab] OR foods[tiab] OR foodborne[tiab] OR egg[tiab] OR eggs[tiab] OR meat[tiab] OR meats[tiab] OR milk[tiab] OR seafood[tiab] OR seafoods[tiab] OR "sea food"[tiab] OR "sea foods"[tiab] OR sellfish[tiab] OR shellfishes[tiab]))

PUBLICATION DATE

("2019/11/01"[Date - Publication] : "3000"[Date - Publication])

WEB OF SCIENCE

TS=(animals OR animal OR mice OR mus OR mouse OR murine OR woodmouse OR rats OR rat OR murinae OR muridae OR cottonrat OR cottonrats OR hamster OR hamsters OR cricetinae OR rodentia OR rodent OR rodents OR pigs OR pig OR swine OR swines OR piglets OR piglet OR boar OR boars OR "sus scrofa" OR ferrets OR ferret OR polecat OR polecats OR "mustela putorius" OR "guinea pigs" OR "guinea pig" OR cavia OR callithrix OR marmoset OR marmosets OR cebuella OR hapale OR octodon OR chinchilla OR chinchillas OR gerbillinae OR gerbil OR gerbils OR jird OR jirds OR merione OR meriones OR rabbits OR rabbit OR hares OR hare OR diptera OR flies OR fly OR dipteral OR drosphila OR drosophilidae OR cats OR cat OR carus OR felis OR nematoda OR nematode OR nematoda OR nematode OR nematodes OR sipunculida OR dogs OR dog OR canine OR canines OR canis OR sheep OR sheeps OR mouflon OR mouflons OR ovis OR goats OR goat OR capra OR capras OR rupicapra OR chamois OR haplorhini OR monkey OR monkeys OR anthropoidea OR anthropoids OR saguinus OR tamarin OR tamarins OR leontopithecus OR hominidae OR ape OR apes OR pan OR paniscus OR "pan paniscus" OR bonobo OR bonobos OR troglodytes OR "pan troglodytes" OR gibbon OR gibbons OR siamang OR siamangs OR nomascus OR symphalangus OR chimpanzee OR chimpanzees OR prosimians OR "bush baby" OR prosimian OR bush babies OR galagos OR galago OR pongidae OR gorilla OR gorillas OR pongo OR pygmaeus OR "pongo pygmaeus" OR orangutans OR pygmaeus OR lemur OR lemurs OR lemuridae OR horse OR horses OR pongo OR equus OR cow OR calf OR bull OR chicken OR chickens OR gallus OR quail OR bird OR birds OR quails OR poultry OR poultries OR fowl OR fowls OR reptile OR reptilia OR reptiles OR snakes OR snake OR lizard OR lizards OR alligator OR alligators OR crocodile OR crocodiles OR turtle OR turtles OR amphibian OR amphibians OR amphibia OR frog OR frogs OR bombina OR salientia OR toad OR toads OR "epidalea calamita" OR salamander OR salamanders OR eel OR eels OR fish OR fishes OR pisces OR catfish OR catfishes OR siluriformes OR arius OR heteropneustes OR sheatfish OR perch OR perches OR percidae OR perca OR trout OR trouts OR char OR chars OR salvelinus OR "fathead minnow" OR minnow OR cyprinidae OR carps OR carp OR zebrafish OR zebrafishes OR goldfish OR goldfishes OR guppy OR guppies OR chub OR chubs OR tinca OR barbels OR barbus OR pimephales OR promelas OR "poecilia reticulata" OR mullet OR mullets OR seahorse OR seahorses OR mugil curema OR atlantic cod OR shark OR sharks OR catshark OR anguilla OR salmonid OR salmonids OR whitefish OR whitefishes OR salmon OR salmons OR sole OR solea OR "sea lamprey" OR lamprey OR lampreys OR pumpkinseed OR sunfish OR sunfishes OR tilapia OR tilapias OR turbot OR turbots OR flatfish OR flatfishes OR sciuridae OR squirrel OR squirrels OR chipmunk OR chipmunks OR suslik OR susliks OR vole OR voles OR lemming OR lemmings OR

