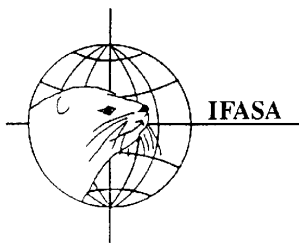
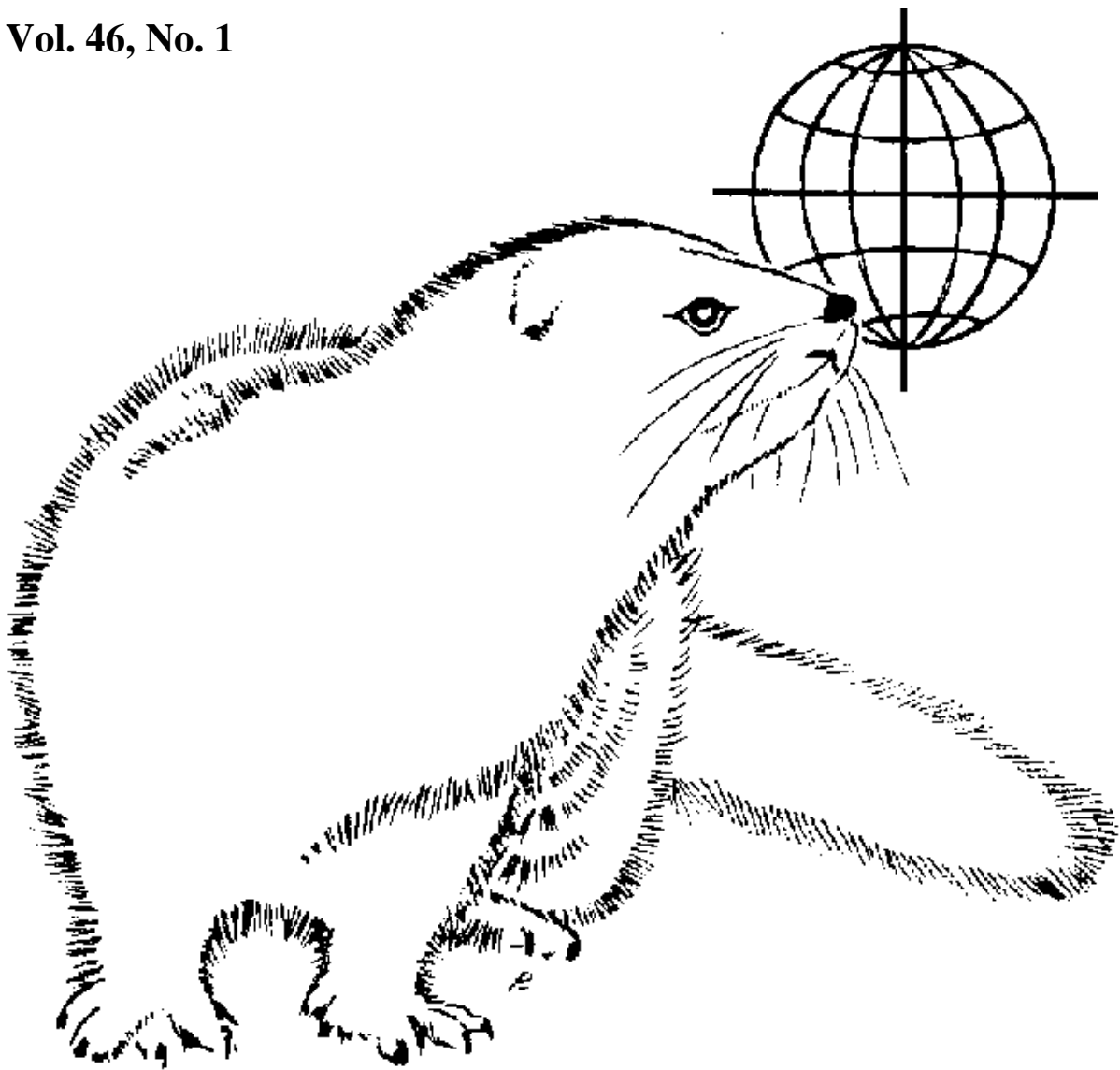


