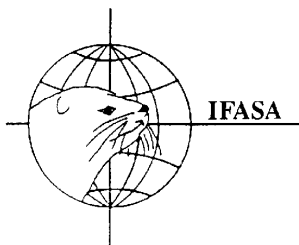
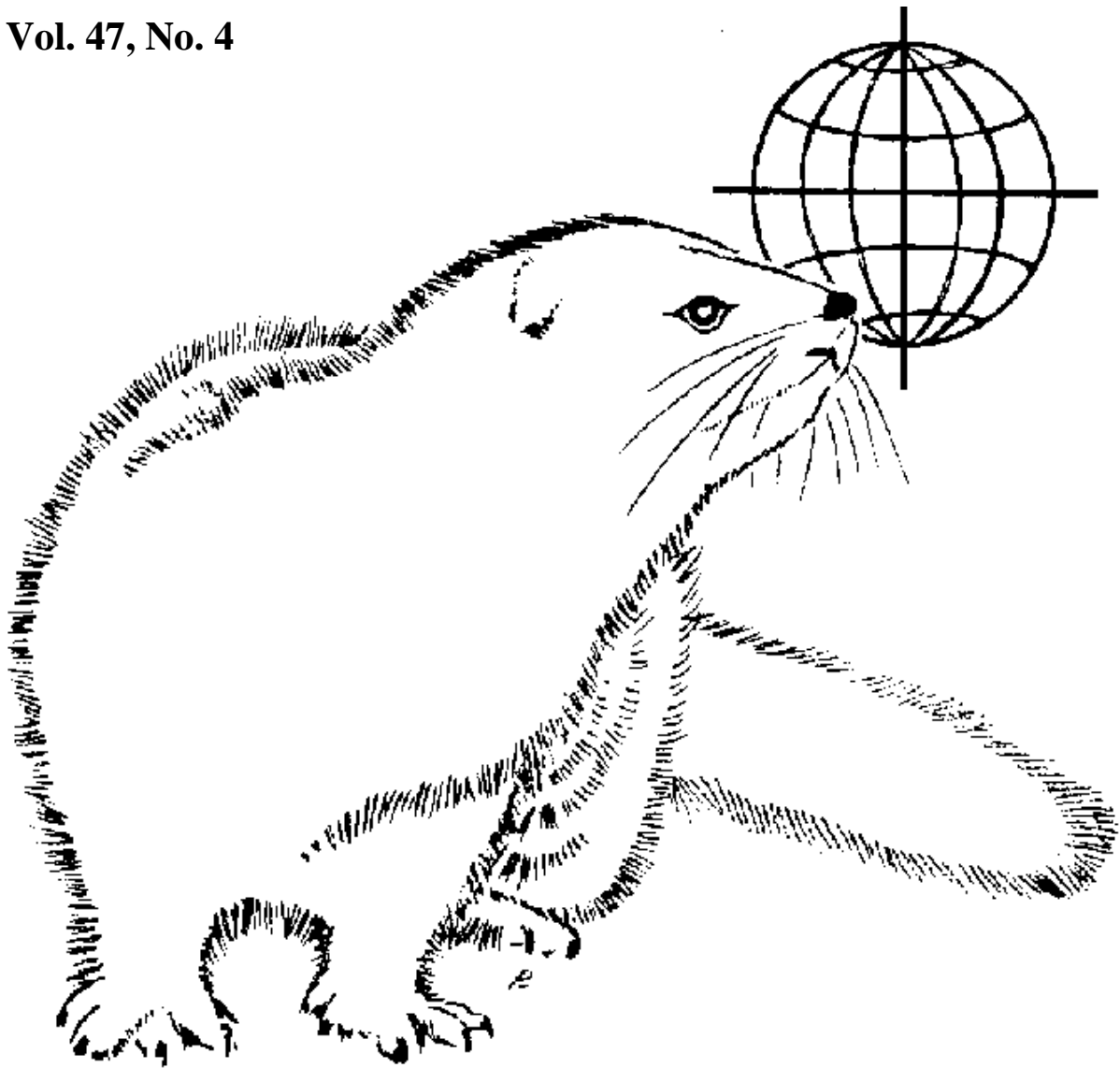