muskrat OR muskrats OR lemmus OR otter OR otters OR marten OR martens OR martes OR weasel OR badger OR badgers OR ermine OR mink OR minks OR sable OR sables OR gulo OR gulos OR wolverine OR wolverines OR minks OR mustela OR llama OR llamas OR alpaca OR alpacas OR camelid OR camelids OR guanaco OR guanacos OR chiroptera OR chiropteras OR bat OR bats OR fox OR foxes OR iguana OR iguanas OR xenopus laevis OR parakeet OR parakeets OR parrot OR parrots OR donkey OR donkeys OR mule OR mules OR zebra OR zebras OR shrew OR shrews OR bison OR bisons OR buffalo OR buffaloes OR deer OR deers OR bear OR bears OR panda OR pandas OR "wild hog" OR "wild boar" OR fitchew OR fitch OR beaver OR beavers OR jerboa OR jerboas OR capybara OR capybaras)

TS=(2019-nCoV OR 2019nCoV OR "2019 nCoV" OR COVID-19 OR COVID19 OR "COVID 19" OR SARS-CoV-2 OR SARSCov2 OR "SARS CoV 2" OR SARS-COV-19 OR SARSCOV19 OR "SARS CoV19" OR ((wuhan OR hubei OR Huanan OR China) AND (betacoronavirus* OR coronavirus* OR CoV OR nCoV OR pneumonia)) OR "severe acute respiratory syndrome coronavirus 2")

TS=(disseminat* OR route OR routes OR transmi*)

Literature extraction as from Annex 7.1:

Authors, Year	Title	Extractions
Abdel-Moneim & Abdelwhab, 2020	Evidence for SARS-CoV-2 Infection of Animal Hosts	SARS-CoV-2 originated from animal reservoir, most likely bats and/or pangolins. **Anthroponotic transmission has been reported in cats, dogs, tigers, lions and minks**. As of now, there is **no a strong evidence for natural animal-to-human transmission or sustained animal-to-animal transmission of SARS-CoV-2**. Experimental infections conducted by several research groups have shown **that monkeys, hamsters, ferrets, cats, tree shrews, transgenic mice and fruit bats were permissive, while dogs, pigs and poultry were resistant**. There is an urgent need to understand the zoonotic potential of different viruses in animals, particularly in bats, before they transmit to humans. Vaccines or antivirals against SARS-CoV-2 should be evaluated not only for humans, but also for the protection of companion animals (particularly cats) and susceptible zoo and farm animals.
Aboubakr et al., 2020	Stability of SARS-CoV-2 and other coronaviruses in the environment and on common touch surfaces and the influence of climatic conditions: A review	SARS-CoV-2 and other human and animal CoVs have remarkably **short persistence on copper, latex and surfaces with low porosity as compared with other surfaces like stainless steel, plastics, glass and highly porous fabrics**. It has also been reported that SARS-CoV-2 is associated with diarrhoea and that it is shed in the faeces of COVID-19 patients. Some CoVs show **persistence in human excrement, sewage and waters for a few days**. These findings suggest a **possible risk of faecal–oral, foodborne and waterborne transmission of SARS-CoV-2** in developing countries that often use sewage polluted waters in irrigation and have poor water treatment systems. CoVs survive longer in the environment at lower temperatures and lower relative humidity.
Alexander et al., 2020	Predicting susceptibility to SARS-CoV-2 infection based on structural differences in ACE2 across species	Certain species, such as **domestic cats and tigers, are susceptible to SARSCoV-2 infection**, while other species such as **mice and chickens are not**. Most animal species, including those in close contact with humans, have unknown susceptibility. Given experimental **evidence for the susceptibility of humans, house cats, tigers, lions, rhesus macaques, and Golden Syrian hamsters to SARS-CoV-2 infection, and experimental evidence for non-susceptibility of mice, ducks, and chickens,**[3-5,7,9-11,39,40] we performed protein sequence alignment of ACE2 from these organisms using MAFFT (Figure S4).[20] We also included species with intermediate susceptibility, including dogs, pigs, and ferrets,[7,9,13,14] as well as species with unknown susceptibility, including camels, horses, Malayan pangolin, and sheep. The degree of similarity of ACE2 protein sequences largely fell along expected phylogenetic relationships among species (Figure S5). Susceptibility to SARS-CoV-2 infection, however, did not match either phylogenetic relationships or ACE2 sequence similarities across species. For example,