# SCIENTIFUR

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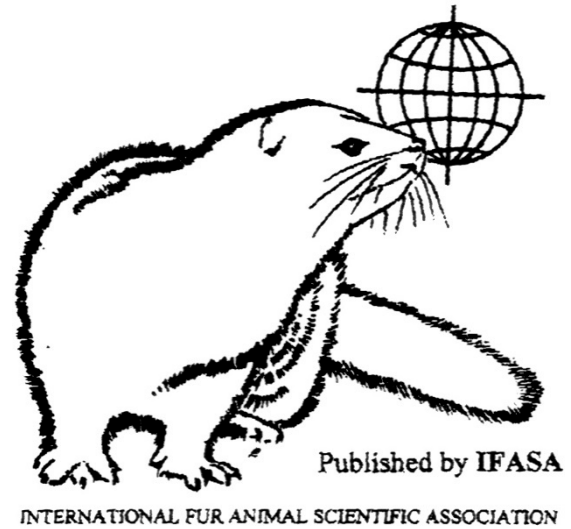
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## Notes from the Editor

A successful XII International Scientific Congress in Fur Production (IFASA Congress) was held on 24 and 25 August 2021. The IFASA Congress was originally scheduled to be held on 25-27 August 2020 in Warsaw in Poland, but this event was canceled due to the COVID-19 pandemic. As the pandemic was still ongoing in 2021, it was decided to hold the XII IFASA Congress virtually.

Following the traditional procedure for the IFASA Congress, publications were presented within behaviour and welfare, breeding, genetics and reproduction, environmental impact of fur farms and health and disease. As a result of the SARS-CoV-2 issues, a special session: “SARSCoV2 impact on fur farming” was held at the Congress. The Proceedings of the XII IFASA Congress can be found at: Congresses (ifasanet.org)

The COVID-19 pandemic has been fatal to mink production in some countries. In Denmark, all mink were culled in November 2020 and a ban of mink production has been imposed until the end of 2022 but with little chance for reestablishment. In the Netherlands, the planned phasing out of mink production in 2024

has been changed to 2021. Other countries like Finland, Russia and Canada (Nova Scotia) have had a different approach to the challenges caused by Corona virus and have rather focused on vaccination of fur animals.

Several abstracts in this issue of *Scientifur* deal with SARS-CoV-2 and its implication for fur animal production. Mink and ferrets are found to be particularly susceptible to infection with Corona virus and the receptor for SARS-CoV-2 is very similar in mink and humans. Abstracts deal with e.g. the consequences of infections with the SARS-CoV-2 on mink farms, the spread of SARS-CoV-2 to feral mink and pets, and human-animal interfaces with zoonotic transmission from humans to mink, among mink and further zoonotic transmission from mink back to humans.

Coping with Aleutian disease on mink farms is a constant challenge. The results of a recent study indicate that Aleutian mink disease virus (AMDV) antigen-based ELISA has the potential to be applied as an indicator trait for genetic selection of Aleutian disease resilient mink.

Vivi Hunnicke Nielsen

Editor *Scientifur*



**BREEDING, GENETICS AND REPRODUCTION****Genetic and phenotypic parameters for Aleutian disease tests and their correlations with pelt quality, reproductive performance, packed-cell volume, and harvest length in mink**

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Aleutian disease (AD), caused by the Aleutian mink disease virus (AMDV), is a major health concern that results in global economic losses to the mink industry. The unsatisfactory outcome of the culling strategy, immunoprophylaxis, and medical treatment in controlling AD have urged mink farmers to select AD resilient mink based on several detection tests, including enzyme-linked immunosorbent assay (ELISA), counterimmunoelectrophoresis (CIEP), and iodine agglutination test (IAT). However, the genetic analysis of these AD tests and their correlations with pelt quality, reproductive performance, packed-cell volume (PCV), and harvest length (HL) have not been investigated. In this study, data on 5,824 mink were used to estimate the genetic and phenotypic parameters of four AD tests, including two systems of ELISA, CIEP, and IAT, and their genetic and phenotypic correlations with two pelt quality, five female reproductive performance, PCV, and HL traits. Significances ( $P < 0.05$ ) of fixed effects (sex, year, dam age, and color type), covariates (age at harvest and blood sampling), and random effects (additive genetic, permanent environmental, and maternal effects) were determined under univariate models using ASReml 4.1 software. The genetic and phenotypic parameters for all traits were estimated under bivariate models using ASReml 4.1 software. Estimated heritabilities ( $\pm$ SE) were  $0.39 \pm 0.06$ ,  $0.61 \pm 0.07$ ,  $0.11 \pm 0.07$ , and  $0.26 \pm 0.05$  for AMDV antigen-based ELISA (ELISA-G), AMDV capsid protein-based ELISA, CIEP, and IAT, respectively. The ELISA-G also showed a moderate repeatability ( $0.58 \pm 0.04$ ) and had significant negative genetic correlations ( $\pm$ SE) with reproductive performance traits (from  $-0.41 \pm 0.16$  to  $-0.49 \pm 0.12$ ), PCV ( $-0.53 \pm 0.09$ ), and HL ( $-0.45 \pm 0.16$ ). These results indicated that ELISA-G had the potential to be applied as an indicator trait for genetic selection of AD resilient

mink in AD endemic ranches and therefore help mink farmers to reduce the adverse effects caused by AD.