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SCIENTIFIC INFORMATION IN FUR ANIMAL PRODUCTION

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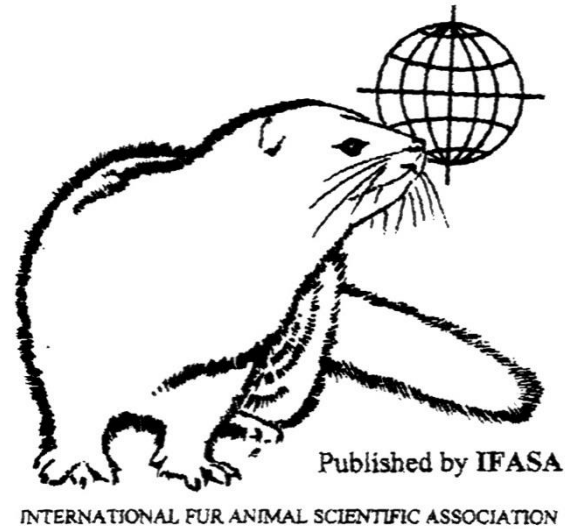
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Notes from the Editor

The Covid 19 pandemic resulted in associated infections with SARS-CoV-2 in mink populations. The populations with a high density of animals pose a risk of serious mutations in the virus and possible subsequent transmission of the mutated SARS-CoV-2 from mink to humans. This possible development has given rise to a great deal of attention and alertness about the conditions.

As reported in this issue of *Scientifur*, recent flue outbreaks - in July 2023 - caused by highly pathogenic avian influenza virus - A(H5N1) - were observed in foxes, American minks and raccoon dogs on 20 farms in Finland. Based on genetic analysis, the source of the infections was ascribed to wild birds scavenging for food at the farms. Given this, the Finnish authorities initiated culling of 120.000 animals on farms affected by the epidemic.

Similarly, the spread of influenza virus from birds, but also from humans to mink in China, is reported. Regarding the virus acquired from

birds, adaptive mammalian mutations were observed in mink. It is considered that mink may serve as an intermediate host or reservoir of emerging pathogens and even generating novel pandemic strains and enhanced surveillance of influenza viruses in mink is pointed at as a required tool for early warning of a potential pandemic.

Optimizing the welfare of Carnivora in captivity is of utmost importance. Research reported here examined the difference between the needs of more sedentary Carnivora such as mink and more itinerant species and points to the need to provide cognitive challenges for improved welfare.

Vivi Hunnicke Nielsen

Editor *Scientifur*

BREEDING, GENETICS AND REPRODUCTION

Epidemiology, pathogenesis, and diagnosis of Aleutian disease caused by Aleutian mink disease virus: A literature review with a perspective of genomic breeding for disease control in American mink (*Neogale vison*)

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Virus Res. 2023 Aug 28; 336:199208.
Doi: 10.1016/j.virusres.2023.199208.
Online ahead of print.

How was genetic diversity transferred with translocations from ex situ to in situ? A case study of the European mink translocation to Hiiumaa Island in Estonia

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Zoo Biol. 2023 Jul-Aug; 42 (4): 557-566.
Doi: 10.1002/zoo.21763. Epub 2023 Mar 15.

Metabolomic Profiling of Female Mink Serum during Early to Mid-Pregnancy to Reveal Metabolite Changes

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Mink embryos enter a period of diapause after the embryo develops into the blastocyst, and its reactivation is mainly caused by an increase in polyamine. The specific process of embryo diapause regulation and reactivation remains largely unexamined. This study aimed to identify changes in metabolites in the early pregnancy of mink by comparing and analyzing in serum metabolites up to twenty-nine days after mating. Blood samples were taken on the first day of mating, once a week until the fifth week. Metabolomic profiles of the serum samples taken during this period were analyzed by ultra-performance liquid chromatography/mass spectrometry. Multivariate statistical analyses identified differential metabolite expression at different time points in both positive and negative ion modes. The levels of dopamine, tyramine, L-phenylalanine, L-tyrosine, tyrosine, L-kynurenine, L-lysine, L-arginine, D-ornithine, and leucine changed significantly. These metabolites may be associated with the process of embryo diapause and subsequent reactivation.

Genes (Basel). 2023 Sep 4; 14 (9): 1759.
Doi: 10.3390/genes14091759.