Authors, Year	Title	Extractions
		mouse (Mus musculus) is not susceptible to infection. However, mouse ACE2 sequence is more similar to a susceptible species, Golden Syrian hamster (Mesocricetus auratus), than non-susceptible species such as duck (Aythya fuligula) or chicken (Gallus gallus).[9,11] In addition, mice are phylogenetically more similar to susceptible species such as humans (Homo sapiens) and rhesus macaques (Macaca mulatta) than non-susceptible species such as ducks and chicken.[9,11] These findings suggest that neither phylogenetic relationships nor overall ACE2 protein sequence similarity across species is able to predict susceptibility to SARS-CoV-2 infection. An alternative approach is to use the experimentally validated differences in infection susceptibility across species to focus on ACE2 amino acids that most differ between susceptible and non-susceptible species. We thus calculated a weighted score of how well the aligned amino acids stratify susceptible vs non-susceptible species, incorporating amino acid similarity. This score, termed GroupSim, permits quantitative determination of which amino acids in the alignment best stratify susceptible from non-susceptible species.[26] We applied our infection susceptibility score to several important species with unknown susceptibility to date. These data suggest that cows (Bos taurus), Malayan pangolin (Manis javanica), and goats (Capra hircus) have intermediate susceptibility to infection, while Chinese horseshoe bats (Rhinolophus sinicus), horses (Equus caballus), and camels (Camelus dromedarius and Camelus bactrianus) have higher susceptibility. *3. APHISpress@usda.gov. (April 6, 2020). USDA Statement on the Confirmation of COVID-19 in a Tiger in New York. United States Department of Agriculture Animal and Plant Health Inspection Service.* https://www.aphis. usda.gov/aphis/newsroom/news/sa_ by_date/sa-2020/ny-zoo-covid -19 *4. APHISpress@usda.gov. (August 13, 2020). Confirmed cases of SARS-CoV-2 in animals in the United States. United States Department of Agriculture Animal and Plant Health Inspection Service.* https://www.aphis.usda.gov/ aphis/ourfocus/anima lheal th/SA_One_Healt h/sars-cov-2-anima ls-us *5. Chan JF, Zhang AJ, Yuan S, et al. Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility. Clin Infect Dis. 2020.* https://doi.org/10.1093/cid/ciaa325 *6. Chandrashekar A, Liu J, Martinot AJ, et al. SARS-CoV-2 infection protects against rechallenge in rhesus macaques. Science. 2020;eabc4776.* *7. Shi J, Wen Z, Zhong G, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science. 2020;368:1016–1020.* *8. Halfmann PJ, Hatta M, Chiba S, et al. Transmission of SARSCoV-2 in domestic cats. N Engl J Med. 2020;383:592–594.* *13. Kim YI, Kim SG, Kim SM, et al. Infection and rapid transmission of SARS-CoV-2 in ferrets. Cell Host Microbe. 2020;27:704-709.e702.* *14. Sit THC, Brackman CJ, Ip SM, et al. Infection of dogs with SARSCoV-2. Nature. 2020.* https://doi.org/10.1038/s41586-020-2334-5
Almendros A., 2020	Can companion animals become infected with Covid-19?	The consensus remains at this time (April 2020) that there is **no evidence that infected pets are a source of infection** for people or other pets
Bosco-Lauth et al., 2020	Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats	Due to concern for **human–pet transmission**, we investigated the susceptibility of domestic cats and dogs to infection and potential for infected cats to transmit to naive cats. We report that cats are highly susceptible to infection, with a prolonged period of oral and nasal viral shedding that is not accompanied by clinical signs, and are capable of direct contact transmission to other cats. These studies confirm that cats are susceptible to productive SARS-CoV-2 infection, but are unlikely to develop clinical disease. Further, we document that cats developed a robust neutralizing antibody response that prevented reinfection following a second viral challenge. Conversely, we