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Doi: 10.1093/jas/skab216.

**Chromosome-level genome assembly of the Arctic fox (*Vulpes lagopus*) using PacBio sequencing and Hi-C technology**

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The Arctic fox (*Vulpes lagopus*) is the only fox species occurring in the Arctic and has adapted to its extreme climatic conditions. Currently, the molecular basis of its adaptation to the extreme climate has not been characterized. Here, we applied PacBio sequencing and chromosome structure capture technique to assemble the first *V. lagopus* genome assembly, which is assembled into chromosome fragments. The genome assembly has a total length of 2.345 Gb with a contig N50 of 31.848 Mb and a scaffold N50 of 131.537 Mb, consisting of 25 pseudochromosomal scaffolds. The *V. lagopus* genome had approximately 32.33% repeat sequences. In total, 21,278 protein-coding genes were predicted, of which 99.14% were

functionally annotated. Compared with 12 other mammals, *V. lagopus* was most closely related to *V. Vulpes* with an estimated divergence time of ~7.1 Ma. The expanded gene families and positively selected genes potentially play roles in the adaptation of *V. lagopus* to Arctic extreme environment. This high-quality assembled genome will not only promote future studies of genetic diversity and evolution in foxes and other canids but also provide important resources for conservation of Arctic species.

*Mol Ecol Resour.* 2021 Aug; 21(6): 2093-2108.  
Doi: 10.1111/1755-0998.13397. Epub 2021 Apr 21.

### Chromosomal Instability at Fragile Sites in Blue Foxes, Silver Foxes, and Their Interspecific Hybrids

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A cytogenetic assay based on fragile sites (FS) enables the identification of breaks, chromatid gaps, and deletions. In healthy individuals, the number of these instabilities remains low. Genome stability in these species is affected by Robertsonian translocations in the karyotype of the blue fox and by B chromosomes in the silver fox. The aims of the study were to characterise the karyotype of blue foxes, silver foxes, and their hybrids and to identify chromosomal fragile sites used to evaluate genome stability. The diploid number of A chromosomes in blue foxes ranged from 48 to 50, while the number of B chromosomes in silver foxes varied from one to four, with a constant number of A chromosomes ( $2n = 34$ ). In interspecific hybrids, both types of karyotypic variation were identified, with the diploid number of A chromosomes ranging from 40 to 44 and the number of B chromosomes varying from 0 to 3. The mean frequency of

FS in foxes was  $4.06 \pm 0.19$ :  $4.61 \pm 0.37$  in blue foxes,  $3.46 \pm 0.28$  in silver foxes, and  $4.12 \pm 0.22$  in hybrids. A relationship was identified between an increased number of A chromosomes in the karyotype of the hybrids and the frequency of chromosomal breaks. The FS assay was used as a biomarker for the evaluation of genomic stability in the animals in the study.

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Doi: 10.3390/ani11061743.

### Fur colour in the Arctic fox: genetic architecture and consequences for fitness

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Genome-wide association studies provide good opportunities for studying the genetic basis of adaptive traits in wild populations. Yet, previous studies often failed to identify major effect genes. In this study, we used high-density single nucleotide polymorphism and individual fitness data from a wild non-model species. Using a whole-genome approach, we identified the *MC1R* gene as the sole causal gene underlying Arctic fox *Vulpes lagopus* fur colour. Further, we showed the adaptive importance of fur colour genotypes through measures of fitness that link ecological and evolutionary processes. We found a tendency for blue foxes that are heterozygous at the fur colour locus to have higher fitness than homozygous white foxes. The effect of genotype on fitness was independent of winter duration but varied with prey availability, with the strongest effect in years of increasing rodent populations. *MC1R* is located in a genomic re-

gion with high gene density, and we discuss the potential for indirect selection through linkage and pleiotropy. Our study shows that whole-genome analyses can be successfully applied to wild species and identify major effect genes underlying adaptive traits. Furthermore, we show how this approach can be used to identify knowledge gaps in our understanding of interactions between ecology and evolution.

*Proc Biol Sci.* 2021 Sep 29; 288 (1959): 20211452.  
Doi: 10.1098/rspb.2021.1452. Epub 2021 Sep 29.