Elaboration of massage technique for semen collection and examination of semen characteristics in chinchilla (*Chinchilla lanigera*)

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The practice of artificial insemination for the long-tailed chinchilla has not been fully elaborated to date, and existing data available regarding their reproduction properties is contradictory. Until now, the collection of semen for chinchillas has been most-commonly obtained using electro-ejaculation methods exclusively. The primary objective of this study was the development of a manual technique for semen collection which meets all animal welfare requirements. An additional aim was to determine the basic spermatological parameters, such as motility, concentration, type and ratio of morphological abnormalities and live/dead cell ratio, under typical northern-hemisphere conditions, in Hungary. Over a 3 month period, a special massage technique was developed for the study, and using this method, the sperm parameters of 46 males were subsequently analyzed weekly for a period of one year. Approximately 66% of chinchillas responded positively to this technique, with the success rate of semen-collection attempts showing no variation between seasons.

Average sperm concentration for the whole year was 935.17 million/ml using this method. Total cell motility was the highest in winter (90.3%), and the lowest in spring (84.3%). The proportion of live, intact cells were above 80% on average for the year, while the ratios of live, morphologically abnormal and dead cells were 6% and 14%, respectively. We found that midpiece abnormalities occurred in the highest proportion (0.95%-3.38%), while the head abnormalities showed the lowest ratio (0.01%-0.15%). Standard deviation among the parameters was relatively high, with the spring season proving to be the weakest in terms of sperm quality. This study has demonstrated that, semen can be successfully collected without the use of electro-ejaculation or anesthesia. Furthermore, although spermatological parameters do exhibit some fluctuation for the different times of the year, semen collected is nonetheless suitable for the purpose of artificial insemination of chinchillas at any time.

*PLoS One. 2023 Aug 31; 18 (8): e0290441.
Doi: 10.1371/journal.pone.0290441.
eCollection 2023.*

NUTRITION, FEEDING AND MANAGEMENT

Tracking pollution from fur farms using forensic paleolimnology

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Environ Pollut. 2023 Oct 15; 335: 122307.

Doi: 10.1016/j.envpol.2023.122307.

Epub 2023 Aug 3.

BEHAVIOUR AND WELFARE

The welfare problems of wide-ranging Carnivora reflect naturally itinerant lifestyles

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Carnivora with naturally small home ranges readily adjust to the evolutionarily new environment of captivity, but wider-ranging species seem prone to stress. Understanding why would advance both collection planning and enclosure design. We therefore investigated which aspects of wide-ranging lifestyles are key. We identified eight correlates of home range size (reflecting energetic needs, movement, intra-specific interactions, and itinerant lifestyles). We systematically assessed whether these correlates predict welfare better than range size *per se*, using data on captive juvenile mortality (from 13 518 individuals

across 42 species) and stereotypic route-tracing (456 individuals, 27 species). Naturally itinerant lifestyles (quantified via ratios of daily to annual travel distances) were found to confer risk, predicting greater captive juvenile losses and stereotypic time-budgets. This finding advances our understanding of the evolutionary basis for welfare problems in captive Carnivora, helping explain why naturally sedentary species (e.g. American mink) may breed even in intensive farm conditions, while others (e.g. polar bears, giant pandas) can struggle even in modern zoos and conservation breeding centres. Naturally itinerant lifestyles involve decision-making, and strategic shifts between locations, suggesting that supplying more novelty, cognitive challenge and/or opportunities for control will be effective ways to meet these animals' welfare needs in captivity.

R Soc Open Sci. 2023 Sep 6; 10 (9): 230437.
Doi: 10.1098/rsos.230437. eCollection 2023 Sep.

Domestication effect of reduced brain size is reverted when mink become feral

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A typical consequence of breeding animal species for domestication is a reduction in relative brain size. When domesticated animals escape from captivity and establish feral populations, the larger brain of the wild phenotype is usually not regained. In the American mink (*Neovison vison*), we found an exception to this rule. We confirmed the previously described reduction in relative braincase size and volume compared to their wild North American ancestors in mink bred for their fur in Poland, in a dataset of 292 skulls. We then also found a significant regrowth of these measures in well-established feral populations in Poland. Closely related, small mustelids are known for seasonal reversible changes in skull and brain size. It seems that these small mustelids are able to regain the brain size, which is adaptive for living in the wild, and flexibly respond to selection accordingly.

R Soc Open Sci. 2023 Jul 5; 10 (7): 230463.
Doi: 10.1098/rsos.230463. eCollection 2023 Jul.