Authors, Year	Title	Extractions
		found that dogs do not shed virus following infection but do seroconvert and mount an antiviral neutralizing antibody response. **There is currently no evidence that cats or dogs play a significant role in human infection**; however, reverse zoonosis is possible if infected owners expose their domestic pets to the virus during acute infection.
Cao et al., 2020	Analysis of ACE2 Gene-Encoded Proteins Across Mammalian Species	The major finding of our predictive analysis suggested **ACE2 gene-encoded proteins** to be **highly homologous across mammals**. Based on their high homology, their possibility of binding the spike-protein of SARS-CoV-2 is quite high and species such as Felis catus, Bos taurus, Rattus norvegicus etc. may be potential susceptible hosts; special monitoring is particularly required for livestock that are in close contact with humans.
Ceylan et al., 2020	Relevance of SARS-CoV-2 in food safety and food hygiene: potential preventive measures, suggestions and nanotechnological approaches	Food products play a key role in virus transmission. There is currently **no scientific evidence to indicate a concern with food products** related to SARS-CoV-2, according to the FDA. However, consumption of meat or organs of infected animals can result in zoonotic foodborne infections. *12. European Commission. Directorate-General for Health and Food Safety. COVID-19 and Food Safety. 2020.* https://ec.europa.eu/food/sites/food/files/ safety/docs/biosafety_crisis_covid19_qandas_en.pdf. *Accessed 23 May 2020. 13. Food and Drug Administration. Coronavirus disease 2019 (COVID-19) frequently asked questions. 2020.* https://www.fda.gov/emergency-prepared ness-and-response/mcm-issues/corona virus-disease-2019-covid-19-freque ntly-asked-questions. *Accessed 13 March 2020.*
Chiocchetti et al., 2020	ACE2 Expression in the Cat and the Tiger Gastrointestinal Tracts	SARS-CoV-2 has been isolated in human and animal faecal samples. The aim of the present study was to investigate the **expression of ACE2 in the gastrointestinal tract of domestic (cat) and wild (tiger) felines**. Samples of the pylorus, duodenum, and distal colon were collected from six cats and one tiger. Risk of the SARS-CoV-2 faecal-oral transmission between cats/felids, and between cats/felids and humans. The localization of ACE2-IR in the gastrointestinal mucosa of the cats and the tiger anatomically suggested that SARS-CoV-2 can bind to its unique receptor on host cells; however, this finding alone did not necessarily mean that SARS-CoV-2 could replicate in the feline GIT. Once again, the positivity of the cat and the tiger faecal samples to SARS-CoV-2 (13, 14) confirmed that the feline GIT could be an important replication site of the virus, which could consequently spread the infection by means of the stool. *Halfmann PJ, Hatta M, Chiba S, Maemura T, Fan S, Takeda M, et al. Transmission of SARS-CoV-2 in domestic cats. NEngl JMed. (2020) 383:592–4.* https://doi.org/10.1056/nejmc2013400
Csiszar et al., 2020	Companion animals likely do not spread COVID-19 but may get infected themselves	The SARS-CoV-2 virus was originally transmitted likely from a bat or a pangolin to humans. Recent evidence suggests that SARS-CoV-2, similar to other coronaviruses, can infect several species of animals, including companion animals such as dogs, cats, and ferrets although their viral loads remain low. While the main source of infection transmission therefore is human to human, there are a few rare cases of pets contracting the infection from a SARS-CoV-2-infected human. There is no evidence that pets actively transmit SARS-CoV-2 via animal-to-human transmission
Ding S. and Liang T. J., 2020	Is SARS-CoV-2 Also an Enteric Pathogen With Potential Faecal– Oral Transmission? A COVID-19 Virological and Clinical Review	The virus may also be an enteric virus that can spread via the **faecal–oral route**

