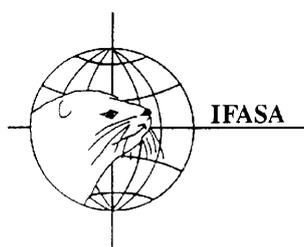
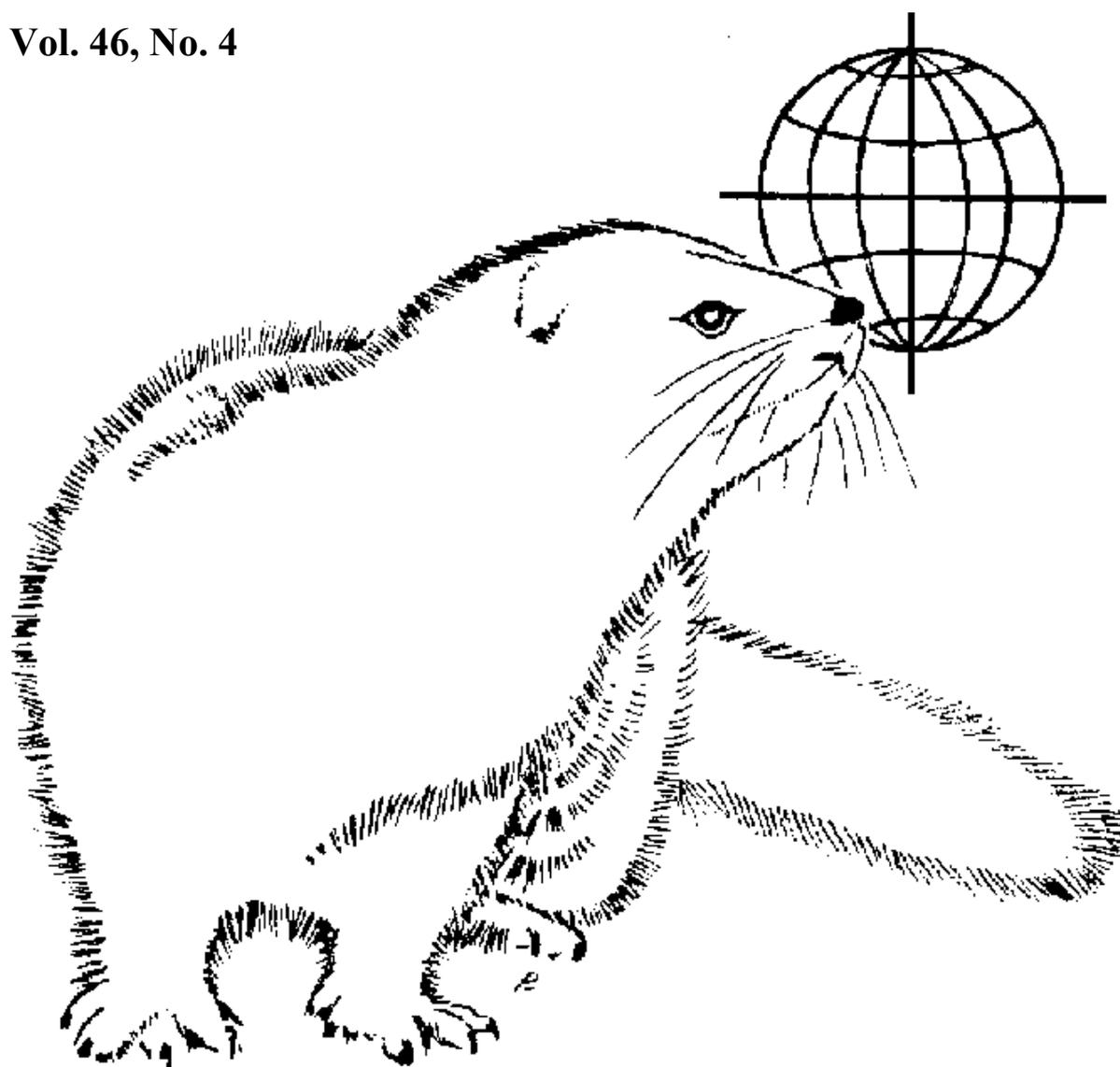