HEALTH AND DISEASE

First identification of canine adenovirus 1 in mink and bioinformatics analysis of its 100 K protein

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Introduction

Animal trade favors the spreading of emerging canine adenovirus 1 (CAV-1) in mink. Because the 100K protein is not exposed to the viral surface at any stage, it can be used to differentiate the vaccine from wild virus infection. However, no related research has been conducted. This study aimed to find evidence of CAV-1 in mink and predict the character of the 100K protein in the current circulating CAV-1 strain of mink.

Method

In this experiment, the identification of CAdV-1, the phylogenetic tree, homology, and bioinformatics analysis of 100K were conducted.

Results

The results showed that the CAdV-1 was identified in the mink and that its Fiber was located in a separate branch. It was closely related to strains isolated from Norwegian Arctic fox and Red fox. 100K was located in a separate branch, which had the closest genetic relationship with skunks, porcupines, raccoons, and hedgehogs and a far genetic relationship with the strains in dogs. 100K protein is an unstable and hydrophobic protein. It had evidence of selective pressure and recombination, 1 glycosylation site, 48 phosphorylation sites, 60 dominant B cell epitopes, and 9 peptides of MHC-I and MHC-II. Its subcellular localization was mainly in the endoplasmic reticulum and mitochondria. The binding sites of 100K proteins were DBP proteins and 33K proteins.

Discussion

The stains in the mink were different from fox. The exploration of its genomic characteristics will provide us with a deeper understanding of the prevention of canine adenovirus.

Front Microbiol. 2023 Aug 17; 14: 1245581.

Doi: 10.3389/fmicb.2023.1245581.

eCollection 2023.

Genetic differences in variants of the AMD virus at the site of a disease outbreak

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Virology. 2023 Jul 28; 587: 109851.

Doi: 10.1016/j.virol.2023.109851.

Online ahead of print.

Genomic characterization and phylogenetic analysis of Aleutian mink disease virus identified in a sudden death mink case

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Comp Immunol Microbiol Infect Dis. 2023 Oct; 101: 102052.

Doi: 10.1016/j.cimid.2023.102052.

Epub 2023 Aug 27.

Application of quantitative real-time PCR to detect Mink Circovirus in minks, foxes and raccoon dogs in northern China

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Mink circovirus disease caused by *Mink Circovirus* (MiCV) is a serious infectious disease of mink that has become prevalent in recent years in China, severely affecting the reproductive performance of mink and causing significant economic losses to farms. To date, there have been few studies on MiCV, its pathogenic mechanism is not clear, and there is no effective vaccine or drug to prevent and control the disease. Therefore, it is necessary to establish a rapid and reliable molecular diagnostic method, which would aid future studies of this novel virus. In our study, we developed a sensitive and specific Taq-Man-based quantitative real-time PCR assay targeting the MiCV Cap gene. The assay showed no cross-reaction with other tested animal viruses. The assay is highly sensitive, with a detection limit of as low as 10 plasmid DNA copies and 2.38×10^{-2} pg of viral DNA. The intra and inter-assay coefficients of variation were both low. The positive detection rate of MiCV in clinical samples from minks, foxes, and raccoon dogs were 58.8% (133/226), 50.7% (72/142), and 42.2% (54/128), respectively, giving a total positive detection rate of 52.2% (259/496). Higher contamination levels were observed in samples from the environment in direct or indirect contact with animals, with a total positive detection rate of 75.1% (220/293). These epidemiological results showed that minks, foxes, and raccoon dogs had high infection rates of MiCV. This was also the first study to detect MiCV on the ground and equipment of fur-bearing animal farms. Our assay is highly sensitive and specific for the diagnosis and quantification of MiCV, and should provide a reliable real-time tool for epidemiological and pathogenetic study of MiCV infection.

Front Microbiol. 2023 Jul 31; 14:1205297.
Doi: 10.3389/fmicb.2023.1205297.
eCollection 2023.