Authors, Year	Title	Extractions
Duda-Chodak et al., 2020	Covid-19 pandemic and food: Present knowledge, risks, consumers fears and safety	There is currently no evidence (scientific publications, WHO, **EFSA** etc.) that COVID-19 disease can spread directly **through food and the human digestive system**. Food can, if not directly, be a carrier of the virus.
Enserink M., 2020	Coronavirus rips through Dutch mink farms, triggering culls	Authorities in the Netherlands began to gas tens of thousands of **minks** on 6 June, most of them being pups born only weeks ago. SARS-CoV-2 has attacked farms that raise the animals for fur, and the Dutch government worries infected mink could become a viral reservoir that could cause new outbreaks in humans. The mink outbreaks are "spillover" from the human pandemic—a zoonosis in reverse that has offered scientists in the Netherlands a unique chance to study how the virus jumps between species. At least two farm workers have caught the virus from mink—the only patients anywhere known to have become **infected by animals**. SARS-CoV-2 can infect other animals, including cats, dogs, tigers, hamsters, ferrets, and macaques, but there are no known cases of transmission from these species back into the human population.
Gan et al., 2020	Research Progress on Coronavirus Prevention and **Control in Animal-Source Foods**	Livestock, poultry and other warm-blooded animals may act as intermediate hosts for CoVs. Consumption of pangolins, illegal but frequent, is present throughout the world that might be an infection pathway of COVID-19. In addition, excessive emphasis on food freshness has resulted in the prevalence of cold foods, raw foods and other consumption methods. However, most bacteria and viruses (e.g Ebola virus, SARS-CoV) have strong infectivity under room temperature and refrigeration conditions, causing possible food poisoning or foodborne infections in humans and providing conditions for viral and bacterial outbreaks in human societies. Due to the risk of virus transmission in raw material procurement, slaughter, division, processing, storage, and transportation in processing companies and the foodborne transmission nature of CoVs, residual viruses may be present in raw materials, intermediate products, and finished products.
Gaudreault et al., 2020	SARS-CoV-2 infection, disease and transmission in domestic cats	Recent SARS-CoV-2 susceptibility and transmission studies in **cats** show that the virus can replicate in these companion animals and transmit to other cats. Here, we present an in-depth study of SARS-CoV-2 infection, disease and transmission dynamics in domestic cats. All animals were clinically asymptomatic during the course of the study and capable of transmitting SARS-CoV-2 to sentinels within 2 days of comingling
Goli M., 2020	Review of novel human β-coronavirus (2019-nCoV or SARSCoV-2) from the food industry perspective —Appropriate approaches to food production technology	The purpose of this study was to review these coronaviruses from the perspective of appropriate **approaches to food production technology**, including following good food safety practices in food production lines; avoidance of underheating in the processing of swine and the other meat products; uncertainty about the safety of frozen or refrigerated meat products; providing unfavourable environmental conditions for coronavirus survival (minimum heat treatment, e.g., low-temperature long time and greater for liquid food products, pH $\leq$ 3, minimum storage relative humidity)
Han et al., 2020	Can the coronavirus disease be transmitted from food? A review of evidence, risks, policies and knowledge gaps	No retrospective study has been reported on foodborne transmission of COVID-19. While studies have shown that low temperature could dramatically prolong the persistence on SARS-CoV-2 and other coronaviruses, **frozen and refrigerated foods have been widely overlooked** as potential vectors in policy frameworks and risk mitigation strategies. Food transmission evidence has been disclosed in China early July 2020 by the detection of SARS-CoV-2 on frozen foods, including their packaging materials and storage environments, with two re-emergent outbreaks linked to contaminated food sources. The contamination risk is augmented by a complex farm-to-table process, which favors exposure to food workers and ambient environments. We therefore hypothesize that contaminated cold-