### **Fitness and fur colouration: Testing the camouflage and thermoregulation hypotheses in an Arctic mammal**

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Selection for crypsis has been recognized as an important ecological driver of animal colouration, whereas the relative importance of thermoregulation is more contentious with mixed empirical support. A potential thermal advantage of darker individuals has been observed in a wide range of animal species. Arctic animals that exhibit colour polymorphisms and undergo seasonal colour moults are interesting study subjects for testing the two alternative hypotheses: demographic performance of different colour morphs might be differentially affected by snow cover with a cryptic advantage for lighter morphs, or conversely by winter temperature with a thermal advantage for darker morphs. In this study, we explored whether camouflage and thermoregulation might explain differences in reproduction and survival between the white and blue colour morphs of the Arctic fox *Vulpes lagopus* under natural conditions. Juvenile and adult survival, breeding propensity and litter size were measured for 798 captive-bred and released or wild-born Arctic foxes monitored during an 11-year period (2007-2017) in two subpopulations in south-central Norway. We investigated the proportion of the two colour morphs and compared their demographic performance in relation to spatial variation in duration of snow cover, onset of snow season and winter temperatures. After population re-establishment, a higher proportion of blue individuals was observed among wild-born Arctic foxes compared to

the proportion of blue foxes released from the captive population. Our field study provides the first evidence for an effect of colour morph on the reproductive performance of Arctic foxes under natural conditions, with a higher breeding propensity of the blue morph compared to the white one. Performance of the two colour morphs was not differentially affected by the climatic variables, except for juvenile survival. Blue morph juveniles showed a tendency for higher survival under colder winter temperatures but lower survival under warmer temperatures compared to white morph juveniles. Overall, our findings do not consistently support predictions of the camouflage or the thermoregulation hypotheses. The higher success of blue foxes suggests an advantage of the dark morph not directly related to disruptive selection by crypsis or thermoregulation. Our results rather point to physiological adaptations and behavioural traits not necessarily connected to thermoregulation, such as stress response, immune function, sexual behaviour and aggressiveness. Our findings highlight the need to explore the potential role of genetic linkage or pleiotropy in influencing the fitness of white and blue Arctic foxes as well as other species with colour polymorphisms.

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### **Comparative transcriptome provides insights into the selection adaptation between wild and farmed foxes**

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The silver fox and blue fox are economically important fur species and were domesticated by humans from their wild counterparts, the arctic fox and red fox, respectively. Farmed foxes show obvious differences from their wild counterparts, including differences in physiology, body size, energy metabolism, and immunity. However, the molecular mechanisms underlying these differences are presently unclear. In this study, we built transcriptome libraries from multiple pooled tissues for each species of farmed fox, used RNA-seq to obtain a comprehensive dataset,

and performed selection analysis and sequence-level analyses of orthologous genes to identify the genes that may be influenced by human domestication. More than 153.3, 248.0, 81.6, and 65.8 million clean reads were obtained and assembled into a total of 118,577, 401,520, 79,900, and 186,988 unigenes with an average length range from 521 to 667 bp for AF, BF, RF, and SF, respectively. Selective pressure analysis showed that 11 and 14 positively selected genes were identified, respectively, in the two groups (AF vs. BF and RF vs. SF). Several of these genes were associated with natural immunity (*CFI* and *LRRFIP1*), protein synthesis (*GOLGA4*, *CEP19* and *SLC35A2*), and DNA damage repair (*MDC1*). Further functional enrichment analyses demonstrated that two positively selected genes (*ACO1* and *ACAD10*) were involved in metabolic process (GO:0008152,  $p$ -value = .032), representing a significant enrichment. Sequence analysis of 117 orthologous genes shared by the two groups showed that the *LEMD2*, *RRBP1*, and *IGBP1* genes might be affected by artificial selection in farmed foxes, with mutation sites located within sequences that are otherwise highly conserved across most mammals. Our results provide a valuable transcriptomic resource for future genetic studies and improvement in the assisted breeding of foxes and other farmed animals.

*Ecol Evol.* 2021 Aug 30; 11 (19): 13475-13486.  
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### **Cranial shape variation in mink: Separating two highly similar species**

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European and American minks (*Mustela lutreola* and *Neovison vison*, respectively) are very similar in their ecology, behavior, and morphology. However, the American mink is a generalist predator and seems to adapt better to anthropized environments, allowing it to outcompete the European mink in areas where it has been introduced, threatening the survival of the native species. To assess whether morphological differences may be contributing to the success of the