High subtelomeric GC content in the genome of a zoonotic *Cryptosporidium* species

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Cryptosporidium canis is a zoonotic species causing cryptosporidiosis in humans in addition to its natural hosts dogs and other fur animals. To understand the genetic basis for host adaptation, we sequenced the genomes of *C. canis* from dogs, minks, and foxes and conducted a comparative genomics analysis. While the genomes of *C. canis* have similar gene contents and organisations, they (~41.0 %) and *C. felis* (39.6 %) have GC content much higher than other *Cryptosporidium* spp. (24.3-32.9 %) sequenced to date. The high GC content is mostly restricted to subtelomeric regions of the eight chromosomes. Most of these GC-balanced genes encode *Cryptosporidium*-specific proteins that have intrinsically disordered regions and are involved in host-parasite interactions. Natural selection appears to play a more important role in the evolution of codon usage in GC-balanced *C. canis*, and most of the GC-balanced genes have undergone positive selection. While the identity in whole genome sequences between the mink- and dog-derived isolates is 99.9 % (9365 SNVs), it is only 96.0 % (362 894 SNVs) between them and the fox-derived isolate. In agreement with this, the fox-derived isolate possesses more subtelomeric genes encoding invasion-related protein families. Therefore, the change in subtelomeric GC content appears to be responsible for the more GC-balanced *C. canis* genomes, and the fox-derived isolate could represent a new *Cryptosporidium* species.

Microb Genom. 2023 Jul; 9 (7): mgen001052.
Doi: 10.1099/mgen.0.001052.

Mink infection with influenza A viruses: an ignored intermediate host?

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Continuously emergence of human infection with avian influenza A virus poses persistent threat to public health, as illustrated in zoonotic H5N1/6 and

H7N9 infections. The recent surge of infection to farmed mink by multiple subtypes of avian influenza A viruses in China highlights the role of mink in the ecology of influenza in this region. Serologic studies suggested that farmed mink in China are frequently infected with prevailing human (H3N2 and H1N1/pdm) and avian (H7N9, H5N6, and H9N2) influenza A viruses. Moreover, genetic analysis from the sequences of influenza viruses from mink showed that several strains acquired mammalian adaptive mutations compared to their avian counterparts. The transmission of SARS-CoV-2 from mink to human alerts us that mink may serve as an intermediate host or reservoir of some emerging pathogens. Considering the high susceptibility to different influenza A viruses, it is possible that mink in endemic regions may play a role as a "mixing vessel" for generating novel pandemic strain. Thus, enhanced surveillance of influenza viruses in mink should be urgently implemented for early warning of potential pandemic.

One Health Adv. 2023;1 (1): 5.
Doi: 10.1186/s44280-023-00004-0.
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Highly pathogenic avian influenza A(H5N1) virus infection on multiple fur farms in the South and Central Ostrobothnia regions of Finland, July 2023

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Since mid-July 2023, an outbreak caused by highly pathogenic avian influenza A(H5N1) virus clade 2.3.4.4b genotype BB is ongoing among farmed animals in South and Central Ostrobothnia, Finland. Infections in foxes, American minks and raccoon dogs

have been confirmed on 20 farms. Genetic analysis suggests introductions from wild birds scavenging for food in farm areas. Investigations point to direct transmission between animals. While no human infections have been detected, control measures are being implemented to limit spread and human exposure.

Euro Surveill. 2023 Aug; 28 (31): 2300400.
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Mink farming poses risks for future viral pandemics

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Mink are widely farmed for fur, including intensive farming in Europe, North America, and China. Much has been written about the cruel, unnecessary, and, some say, unethical practice of fur farming. Less has been discussed regarding the dangers to human and animal health that fur farming poses, mink farming in particular. As with any intensive farming, fur farming takes place in a high-density animal environment that allows for rapid spread of viruses with pandemic potential—and for virus adaptation to animals that would be unlikely to occur in nature. This is particularly true for normally solitary, undomesticated carnivores, such as mink. Here, we argue that mink, more so than any other farmed species, pose a risk for the emergence of future disease outbreaks and the evolution of future pandemics.