Authors, Year	Title	Extractions
		storage foods may present a systematic risk for SARS-CoV-2 transmission between countries and regions. Here, we review the evidence, risk factors, current policy and knowledge gaps, on food contamination and foodborne transmission of SARS-CoV-2. Although **the likelihood of food-to-human transmission is considered lower** when compared with other routes such as respiratory droplets and fomites, these should not be neglected as a risk factor given the large volumes of refrigerated foods being transported across different countries and regions, the personnel and complex environments they could be exposed to through the "farm-to-table" lifecycle, and their eventual human contact with a large consumer base. **The presence of SARS-CoV-2 was detected on frozen foods, including packaging materials and storage environments, with 9 incidents reported by health authorities across the country between early July and mid-August 2020.** Further, latest laboratory studies found new evidence that SARS-CoV-2 remained highly stable on meat, fish, and animal skin for the entire duration of studies (14–21 days) at both refrigerated (4 °C) and freezing temperatures ($-$ 20 and $-$ 80 °C).
Hayashi et al., 2020;	Highly conserved binding region of ACE2 as a receptor for SARS-CoV-2 between humans and mammals	Several cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection transmitted from humans to animals
Hernández et al., 2020	Are Animals a Neglected Transmission Route of SARS-CoV-2?	There is evidence that SARS-CoV-2 can infect felines, dogs and minks, and there is evidence of human-to-animal infection. Likewise, the S protein nucleotide sequence of the SARS-CoV-2 virus isolated in domestic animals and humans is identical, and the replication of the SARS-CoV-2 in cats is efficient.
Huang et al., 2020	SARS-CoV-2 failure to infect or replicate in mosquitoes: an extreme challenge	The WHO has stated "To date there has been no information nor evidence to suggest that the new coronavirus could be transmitted by mosquitoes". Here we provide the first experimental data to investigate the capacity of SARS-CoV-2 to infect and be transmitted by mosquitoes. Three widely distributed species of mosquito; Aedes aegypti, Ae. albopictus and Culex quinquefasciatus were tested. We demonstrate that even under extreme conditions, **SARS-CoV-2 virus is unable to replicate in these mosquitoes** and therefore cannot be transmitted to people even in the unlikely event that a mosquito fed upon a viremic host.
Kim et al., 2020;	Infection and Rapid Transmission of SARS-CoV-2 in Ferrets	**Ferrets** are highly susceptible to SARSCoV-2 infection and effectively transmit the virus by direct or indirect contact, recapitulating human infection and transmission.
Kiros et al., 2020	COVID-19 pandemic: current knowledge about the role of pets and other animals in disease transmission	SARS-CoV-2 can affect not only humans but also pets and other domestic and wild animals, making it a one health global problem. Several published scientific evidence has shown that bats are the initial reservoir hosts of SARS-CoV-2, and pangolins are suggested as an intermediate hosts. So far, little is known concerning the role of pets and other animals in the transmission of COVID19. Review of available data
Kumar et al., 2020	Predicting susceptibility for SARS-CoV-2 infection in domestic and wildlife animals using ACE2 protein sequence homology	The article is presenting a bioinformatics based method predicting susceptibility for SARS-CoV-2 infection in domestic and wildlife animals.