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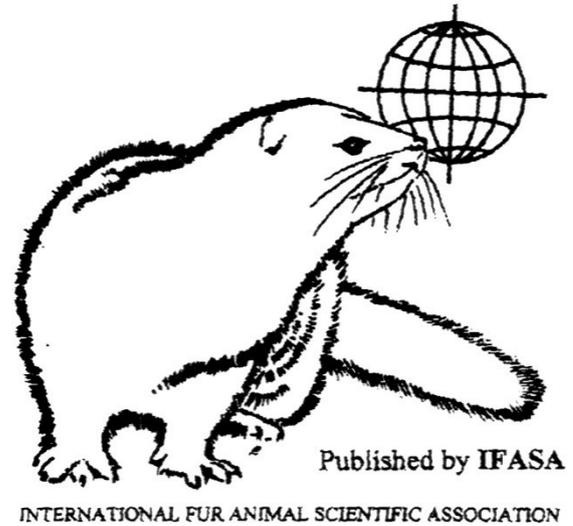
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Notes from the Editor

Transmission of coronavirus 2 (SARS-CoV-2) between mink and between mink and humans remain a focal point for research. Reported studies deal with understanding of the cross-species transmission of SARS-CoV-2 including mechanisms facilitating the transmission, the pathology and pathogenesis of infections in mink as well as recommended precautions for safe mink farming.

Denmark's mink production was among the world's largest, but in November 2020 all mink were killed due to infection with the coronavirus on many farms, and a ban on mink production was introduced until the end of 2022. The Danish government has now announced that mink production in Denmark will be allowed again from January 2023. Farmers must, however, comply with a number of infection prevention measures, including testing of mink, use of protective equipment and hygiene courses.

Feed costs are a problem for the farmer and inefficient uptake of nutrients by the animals can result in harmful emissions of e.g. nitrogen and phosphorus to the environment. Outcomes of presented research show that breeding for feed utilization and optimal

feed composition can mitigate these undesirable effects.

Antibiotic use raises concerns about antibiotic-resistant bacteria in a One Health perspective. A new study shows the complex interplay between antibiotic use and resistance. The obvious link between antibiotic use and antibiotic resistance was shown, but antibiotic resistance was also shown in farms without antibiotic use.

Whole-genome sequencing is an important research tool in most domestic animal species for understanding of genetic diversity. Whole-genome sequencing was performed in mink for the first genome-wide copy number variation analyses. Copy number variations are potentially a tool for use in genome analyses, including mapping of genomic structural variations. Cryobanking as a basis for ex situ conservation has been documented as a tool for the conservation of genetic variation in the European mink (*Mustela lutreola*).

Vivi Hunnicke Nielsen

Editor Scientifur

BREEDING, GENETICS AND REPRODUCTION

Genome-wide detection of copy number variation in American mink using whole-genome sequencing

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Background

Copy number variations (CNVs) represent a major source of genetic diversity and contribute to the phenotypic variation of economically important traits in livestock species. In this study, we report the first genome-wide CNV analysis of American mink using whole-genome sequence data from 100 individuals. The analyses were performed by three complementary software programs including CNVpytor, DELLY and Manta.

Results

A total of 164,733 CNVs (144,517 deletions and 20,216 duplications) were identified representing 5378 CNV regions (CNVR) after merging overlapping CNVs, covering 47.3 Mb (1.9%) of the mink autosomal genome. Gene Ontology and KEGG pathway enrichment analyses of 1391 genes that overlapped CNVR revealed potential role of CNVs in a wide range of biological, molecular and cellular functions, e.g., pathways related to growth (regulation of actin cytoskeleton, and cAMP signaling pathways), behavior (axon guidance, circadian entrainment, and glutamatergic synapse), lipid metabolism (phospholipid binding, sphingolipid metabolism and regulation of lipolysis in adipocytes), and immune response (Wnt signaling, Fc receptor signaling, and GTPase regulator activity pathways). Furthermore, several CNVR-harbored genes associated with fur characteristics and development (MYO5A, RAB27B, FGF12, SLC7A11, EXOC2), and immune system processes (SWAP70, FYN, ORAI1, TRPM2, and FOXO3).

Conclusions

This study presents the first genome-wide CNV map of American mink. We identified 5378 CNVR in the mink genome and investigated genes that overlapped with CNVR. The results suggest potential links with mink behaviour as well as their possible impact on fur quality and immune response. Overall, the results provide new resources for mink genome analysis, serving as a guideline for future investigations in which genomic structural variations are present.

BMC Genomics 2022 Sep 13;23 (1): 649.

Doi: 10.1186/s12864-022-08874-1.

Genetic and phenotypic parameters for feed efficiency and component traits in American mink

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J Anim Sci. 2022 Aug 1;100 (8): skac216.

Doi: 10.1093/jas/skac216.