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Mathematical Modeling of SARS-CoV-2 Transmission between Minks and Humans Considering New Variants and Mink Culling

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We formulated and studied mathematical models to investigate control strategies for the outbreak of the disease caused by SARS-CoV-2, considering the transmission between humans and minks. Two novel models, namely SEIR and SVEIR, are proposed to incorporate human-to-human, human-to-mink, and mink-to-human transmission. We derive formulas for the reproduction number R_0 for both models using the next-generation matrix technique. We fitted our model to the daily number of COVID-19-infected cases among humans in Denmark as an example, and using the best-fit parameters, we calculated the values of R_0 to be 1.58432 and 1.71852 for the two-strain and single-strain models, respectively. Numerical simulations are conducted to investigate the impact of control measures, such as mink culling or vaccination strategies, on the number of infected cases in both humans and minks. Additionally, we investigated the possibility of the mutated virus in minks being transmitted to humans. Our results indicate that to control the disease and spread of SARS-CoV-2 mutant strains among humans and minks, we must minimize the transmission and contact rates between mink farmers and other humans by quarantining such individuals. In order to reduce the virus mutation rate in minks, culling or vaccination strategies for infected mink farms must also be implemented. These measures are essential in managing the spread of SARS-CoV-2 and its variants, protecting public health, and mitigating the potential risks associated with human-to-mink transmission.

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Risk assessment of SARS-CoV-2 replicating and evolving in animals

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Analysis of RBD-ACE2 interactions in livestock species as a factor in the spread of SARS-CoV-2 among animals

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The high mutation rate of SARS-CoV-2, which has led to the emergence of a number of virus variants, creates risks of transmission from humans to animal species and the emergence of new animal reservoirs of COVID-19. This study aimed to identify animal species among livestock susceptible to infection and develop an approach that would be possible to use for assessing the hazards caused by new SARS-CoV-2 variants for animals. Bioinformatic analysis was used to evaluate the ability of receptor-binding domains (RBDs) of different SARS-CoV-2 variants to interact with ACE2 receptors of livestock species. The results indicated that the stability of RBD-ACE2 complexes

depends on both amino acid residues in the ACE2 sequences of animal species and on mutations in the RBDs of SARS-CoV-2 variants, with the residues in the interface of the RBD-ACE2 complex being the most important. All studied SARS-CoV-2 variants had high affinity for ferret and American mink receptors, while the affinity for horse, donkey, and bird species' receptors significantly increased in the highly mutated Omicron variant. Hazards that future SARS-CoV-2 variants may acquire specificity to new animal species remain high given the mutability of the virus. The continued use and expansion of the bioinformatic approach presented in this study may be relevant for monitoring transmission risks and preventing the emergence of new reservoirs of COVID-19 among animals.

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Investigations into SARS-CoV-2 and other coronaviruses on mink farms in France late in the first year of the COVID-19 pandemic

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Soon after the beginning of the COVID-19 pandemic in early 2020, the Betacoronavirus SARS-CoV-2 infection of several mink farms breeding American minks (*Neovison vison*) for fur was detected in various European countries. The risk of a new reservoir being formed and of a reverse zoonosis from minks quickly became a major concern. The aim of this study was to investigate the four French mink farms to see whether SARS-CoV-2 was circulating there in late 2020. The investigations took place during the slaughtering period, thus facilitating different types of sampling (swabs and blood). On one of the four mink farms, 96.6 % of serum samples were positive when tested with a SARS-CoV-2 ELISA coated with purified N protein recombinant antigen, and 54 out of 162 (33%) pharyngo-tracheal swabs were positive by RT-qPCR. The genetic variability among 12 SARS-CoV-2 genomes sequenced from this farm indicated the co-circulation of several lineages at the time of sampling. All the SARS-CoV-2 genomes detected were nested within the 20A clade (Nextclade), together with SARS-CoV-2 genomes from humans sampled during the same period. The percentage of SARS-CoV-2 seropositivity by ELISA varied between 0.3 and 1.1% on the other three farms. Interestingly, among these three farms, 11 pharyngo-tracheal swabs and 3 fecal pools from two farms were positive by end-point RT-PCR for an Alphacoronavirus very similar to a mink coronavirus sequence observed on Danish farms in 2015. In addition, a mink Caliciviridae was identified on one of the two farms positive for Alphacoronavirus. The clinical impact of these inapparent viral infections is not known. The co-infection of SARS-CoV-2 with other viruses on mink farms could help explain the diversity of clinical symptoms noted on different infected farms in Europe. In addition, the co-circulation of an Alphacoronavirus and SARS-CoV-2 on a mink farm would potentially increase the risk of viral recombination between alpha and betacoronaviruses as already suggested in wild and domestic animals, as well as in humans.

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Multiple genotypes infection and molecular characterization of Torque teno neovison virus: A novel Anelloviridae of mink in China

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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.