American mink relative to the European mink, we analyzed shape variation in the cranium of both species using 3D geometric morphometrics. A set of 38 landmarks and 107 semilandmarks was used to study shape variation between and within species, and to assess how differences in size factored into that variation. Sexual dimorphism in both size and shape was also studied. Significant differences between species were found in cranial shape, but not in size. Relative to American mink, European mink have a shorter facial region with a rounder forehead and wider orbits, a longer neurocranium with less developed crests and processes, and an antero-medially placed tympanic bullae with an anteriorly expanded cranial border. Within species, size-related sexual dimorphism is highly significant, but sexual dimorphism in shape is only significant in American mink, not in European mink. Additionally, two trends common to both species were discovered, one related to allometric changes and another to sexual size dimorphism. Shape changes related to increasing size can be subdivided into two, probably related, groups: increased muscle force and growth. The first group somewhat parallels the differences between both mink species, while the second group of traits includes an antero-dorsal expansion of the face, and the neurocranium shifting from a globous shape in small individuals to a dorsoventrally flattened ellipse in the largest ones. Finally, the sexual dimorphism trend, while also accounting for differences in muscle force, seems to be related to the observed dietary differences between males and females. Overall, differences between species and sexes, and shape changes with increasing size, seem to mainly relate to differences in masticatory-muscle volume and therefore muscle force and bite force, which, in turn, relate to a wider range of potential prey (bigger prey, tougher shells). Thus, muscle force (and dietary range) would be larger in American mink than in European mink, in males than in females, and in larger individuals than in smaller ones.

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### **Transcriptomic analysis of ovarian signaling at the emergence of the embryo from obligate diapause in the American mink (*Neovison vison*)**

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**The Effects of Prenatal Supplementation with  $\beta$ -Hydroxy- $\beta$ -Methylbutyrate and/or Alpha-Ketoglutaric Acid on the Development and Maturation of Mink Intestines Are Dependent on the Number of Pregnancies and the Sex of the Offspring**

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Prenatal and postnatal supplementation with  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB) and alpha-ketoglutaric acid (AKG) affects the development and maturation of offspring. Both substances have the potential to stimulate cell metabolism via different routes. However, parity affects development and may alter the effects of dietary supplementation. This study aimed to evaluate the effect of gestational supplementation with HMB and/or AKG to primiparous and multiparous minks on the structure and maturation of the offspring's small intestine. Primiparous and multiparous American minks (*Neovison vison*), of the standard dark brown type, were supplemented daily with HMB (0.02 g/kg b.w.) and/or AKG (0.4 g/kg b.w.) during gestation ( $n = 7$  for each treatment). Supplementation stopped when the minks gave birth. Intestine samples were collected from 8-month-old male and female offspring during autopsy and histology and histomorphometry analysis was conducted (LAEC approval no 64/2015). Gestational supplementation had a long-term effect, improving the structure of the offspring's intestine toward facilitating absorption and passage of intestinal contents. AKG supplementation affected intestinal absorption (enterocytes, villi and absorptive surface), and HMB affected intestinal peristalsis and secretion (crypts and Goblet cells). These effects were strongly dependent on parity and offspring gender. Present findings have important nutritional implications and should be considered in feeding practices and supplementation plans in animal reproduction.

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*Doi: 10.3390/ani11051468.*

### Severe asynapsis in spermatocytes of interspecific hybrids of the silver fox (*Vulpes vulpes*) and the blue fox (*Alopex lagopus*) leads to pachytene I arrest as a result of sustained H2AX $\gamma$ phosphorylation

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### Molecular Identification and Antibacterial Activity Analysis of Blue Fox (*Vulpes lagopus*) $\beta$ -Defensins 108 and 122

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The blue fox (*Vulpes lagopus*), a fur-bearing animal, is an important component of the breeding industry in China. Semen quality is a key factor for the reproductive process and the breeding effectiveness of the farmed blue fox. However, bacterial contamination in semen samples utilized in artificial fertilization is very common. The  $\beta$ -defensins, a class of important antimicrobial peptides in mammals, could protect the reproductive system of male animals from bacterial invasion, maintain the stability of the genital tract microenvironment and improve semen quality. In this study, molecular cloning and bioinformatics analysis were conducted to analyze the protein structure and function of blue fox  $\beta$ -defensin 108 (*Vulpes lagopus* beta-defensin 108, vBD108) and 122 (*Vulpes*

*lagopus* beta-defensin 122, vBD122). To evaluate the bacteriostatic effect of recombinant vBDs (*Vulpes lagopus* beta-defensins) protein, varying concentrations (0, 25, 50, 100, 200  $\mu$ g/mL) were taken to evaluate the effects on *Escherichia coli* and *Staphylococcus aureus* at different times (0, 2, 4, 6, 8, 12 h). The results showed that vBD108 and vBD122 existed in different forms in protein structure and had antibacterial activity. Both proteins, at 50  $\mu$ g/mL, had efficacious bacteriostatic activity. This study shows that recombinant vBD108 and vBD122 proteins have good antibacterial activity in vitro. This implies a potential role in improving semen quality and hygienic measures in the process of artificial insemination as an extender of semen dilution with antibacterial activity.