Cryobanking European Mink (*Mustela lutreola*) Mesenchymal Stem Cells and Oocytes

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The European mink (*Mustela lutreola*) is one of Europe's most endangered species, and it is on the brink of extinction in the Iberian Peninsula. The species' precarious situation requires the application of new ex situ conservation methodologies that complement the existing ex situ and in situ conservation measures. Here, we report for the first time the establishment of a biobank for European mink mesenchymal stem cells (emMSC)

and oocytes from specimens found dead in the Iberian Peninsula, either free or in captivity. New emMSC lines were isolated from different tissues: bone marrow (emBM-MSC), oral mucosa (emOM-MSc), dermal skin (emDS-MSC), oviduct (emO-MSc), endometrium (emE-MSC), testicular (emT-MSC), and adipose tissue from two different adipose depots: subcutaneous (emSCA-MSC) and ovarian (emOA-MSC). All eight emMSC lines showed plastic adhesion, a detectable expression of characteristic markers of MSCs, and, when cultured under osteogenic and adipogenic conditions, differentiation capacity to these lineages. Additionally, we were able to keep 227 Cumulus-oocyte complexes (COCs) in the biobank, 97 of which are grade I or II. The European mink MSC and oocyte biobank will allow for the conservation of the species' genetic variability, the application of assisted reproduction techniques, and the development of in vitro models for studying the molecular mechanisms of infectious diseases that threaten the species' precarious

Int J Mol Sci. 2022 Aug 18; 23 (16): 9319.
Doi: 10.3390/ijms23169319.

Effects of Dietary Inclusion of *Spirulina platensis* on the Reproductive Performance of Female Mink

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The objective of this study was to investigate the impact of providing *Spirulina platensis* (Spirulina) on reproductive performance of female mink. A total of 100 adult brown female mink (*Mustela vison*) were randomly and equally allocated to control group (C group), in which mink were fed basal diet and Spirulina group (Sp group), where mink received basal diet supplemented with 100 mg of Spirulina/kg of body weight. The experiment lasted 5 months, starting from 1 month prior to mating till kit weaning. Weight gain during pre-

mating period was higher in Sp group compared to C group ($p < 0.001$). Sp group remained heavier until the onset of lactation. Subsequently, mink of Sp group lost more weight than C group ($p < 0.001$) but without an adverse effect on kit survival. A tendency for a higher whelping rate was detected in Sp group (93.61%) compared to C group (81.25%) ($p = 0.07$). Litter size, as well as weight of kits at weaning, did not differ between groups ($p > 0.10$). Finally, Sp group weaned numerically more kits compared to C group. Results obtained here showed that Spirulina treated animals tended to an increased whelping rate.

Vet Sci. 2022 Aug 12; 9 (8): 428.
Doi: 10.3390/vetsci9080428.

NUTRITION, FEEDING AND MANAGEMENT

Effects of Dietary Protein and Energy Levels on Growth Performance, Nutrient Digestibility, and Serum Biochemical Parameters of Growing Male Mink (*Neovison vison*)

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The objective of this experiment was to determine the optimum dietary metabolic energy (ME) and crude protein (CP) levels of growing male mink. One hundred forty-four healthy male minks at 75 days were randomly allocated into the six groups with 24 replicates, which was one mink for each replicate. The mink were fed six experimental diets with two CP levels (31.59 and 35.63%) and three ME levels (14.17, 15.96, and 17.73 MJ/kg) for a 7-day preliminary period and then for an 88-day experimental period. The final body weight (BW), average daily gain (ADG), feed conversion ratio (FCR), fat digestibility, energy intake, the concentration of glucose (GLU), and low-density lipoprotein (LDL) of the mink were significantly increased by the CP or ME levels ($P < 0.05$). In addition, CP levels significantly ($P < 0.01$) increased the N intake and N retention. Dietary ME levels increased the utilization of gross energy. Obviously, there were significant CP \times ME interactions for the final BW, ADG, fat digestibility, energy utilization, GLU, LDL ($P < 0.01$), and triglyceride contents ($P < 0.05$). Therefore, the optimum

CP and ME levels were 35.97% and 18.18 MJ/kg, which can improve growth, enhance nutrient digestion, and promote blood lipid metabolism in growing mink. *Front Vet Sci.* 2022 Jul 18; 9: 961461.

Doi: 10.3389/fvets.2022.961461. eCollection 2022.

Effect of Dietary Supplementation with Calcium, Phosphorus and Vitamin D₃ on Growth Performance, Nutrient Digestibility, and Serum Biochemical Parameters of Growing Blue Foxes

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Based on the randomized design, a 3 × 3 factorial experiment was designed to examine the effects of dietary calcium (Ca), phosphorus (P), and vitamin D₃ (VD₃) supplemental levels with a fixed 1.5/1 ratio of Ca to P on the growth performance, nutrient digestibility, and serum biochemical indices blue foxes' growth. In total, 135 male blue foxes with the age of 60 days were randomly divided into 9 groups each with 15 blue foxes. The blue foxes belonging to the nine treatment groups were fed Ca supplementation (0%, 0.4%, or 0.8%) and VD₃ supplementation (1000, 2000, or 4000 IU/kg DM). The base diet contained 0.8% Ca and 327 IU/kg VD₃. The dosage of VD₃ in blue foxes showed a significant impact on their growth performance ($p < 0.05$). The Ca dosage had a linear effect on the digestibility of the CP and carbohydrates (CHO) ($p < 0.05$). In conclusion, the results indicated that the Ca and VD₃ doses showed promising effects on growth performance and nutrient digestibility in growing blue foxes and could reduce fecal N and P via improvement in protein and P utilization.