Authors, Year	Title	Extractions
McNamara et al., 2020	A Critical Needs Assessment for Research in Companion Animals and Livestock Following the Pandemic of COVID-19 in Humans	The susceptibility of **livestock and poultry** that could act as virus reservoirs, might serve as animal models for COVID-19 or are possibly in close contact with infected humans, is still understudied. Recent work at the Harbin Veterinary Research Institute (Shi et al. 2020b) in China and the Friedrich-LoefflerInstitut (Swine Health Information Center 2020, FriedrichLoeffler-Institut 2020) in Germany examined SARS-CoV-2 susceptibility of pigs, chickens, and ducks (only studied by Shi et al. 2020b). They reported that **pigs, chickens, and ducks could not be productively infected by SARS-CoV-2** under the experimental conditions used in their work. This raises many questions: What is the susceptibility of livestock and poultry to SARS-CoV-2? Do we know the potential transmission of SARS-CoV-2 from humans to livestock/poultry and among different livestock/poultry species? Do we have methods for detection and surveillance of SARS-CoV-2 in livestock/poultry?
Meekins et al., 2020	Susceptibility of swine cells and domestic pigs to SARS-CoV-2	We determined the ability of SARS-CoV-2 to (i) replicate in porcine cell lines, (ii) establish infection in domestic pigs via experimental oral/intranasal/ intratracheal inoculation, and (iii) transmit to co-housed naive sentinel pigs. SARS-CoV-2 was able to replicate in two different porcine cell lines with cytopathic effects. Interestingly, none of the SARS-CoV-2-inoculated pigs showed evidence of clinical signs, viral replication or SARS-CoV-2-specific antibody responses. Moreover, none of the sentinel pigs displayed markers of SARS-CoV-2 infection. These data indicate that although different porcine cell lines are permissive to SARS-CoV-2, five-week-old pigs are not susceptible to infection via oral/intranasal/intratracheal challenge. **Pigs are therefore unlikely to be significant carriers of SARS-CoV-2** and are not a suitable pre-clinical animal model to study SARS-CoV-2 pathogenesis or efficacy of respective vaccines or therapeutics.
Munir et al., 2020	Zoonotic and reverse zoonotic events of SARS-CoV-2 and their impact on global health	Recent detection of SARS-CoV-2 in pet, zoo and certain farm animals has highlighted its **potential for reverse zoonosis**. This scenario is particularly alarming, since these animals could be potential reservoirs for secondary zoonotic infections. In this article, we highlight interspecies SARS-CoV-2 infections and focus on the reverse zoonotic potential of this virus. We also emphasize the importance of potential secondary zoonotic events and the One-Health and One-World approach to tackle such future pandemics.
Richard et al., 2020;	SARS-CoV-2 is transmitted via contact and via the air between ferrets	
Salajegheh Tazerji et al., 2020	Transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to animals: an updated review	Review of cases of human to animal transmissions
Schlottau et al., 2020;	SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study	We intranasally inoculated nine fruit bats (Rousettus aegyptiacus), ferrets (Mustela putorius), pigs (Sus scrofa domesticus), and 17 chickens (Gallus gallus domesticus) with 10^5 TCID50 of a SARS-CoV-2 isolate per animal. Direct contact animals (n=3) were included 24 h after inoculation to test viral transmission. **Pigs and chickens were not susceptible to SARS-CoV-2.** All swabs, organ samples, and contact animals were negative for viral RNA, and none of the pigs or chickens seroconverted. Seven (78%) of nine fruit bats had a transient infection