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### Spermatozoon head size - the main differentiating feature between spermatozoa of blue and white Arctic fox (*Vulpes lagopus*)

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Morphology and sperm morphometry, this is an important determinant of male reproductive capacity. Morphometric data may provide relevant information in studies focused on evolutionary biology, sperm quality assessment, including prediction of the potential fertility, semen cryopreservation, or the effect of reprotoxicants. The paper presents the morphometric analysis of spermatozoa from two colour morphs of Arctic fox (*Vulpes lagopus*), and attempts to determine the relationship between selected quality indicators and dimensions and shape of spermatozoa. The research material consisted of ejaculates collected once by manual stimulation from 20 one-year-old Arctic foxes (10 individuals of the blue morph and 10 of the white morph). Ejaculates were analysed for standard parameters (volume, sperm concentration, total number of spermatozoa in the ejaculate) and used for the preparation of microscopic specimens. It was found that, the dimensions of spermatozoa from Arctic foxes depend on the male colour morphs. Spermatozoa from white Arctic foxes were significantly longer (by 1.82  $\mu$ m) and had larger heads (0.32

µm longer and 0.15 µm wider) compared to spermatozoa from blue Arctic foxes ( $P < 0.05$ ). The interactions between particular sperm dimensions indicated the occurrence of gametes differing in shape. The all correlation coefficients between the morphometric traits of spermatozoa were statistically significant. Our research proved that in the blue Arctic foxes, sperm dimensions (tail length and total sperm length) can be related to the percentage of spermatozoa with primary changes (respectively:  $r = -0.68$  and  $r = -0.75$ ; at  $P < 0.05$ ). However, in the case of white Arctic foxes, these characteristics depend on the ejaculate volume (respectively:  $r = 0.65$  and  $r = 0.68$ ; at  $P < 0.05$ ).

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Doi: 10.1590/1984-3143-AR2021-0015.  
eCollection 2021.

### **A Retrospective Survey of the Abortion Outbreak Event Caused by Brucellosis at a Blue Fox Breeding Farm in Heilongjiang Province, China**

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Brucellosis is a common zoonosis in China, resulting in abortion in animals. Outbreaks of abortion in blue foxes caused by *Brucella* infection have rarely been reported. In the present study, 3-5 mL blood samples collected from the femoral veins of 10 abortuses of blue foxes were assessed by RBPT (Rose Bengal plate test) and SAT (serum tube agglutination test) to preliminarily investigate the source of infection for the clustering of abortion events at a blue fox farm in Heilongjiang Province. Screening experiments showed that all 10 blood samples were positive in the RBPT, while only eight blood samples out of the 10 were positive in the SAT. Subsequently, 10 tissue

samples (spleen, lungs, stomach contents, and after-birth) from the same 10 foxes were assessed using AMOS (acronym for *B. abortus*, *melitensis*, *ovis*, and *suis*)-PCR (polymerase chain reaction), and sequencing analysis was performed on amplification products to verify the results of the serology survey. Results showed a spectral band of ~731 bp in these samples. BLAST showed sequences of AMOS-PCR products in this study to be 100% similar ( $E = 0.0$ ) to sequences in *B. melitensis* strain from GenBank. These data preliminarily indicated that the blue fox's outbreak of abortion events was caused by brucellosis via the *B. melitensis* strain. Then 726 serum samples were tested by RBPT and SAT to determine the prevalence of brucellosis on the farm. A comprehensive epidemiological and reproductive status survey of the infected blue fox population was performed. The seropositive rate was found to be 67.90% (493/726) by RBPT and 41.32% (300/726) by SAT. The technicians had stopped feeding the foxes with chicken carcasses and instead fed them raw ground sheep organs (lungs, tracheae, placentae, and dead sheep fetuses) infected by *B. melitensis* strains, and that this change in diet caused the outbreak of abortion events. The high abortion rate (55%) and low cub survival rate (65%) were the most distinctive features of the outbreak; these factors led to severe economic losses. Feeding cooked sheep/goat offal and strict breeding management is necessary for disease prevention.