Animals (Basel). 2022 Jul 15; 12 (14): 1814.

Doi: 10.3390/ani12141814.

A three-dimensional reconstructive study of pelvic cavity in the red fox (*Vulpes vulpes*)

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Anat Histol Embryol. 2022 Mar; 51(2): 215-220.

Doi: 10.1111/ah.12780. Epub 2021 Dec 26.

Morphometric analysis of the red fox molar teeth in Lithuania

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Anat Histol Embryol. 2022 Jul; 51 (4): 452-458.

Doi: 10.1111/ah.12809. Epub 2022 May 3.

HEALTH AND DISEASE

Association between Antibiotic Consumption and Resistance in Mink Production

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Antibiotic consumption is considered to be a main driver of antibiotic resistant bacteria. Mink breeding

follows a distinctive seasonal reproduction cycle, and all of the mink produced in the northern hemisphere are bred, born, and pelted around the same time of year. Some of the diseases are age-related, which is reflected in the seasonal variation of antibiotic consumption. The seasonality makes mink a good model for the investigation of the association between antibiotic consumption and resistance. The objectives of this study were (1) to monitor the farm level of antibiotic resistance during one production cycle and (2) to assess the potential associations between antibiotic consumption and resistance. Twenty-four farms were included in this study (Denmark n = 20, Iceland n = 2, and The Netherlands n = 2), following a cohort of animals born in 2018. *Staphylococcus delphini* and *Escherichia coli* were isolated from samples of the carcasses and faeces and were collected randomly. The isolates were susceptibility tested and subsequently divided into the sensitive wildtype (WT) and the resistant non-wildtype (NWT) populations. The antibiotic consumption relative to the sampling periods was assessed as having a short-term or a long-term impact, i.e., in two explanatory factors. For both *S. delphini* and *E. coli*, a large between-farm variation of NWT profiles was detected. In the final multivariable, generalized linear mixed models, significant associations between NWT isolates and the consumption of specific antibiotics were found: the short-term use of tetracyclines in the growth period was associated with the occurrence of tetracycline NWT *E. coli* in the growth period (OR: 11.94 [1.78; 89.28]), and the long-term use of macrolide and tetracyclines was associated with the occurrence of erythromycin NWT *S. delphini* in the weaning period (OR: 18.2 [2.26; 321.36]) and tetracycline NWT *S. delphini* in the growth period (OR: 8.2 [1.27; 63.31]), respectively. Farms with zero consumption in the study years prior to sampling also had a substantial proportion of NWT isolates, indicating that NWT isolates are persistent and/or widely spread in the environment. Generally, a high occurrence of tetracycline NWTs was observed. NWT isolates with resistance against the most commonly used antibiotics were found on all the farms, stressing the need for routine surveillance and the prudent use of antibiotics. The results offer a preview of the complex relationship between consumption and resistance, demonstrating some significant associations between use and resistance. Moreover, antibiotic-resistant bacteria are present even on farms with no antibiotic consumption over extended periods, and theoretical explanations supported by the data are offered.

Antibiotics (Basel) 2022, Jul 9; 11 (7): 927.
Doi: 10.3390/antibiotics11070927.

The first evidence of shaking mink syndrome (SMS)-astrovirus associated encephalitis in farmed minks, China

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Transbound Emerg Dis. 2022 Sep 4.

Doi: 10.1111/tbed.14693. Online ahead of print.

Effect of vitamin C supplementation on some leukocyte parameters in American mink (*Neovison vison*) with abnormal granulogenesis

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Tissue Cell. 2022 Aug; 77: 101870.

Doi: 10.1016/j.tice.2022.101870. Epub 2022 Jul 15.