Authors, Year	Title	Extractions
Shi et al., 2020;	Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2	We investigated the susceptibility of ferrets and animals in close contact with humans to SARS-CoV-2. We found that SARS-CoV-2 replicates poorly in dogs, pigs, chickens, and ducks, but ferrets and cats are permissive to infection. Additionally, cats are susceptible to airborne transmission. Our study provides insights into the animal models for SARS-CoV-2 and animal management for COVID-19 control.
Sreenivasan et al., 2020	Susceptibility of livestock and companion animals to COVID-19	Here, we explore the **susceptibility of companion and agricultural animals**, in light of the existing information on natural infections, experimental infections, serosurveillance, and in vitro protein-homology binding interaction studies of the SARS-CoV-2 with the proposed receptor angiotensin-converting enzyme 2 from diverse animal species. Luan et al. performed homology modeling of SARS-CoV-2 spike protein with ACE2 of several mammalian species and predicted that SARS-CoV-2 could bind to ACE2 of oldworld monkeys, orangutans, baboons, mustelids, civets, several species of horseshoe bats, pigs, ferrets, dogs, cats, pangolin, Malayan fruit bats, horse, cow, rabbits, red fox, sheep, Chinese hamster and other hamster spp., marmoset, naked mole-rat, and ground squirrel to SARS-CoV-2. Species that could not bind to the virus include camels, raccoon, Greater horseshoe bat, rat, mice, platypus, African bush elephant, European hedgehog, mongoose, kangaroo rat, and guinea pigs. There is **no concrete evidence as yet to indicate that livestock or companion animals could transmit SARS-CoV-2 to humans.** Minks are the only domesticated species, which were largely affected by the SARS-CoV-2 on a global scale.
Stout et al., 2020	Coronaviruses in cats and other companion animals: Where does SARS-CoV2/COVID-19 fit?	With a focus on felines, we review here the evidence for SARS-CoV-2 infection in cats, ferrets and dogs, describe the relationship between SARS-CoV-2 and the natural coronaviruses known to infect these species, and provide a rationale for the relative susceptibility of these species to SARS-CoV-2 through comparative analysis of the ACE2 receptor. Both cats and ferrets are known hosts of human (and avian) influenza viruses yet are not considered to be a significant risk for human infections. In contrast, dogs maintain their own pool of influenza viruses. Overall, both cats and ferrets may be part of a common pool of human respiratory infections.
Vergara-Alert et al., 2020	Pigs are not susceptible to SARS-CoV-2 infection but are a model for viral immunogenicity studies	Conventional piglets were inoculated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through different routes, including intranasal, intratracheal, intramuscular and intravenous ones. Although **piglets were not susceptible to SARS-CoV-2** and lacked lesions or viral RNA in tissues/ swabs, seroconversion was observed in pigs inoculated parenterally (intramuscularly or intravenously). Multiple experimental infections have already shown a broad range of susceptible animals. Specifically, Egyptian fruit bat, ferret, golden Syrian hamster, cat, mice expressing humanized angiotensin-converting enzyme 2 (ACE2), BALB/c mice (using a mutated SARS-CoV-2 by several cell culture passages) and some nonhuman primate species are permissive to viral infection, developing from subclinical to mild-to moderate respiratory disease (Bao et al., 2020; Halfmann et al., 2020; Kim et al., 2020; Rockx et al., 2020; Shi et al., 2020; Yu et al., 2020). From an experimental point of view, dog susceptibility to SARS-CoV-2 is limited, since inoculated animals can partly seroconvert (Shi et al., 2020). In contrast, the intranasal inoculation of **chicken, duck and pig resulted in no evidence of infection** (Schlottau et al., 2020; Shi et al., 2020).

Authors, Year	Title	Extractions
Westhaus et al., 2020	Detection of SARS-CoV-2 in raw and treated **wastewater** in Germany – Suitability for COVID-19 surveillance and potential transmission risks	**Table 1** Overview of selected SARS-CoV2 studies in wastewater conducted in 2020.

Study region	Genetic traces/genes analysed	Results
Paris (France)	RdRp	Samples in three WWTPs positive between 5th March and 23th April
Milan and Rome (Italy)	ORF1ab, S	6 out of 12 samples positive in raw wastewater
Netherlands	N1, N2, N3, E	4 out of 7 wastewater samples positive at the beginning of March. 9 out 9 wastewater samples positive in the middle of March
Milan (Italy) (WWTP and river)	ORF1ab, N, E	First sampling: 3 out of 4 raw wastewater samples positive 2 out of 2 effluent samples negative 2 out of 2 river samples positive
Brisbane (Australia)	N and confirmation via Sanger and MiSeq Illumina sequencing	2 out of 9 samples positive in raw wastewater
Massachusetts (USA)	N1, N2, N3,	10 out of 10 raw wastewater samples positive
Israel (different cities and facilities)	E	2 out of 15 samples positive in March 2020 9 out of 11 samples positive in April 2020
Bozeman, Montana (USA)	N1, N2	7 out of 7 positive in March/April 2020
Istanbul (Turkey)	RdRp	9 out of 9 sludge samples positive
Valencia (Spain)	N1, N2, N3	35 out of 42 influent samples positive 2 out of 18 secondary treated samples positive 0 out of 12 tertiary effluent samples positive
Yamanashi Prefecture, Japan	N1, N2	None of 5 influent samples positive 1 out of 5 secondary effluent samples positive None out of 3 river water samples positive
Ahmedabad, Gujara, India	ORF1ab, N and S	2 out of 2 influent samples positive 2 out 2 effluent samples negative
Louisiana, USA	N1 and N2	2 out of 15 raw wastewater samples positive Effluent samples all negative
Quito, Ecuador	N1 and N2	3 out of 3 river water samples positive