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### **NUTRITION, FEEDING AND MANAGEMENT**

#### **Effect of Vitamin A Supplementation on Growth Performance, Serum Biochemical Parameters, Intestinal Immunity Response and Gut Microbiota in American Mink (*Neovison vison*)**

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This experiment investigated the effect of vitamin A supplementation on growth, serum biochemical parameters, jejunum morphology and the microbial community in male growing-furring mink. Thirty healthy male mink were randomly assigned to three treatment groups, with 10 mink per group. Each mink was housed in an individual cage. The mink in the three groups were fed diets supplemented with vitamin A acetate at dosages of 0 (CON), 20,000 (LVitA) and 1,280,000 IU/kg (HVitA) of basal diet. A 7-day pretest period preceded a formal test period of 45 days. The results show that 20,000 IU/kg vitamin A increased the ADG, serum T-AOC and GSH-Px activities, villus height and villus height/crypt depth ratio ( $p < 0.05$ ). The mRNA expression levels of *IL-22*, *Occludin* and *ZO-1* in the jejunum of mink were significantly higher in the LVitA group than those in the CON and HVitA groups ( $p < 0.05$ ). Vitamin A supplementation increased the diversity of jejunum bacteria, decreased the ratio of *Firmicutes* to *Bacteroidetes* and increased the relative abundance of *Akkermansia*, *uncultured bacterium f Muribaculaceae*, *Allobaculum*, *Lachnospiraceae NK4A136 group*, *Rummeliibacillus* and *Parasutterella*. The comparison of potential functions also showed enrichment of glycan biosynthesis and metabolism, transport and catabolism pathways in the vitamin A supplementation groups compared with the CON group. In conclusion, these results indicate that dietary vitamin A supplementation could mediate host growth by improving intestinal development, immunity and the relative abundance of the intestinal microbiota.

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### **Fermented Rapeseed Meal as a Component of the Mink Diet (*Neovison vison*) Modulating the Gastrointestinal Tract Microbiota**

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Fermented rapeseed meal (FRSM) was used in the diet of American mink (*Neovison vison*). An advantage of this product is its prebiotic and functional properties, which can modify the bacterial microbiota of the GIT. A control group and three experimental groups were formed, with 60 animals in each group. The control group received a basal diet and the experimental groups received a diet with a 2%, 4% or 6% of FRSM as a replacement of extruded wheat. *Bacillus subtilis* strain 87Y was used to ferment the rapeseed meal (RSM). The study was conducted on mink from the age of 16-17 weeks until slaughter. Changes in the microbiota were analysed in samples of the animals' faeces and intestinal contents. The analyses included determination of the total number of bacteria and fungi, the number of coliforms and *Escherichia coli*, the total number of anaerobic *Clostridium perfringens*, and the presence of *Salmonella* spp. In animals receiving 4% and 6% FRSM (groups II and III), the content of microscopic fungi and the number of *C. perfringens* bacteria was significantly ( $p \leq 0.05$ ) lower than in the animals from the control group (group 0). A decrease in *E. coli* was observed in all experimental groups (I, II and III), although these differences were not statistically significant. The inclusion of FRSM in the feed ration did not affect the number of lactic acid intestinal bacteria. Analysis of the results obtained from the stool samples showed that the inclusion of FRSM in the ration did not significantly affect the number of microorganisms in each group. However, as in the case of the intestinal contents, in these samples there was a decrease in the total number of *C. perfringens* in the experimental groups (I, II and III), with a simultaneous increase in the number of mesophilic bacteria in relation to the control. There was no detection of *Salmonella* bacteria in any of the analysed material.

*Animals (Basel)*. 2021 May 8; 11 (5): 1337.  
Doi: 10.3390/ani11051337.

**Effects of health related farm-level factors on skin size and quality in commercial mink (*Neovison vison*) production**

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**BEHAVIOUR AND WELFARE**

**Animal Transmission of SARS-CoV-2 and the Welfare of Animals during the COVID-19 Pandemic**

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The accelerated pace of research into Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) necessitates periodic summaries of current research. The present paper reviews virus susceptibilities in species with frequent human contact, and factors that are best predictors of virus susceptibility. Species reviewed were those in contact with humans through entertainment, pet, or agricultural trades, and for whom reports (either anecdotal or published) ex-

ist regarding the SARS-CoV-2 virus and/or the resulting disease state COVID-19. Available literature was searched using an artificial intelligence (AI)-assisted engine, as well as via common databases, such as Web of Science and Medline. The present review focuses on susceptibility and transmissibility of SARS-CoV-2, and polymorphisms in transmembrane protease serine 2 (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2) that contribute to species differences. Dogs and pigs appear to have low susceptibility, while ferrets, mink, some hamster species, cats, and nonhuman primates (particularly Old World species) have high susceptibility. Precautions may therefore be warranted in interactions with such species, and more selectivity practiced when choosing appropriate species to serve as models for research.