Molecular Basis of Mink ACE2 Binding to SARS-CoV-2 and Its Mink-Derived Variants

Chao Su^{1,2}, Juanhua He^{2,3}, Pengcheng Han^{2,4}, Bin Bai^{2,5}, Dedong Li^{2,6}, Jian Cao², Mingxiong Tian^{2,7}, Yu Hu^{2,8}, Anqi Zheng^{2,4}, Sheng Niu^{2,9}, Qian Chen^{2,10}, Xiaoyu Rong^{2,11}, Yanfang Zhang², Weiwei Li², Jianxun Qi², Xin Zhao^{2,12}, Mengsu Yang¹, Qihui Wang², George Fu Gao^{1,2,3}

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is transmitted between humans and minks, and some mutations in the spike (S) protein, especially in the receptor-binding domain (RBD), have been identified in mink-derived viruses. Here, we examined binding of the mink angiotensin-converting enzyme 2 (ACE2) receptor to mink-derived and important human-originating variants, and we demonstrated that most of the RBD variants increased the binding affinities to mink ACE2 (mkACE2). Cryo-electron microscopy structures of the mkACE2-RBD Y453F (with a Y-to-F change at position 453) and mkACE2-RBD F486L complexes helped identify the key residues that facilitate changes in mkACE2 binding affinity. Additionally, the data indicated that the Y453F and F486L mutations reduced the binding affinities to some human monoclonal antibodies, and human vaccinated sera efficiently prevented infection of human cells by pseudoviruses expressing Y453F, F486L, or N501T RBD. Our findings provide an important molecular mechanism for the rapid adaptation of SARS-CoV-2 in minks and highlight the potential influence of the main mink-originating variants for humans. **IMPORTANCE** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has a broad range of hosts. Mink-derived SARS-CoV-2 can transmit back to humans. There is an urgent need to understand the binding mechanism of mink-derived SARS-CoV-2 variants to

mink receptor. In this study, we identified all mutations in the receptor-binding domain (RBD) of spike (S) protein from mink-derived SARS-CoV-2, and we demonstrated the enhanced binding affinity of mink angiotensin-converting enzyme 2 (ACE2) to most of the mink-derived RBD variants as well as important human-originating RBD variants. Cryo-electron microscopy structures revealed that the Y453F and F486L mutations enhanced the binding forces in the interaction interface. In addition, Y453F and F486L mutations reduced the binding affinities to some human monoclonal antibodies, and the SARS-CoV-2 pseudoviruses with Y453F, F486L, or N501T mutations were neutralized by human vaccinated sera. Therefore, our results provide valuable information for understanding the cross-species transmission mechanism of SARS-CoV-2.

J Virol. 2022 Sep 14; 96 (17): e0081422.

Doi: 10.1128/jvi.00814-22. Epub 2022 Aug 24.

Histopathology and localization of SARS-CoV-2 and its host cell entry receptor ACE2 in tissues from naturally infected US-farmed mink (*Neovison vison*)

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes respiratory disease in mink similar to human COVID-19. We characterized the pathological findings in 72 mink from US farms with SARS-CoV-2 outbreaks, localized SARS-CoV-2 and its host cellular receptor angiotensin-converting enzyme 2 (ACE2) in mink respiratory tissues, and evaluated the utility of various test methods and specimens for SARS-CoV-2 detection in necropsy tissues. Of SARS-

CoV-2-positive animals found dead, 74% had bronchiolitis and diffuse alveolar damage (DAD). Of euthanized SARS-CoV-2-positive animals, 72% had only mild interstitial pneumonia or minimal nonspecific lung changes (congestion, edema, macrophages); similar findings were seen in SARS-CoV-2-negative animals. Suppurative rhinitis, lymphocytic perivascular inflammation in the lungs, and lymphocytic infiltrates in other tissues were common in both SARS-CoV-2-positive and SARS-CoV-2-negative animals. In formalin-fixed paraffin-embedded (FFPE) upper respiratory tract (URT) specimens, conventional reverse transcription-polymerase chain reaction (cRT-PCR) was more sensitive than in situ hybridization (ISH) or immunohistochemistry (IHC) for detection of SARS-CoV-2. FFPE lung specimens yielded less detection of virus than FFPE URT specimens by all test methods. By IHC and ISH, virus localized extensively to epithelial cells in the nasal turbinates, and prominently within intact epithelium; olfactory mucosa was mostly spared. The SARS-CoV-2 receptor ACE2 was extensively detected by IHC within turbinate epithelium, with decreased detection in lower respiratory tract epithelium and alveolar macrophages. This study expands on the knowledge of the pathology and pathogenesis of natural SARS-CoV-2 infection in mink and supports their further investigation as a potential animal model of SARS-CoV-2 infection in humans.

Vet Pathol. 2022 Jul; 59 (4): 681-695.

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Role of spike compensatory mutations in the interspecies transmission of SARS-CoV-2

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SARS-CoV-2, the virus responsible for COVID-19 in humans, can efficiently infect a large number of animal species. Like any virus, and particularly RNA viruses, SARS-CoV-2 undergoes mutations during its life cycle some of which bring a selective advantage, leading to the selection of a given lineage. Minks are very susceptible to SARS-CoV-2 and owing to their presence in

mass rearing, they make a good model for studying the relative importance of mutations in viral adaptation to host species. Variants, such as the mink-selected SARS-CoV-2 Y453F and D614G or H69del/V70del, Y453F, I692V and M1229I were identified in humans after spreading through densely caged minks. However, not all mink-specific mutations are conserved when the virus infects human populations back. Many questions remain regarding the interspecies evolution of SARS-CoV-2 and the dynamics of transmission leading to the emergence of new variant strains. We compared the human and mink ACE2 receptor structures and their interactions with SARS-CoV-2 variants. In minks, ACE2 presents a Y34 amino acid instead of the H34 amino acid found in the human ACE2. H34 is essential for the interaction with the Y453 residue of the SARS-CoV-2 Spike protein. The Y453F mink mutation abolishes this conflict. A series of 18 mutations not involved in the direct ACE2 interaction was observed in addition to the Y453F and D614G in 16 different SARS-CoV-2 strains following bidirectional infections between humans and minks. These mutations were not random and were distributed into five different functional groups having an effect on the kinetics of ACE2-RD interaction. The interspecies transmission of SARS-CoV-2 from humans to minks and back to humans, generated specific mutations in each species which improved the affinity for the ACE2 receptor either by direct mutation of the core 453 residue or by associated compensatory mutations.

One Health. 2022 Dec; 15: 100429.

Doi: 10.1016/j.onehlt.2022.100429.

Epub 2022 Aug 29.

Genome Similarities between Human-Derived and Mink-Derived SARS-CoV-2 Make Mink a Potential Reservoir of the Virus

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SARS-CoV-2 has RNA as the genome, which makes the virus more prone to mutations. Occasionally, mutations help a virus to cross the species barrier. SARS-CoV-2 infections in humans and minks (*Neovison vison*) are examples of zoonotic spillover. Many studies on the mutational analysis of human-derived SARS-

CoV-2 have been published, but insight into the mink-derived SARS-CoV-2 genome of mutations is still required. Here, we performed a mutation analysis of the mink-derived SARS-CoV-2 genome sequences. We analyzed all available full-length mink-derived SARS-CoV-2 genome sequences on GISAID (214 genome sequences from the Netherlands and 133 genome sequences from Denmark). We found a striking resemblance between human-derived and mink-derived SARS-CoV-2. Our study showed that mutation patterns in the SARS-CoV-2 genome samples from the Netherlands and Denmark were different. Out of the 201 mutations we found, only 13 mutations were shared by the Netherlands' and Denmark's mink-derived samples. We found that six mutations were prevalent in the mink-derived SARS-CoV-2 genomes, and these six mutations are also known to be prevalent in human-derived SARS-CoV-2 variants. Our study reveals that the G27948T mutation in SARS-CoV-2 leads to truncation of ORF8, which was also reported in human-derived SARS-CoV-2, thus indicating that the virus can replicate without the full-length ORF8. These resemblances between mink-derived and human-derived SARS-CoV-2 enable the virus to cross the species barrier and suggest mink a potential reservoir for the virus.

Vaccines (Basel). 2022 Aug 19; 10 (8): 1352.
Doi: 10.3390/vaccines10081352.

SARS-CoV-2 in a Mink Farm in Italy: Case Description, Molecular and Serological Diagnosis by Comparing Different Tests

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This study described a SARS-CoV-2 infection in minks on an Italian farm. Surveillance was performed based on clinical examination and a collection of 1879 swabs and 74 sera from dead and live animals. The farm was placed under surveillance for 4.5 months, from the end of July 2020, when a man working on the farm tested positive by RT-PCR, till mid-December 2020 when all the animals were sacrificed. Clinical examination revealed no clinical signs or increased mortality rates attributable to SARS-CoV-2, while diagnostic tests detected only four weak PCR-positive samples, but 100% of sera were positive for SARS-CoV-2 anti-S antibodies. The phylogenetic analysis of two SARS-CoV-2 sequences from two minks and the sequence of the worker showed that they belonged to different clades. It could be therefore assumed that two distinct introductions of the virus occurred on the farm, and that the first introduction probably occurred before the start of the surveillance period. From the data collected, and especially from the detection of specific antibodies through the combination of different tests, it can be postulated that syndromic surveillance combined with genome detection by PCR may not be sufficient to achieve a diagnosis in asymptomatic animals. In particular, the serological approach, especially when using tests directed towards the S protein, may be useful for improving the traceability of virus circulation in similar environments.

Viruses. 2022 Aug 8; 14 (8): 1738.
Doi: 10.3390/v14081738.

Risks of SARS-CoV-2 transmission between free-ranging animals and captive mink in the Netherlands

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In the Netherlands, 69 of the 126 (55%) mink farms in total became infected with SARS-CoV-2 in 2020. Despite strict biosecurity measures and extensive epidemiological investigations, the main transmission route remained unclear. A better understanding of SARS-CoV-2 transmission between mink farms is of relevance for countries where mink farming is still common practice and can be used as a case study to improve future emerging disease preparedness. We assessed whether SARS-CoV-2 spilled over from mink to free-ranging animals, and whether free-ranging animals may have played a role in farm-to-farm transmission in the Netherlands. The study encompassed farm visits, farm questionnaires, expert workshops and SARS-CoV-2 RNA and antibody testing of samples from target animal species (bats, birds and free-ranging carnivores). In this study, we show that the open housing system of mink allowed access to birds, bats and most free-ranging carnivores, and that direct and indirect contact with mink was likely after entry, especially for free-ranging carnivores and birds. This allowed SARS-CoV-2 exposure to animals entering the mink farm, and subsequent infection or mechanical carriage by the target animal species. Moreover, mink can escape farms in some cases, and two SARS-CoV-2-positive mink were found outside farm premises. No other SARS-CoV-2-RNA-positive free-ranging animals were detected, suggesting there was no abundant circulation in the species tested during the study period. To investigate previous SARS-CoV-2 infections, SARS-CoV-2 antibody detection using lung extracts of carcasses was set up and validated. One tested beech marten did have SARS-CoV-2 antibodies, but the closest SARS-CoV-2-infected mink farm was outside of its home range, making infection at a mink farm unlikely. Knowing that virus exchange between different species and the formation of animal reservoirs affects SARS-CoV-2 evolution, continued vigilance and monitoring of mink farms and surrounding wildlife remains vital.

Transbound Emerg Dis. 2022 Aug 21.

Doi: 10.1111/tbed.14686. Online ahead of print.

Manifestation of SARS-CoV-2 Infections in Mink Related to Host-, Virus- and Farm-Associated Factors, The Netherlands 2020

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SARS-CoV-2 outbreaks on 69 Dutch mink farms in 2020 were studied to identify risk factors for virus introduction and transmission and to improve surveillance and containment measures. Clinical signs, laboratory test results, and epidemiological aspects were investigated, such as the date and reason of suspicion, housing, farm size and distances, human contact structure, biosecurity measures, and presence of wildlife, pets, pests, and manure management. On seven farms, extensive random sampling was performed, and age, coat color, sex, and clinical signs were recorded. Mild to severe respiratory signs and general diseases such as apathy, reduced feed intake, and increased mortality were detected on 62/69 farms. Throat swabs were more likely to result in virus detection than rectal swabs. Clinical signs differed between virus clusters and were more severe for dark-colored mink, males, and animals infected later during the year. Geographical clustering was found for one virus cluster. Shared personnel could explain some cases, but other transmission routes explaining farm-to-farm spread were not elucidated. An early warning surveillance system, strict biosecurity measures, and a (temporary) ban on mink farming and vaccinating animals and humans can contribute to reducing the risks of the virus spreading and acquisition of potential mutations relevant to human and animal health.

Viruses. 2022 Aug 11; 14 (8): 1754.
Doi: 10.3390/v14081754.

SARS-CoV-2 Outbreak on a Spanish Mink Farm: Epidemiological, Molecular, and Pathological Studies

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Farmed minks have been reported to be highly susceptible to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and may represent a risk to humans. In this study, we describe the first outbreak of SARS-CoV-2 occurred on a mink farm in Spain, between June and July 2020, involving 92,700 animals. The outbreak started shortly after some farm workers became seropositive for SARS-CoV-2. Minks showed no clinical signs compatible with SARS-CoV-2 infection throughout the outbreak. Samples from 98 minks were collected for histopathological, serological, and molecular studies. Twenty out of 98 (20.4%) minks were positive by RT-qPCR and 82 out of 92 (89%) seroconverted. This finding may reflect a rapid spread of the virus at the farm with most of the animals overcoming the infection. Additionally, SARS-CoV-2 was detected by RT-qPCR in 30% of brain samples from positive minks. Sequencing analysis showed that the mink sequences were not closely related with the other mink SARS-CoV-2 sequences available, and that this mink outbreak has its probable origin in one of the genetic variants that were prevalent in Spain during the first COVID-19 epidemic wave. Histological studies revealed bron-

chointerstitial pneumonia in some animals. Immunostaining of viral nucleocapsid was also observed in nasal turbinate tissue. Farmed minks could therefore constitute an important SARS-CoV-2 reservoir, contributing to virus spread among minks and humans. Consequently, continuous surveillance of mink farms is needed.

Front Vet Sci. 2022 Jan 21; 8: 805004.

Doi: 10.3389/fvets.2021.805004. eCollection 2021.

Ferrets are valuable models for SARS-CoV-2 research

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), resulted in an ongoing pandemic with millions of deaths worldwide. Infection of humans can be asymptomatic or result in fever, fatigue, dry cough, dyspnea, and acute respiratory distress syndrome with multiorgan failure in severe cases. The pathogenesis of COVID-19 is not fully understood, and various models employing different species are currently applied. Ferrets can be infected with SARS-CoV-2 and efficiently transmit the virus to contact animals. In contrast to hamsters, ferrets usually show mild disease and viral replication restricted to the upper airways. Most reports have used the intranasal inoculation route, while the intratracheal infection model is not well characterized. Herein, we present clinical, virological, and pathological data from young ferrets intratracheally inoculated with SARS-CoV-2. Infected animals showed no significant clinical signs, and had transient infection with peak viral RNA loads at 4 days postinfection, mild to moderate rhinitis, and pulmonary endothelialitis/vasculitis. Viral antigen was exclusively found in the respiratory epithelium of the nasal cavity, indicating a particular tropism for cells in this location. Viral antigen was associated with epithelial damage and influx of inflammatory cells, including activated neutrophils releasing neutrophil extracellular traps. Scanning electron microscopy of the nasal respiratory mucosa revealed loss of cilia, shedding, and rupture of epithelial cells. The

currently established ferret SARS-CoV-2 infection models are comparatively discussed with SARS-CoV-2 pathogenesis in mink, and the advantages and disadvantages of both species as research models for zoonotic betacoronaviruses are highlighted.

Vet Pathol. 2022 Jul; 59 (4): 661-672.

Doi: 10.1177/03009858211071012. Epub 2022 Jan 8.

IIIeA17G2R1) of *C. meleagridis* and two novel subtype families Xf and Xg of the *Cryptosporidium* mink genotype. The presence of zoonotic *C. canis* subtypes in raccoon dogs and *C. meleagridis* subtypes in foxes suggests that these fur animals might be potential reservoirs for human-pathogenic *Cryptosporidium* spp.

Front Cell Infect Microbiol. 2022 Aug 22; 12: 980917.

Doi: 10.3389/fcimb.2022.980917. eCollection 2022.

Divergent *Cryptosporidium* species and host-adapted *Cryptosporidium canis* subtypes in farmed minks, raccoon dogs and foxes in Shandong, China

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Cryptosporidium spp. are common parasitic pathogens causing diarrhea in humans and various animals. Fur animals are widely farmed in Shandong Province, China, but the prevalence and genetic identity of *Cryptosporidium* spp. in them are unclear. In this study, 1,211 fecal samples were collected from 602 minks, 310 raccoon dogs and 299 foxes on two farms in Shandong and analyzed for *Cryptosporidium* spp. by nested PCR and sequence analyses of the small subunit rRNA gene. The overall infection rate of *Cryptosporidium* spp. was 31.5% (381/1,211), with a higher infection rate in raccoon dogs (37.7%, 117/310) than in foxes (32.4%, 97/299) and minks (27.7%, 167/602). By age, the highest infection rates of *Cryptosporidium* spp. were observed in raccoon dogs of 1-2 months, minks of 5-6 months, and foxes of > 12 months. Three *Cryptosporidium* species and genotypes were detected, including *C. canis* ($n = 279$), *C. meleagridis* ($n = 65$) and *Cryptosporidium* mink genotype ($n = 37$). Among the three major host species, raccoon dogs were infected with *C. canis* only ($n = 117$), while foxes were infected with both *C. canis* ($n = 32$) and *C. meleagridis* ($n = 65$), and minks with *C. canis* ($n = 130$) and *Cryptosporidium* mink genotype ($n = 37$). Subtyping of *C. canis* by sequence analysis of the 60 kDa glycoprotein gene identified eight subtypes. They belonged to two known subtype families, XXa and XXd, and two novel subtype families XXf and XXg, with host adaptation at the subtype family level. Notably, *C. canis* from foxes was genetically distant from those in other hosts. Further subtyping analysis identified three subtypes (IIIeA21G2R1, IIIeA19G2R1 and

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Nielsen, V.H., Møller, S.H., Hansen, B.K. & Berg, P. (2007). Genotype - environment interaction in mink. *Scientifur*, 31 (3), 89.

Shirali, M., Nielsen, V.H., Møller S.H. & Jensen, J. (2015). Longitudinal analysis of residual feed intake and BW in mink using random regression with heterogeneous residual variance. *Animal*, 8 (10), 1597-1604.