*Animals (Basel)*. 2021 Jul 8; 11 (7): 2044.

Doi: 10.3390/ani11072044.

**Surrounded by challenges: The simulated presence of competitors and predators modulates perianal secretion marking behaviour in the European mink (*Mustela lutreola*)**

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**Faecal Cortisol Metabolites as an Indicator of Adrenocortical Activity in Farmed Blue Foxes**

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Welfare studies of blue foxes would benefit from a measurement of faecal cortisol metabolites (FCMs) as a non-invasive, physiological stress parameter reflecting hypothalamus-pituitary-adrenal (HPA) axis activity. Before implementation, a species-specific validation of such a method is required. Therefore, we conducted a physiological validation of an enzyme immunoassay (EIA) to measure FCMs in blue foxes. Twenty individuals (nine males and eleven females) were injected with synthetic adrenocorticotrophic hormone (ACTH) and faecal samples were collected every third h for two days. The FCM baseline levels were assessed based on the first sampling day (control period, 144 samples), followed by the ACTH injection and the second day of sampling (treatment period, 122 samples). FCMs were analysed with a 5 $\alpha$ -pregnane-3 $\beta$ ,11 $\beta$ ,21-triol-20-one EIA. We compared the estimated mean FCM concentrations of the treatment samples to the baseline average. All samples for the two periods were collected at the same time of the day, which enabled to test the data also with an hourly pairwise comparison. With the two statistical approaches, we tested whether a possible diurnal fluctuation in the FCM concentrations affected the interpretation of the results. Compared to the baseline levels, both approaches showed 2.4-3.2 times higher concentrations on time points sampled 8-14 h after the ACTH injection ( $p < 0.05$ ). The estimated FCM concentrations also fluctuated slightly within the control period ( $p < 0.01$ ). Inter-individual variations in FCM levels were marked, which highlights the importance of having a sufficient number of animals in experiments utilising FCMs. The sampling intervals of 3 h enabled forming of informative FCM curves. Taken together, this study proves that FCM analysis with a 5 $\alpha$ -pregnane-3 $\beta$ ,11 $\beta$ ,21-triol-20-one EIA is a valid measurement of adrenocortical activity in the farmed blue foxes. Therefore, it can be utilised as a non-invasive stress indicator in future animal welfare studies of the species.

*Animals (Basel)*. 2021 Sep 7; 11 (9): 2631.  
Doi: 10.3390/ani11092631.

## HEALTH AND DISEASE

### AMDV Vaccine: Challenges and Perspectives

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Aleutian mink disease virus (AMDV) is known to cause the most significant disease in the mink industry. It is globally widespread and manifested as a deadly plasmacytosis and hyperglobulinemia. So far, measures to control the viral spread have been limited to manual serological testing for AMDV-positive mink. Further, due to the persistent nature of this virus, attempts to eradicate Aleutian disease (AD) have largely failed. Therefore, effective strategies to control the viral spread are of crucial importance for wildlife protection. One potentially key tool in the fight against this disease is by the immunization of mink against AMDV. Throughout many years, several researchers have tried to develop AMDV vaccines and demonstrated varying degrees of protection in mink by those vaccines. Despite these attempts, there are currently no vaccines available against AMDV, allowing the continuation of the spread of Aleutian disease. Herein, we summarize previous AMDV immunization attempts in mink as well as other preventative measures with the purpose to shed light on future studies designing such a potentially crucial preventative tool against Aleutian disease.

*Viruses*. 2021 Sep 14; 13 (9): 1833.

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### Seroprevalence and Molecular Epidemiology of Aleutian Disease in Various Countries during 1972-2021: A Review and Meta-Analysis

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Aleutian disease (AD) poses a serious threat to both free-ranging and farmed mink around the world. The disease is caused by the Aleutian mink disease virus (AMDV), which also poses a health risk for other members of the family Mustelidae, including wild mink, weasels, badgers and other animal species. This article analyses the seroprevalence of AMDV infections in mink and other species around the world, and reviews recent knowledge relating to the molecular epidemiology of the AMDV. Depending on the applied diagnostic technique and the country, the prevalence of anti-AMDV antibodies or AMDV DNA was established at 21.60-100.00% in farmed American mink, 0.00-93.30% in free-ranging American mink and 0.00-25.00% in European mink. Anti-AMDV antibodies or AMDV DNA were also detected in other free-living fur-bearing animals in Europe and Canada, where their prevalence was determined at 0.00-32.00% and 0.00-70.50%, respectively. This may indicate a potential threat to various animal species. AMDV strains are not clustered into genotypes based on the geographic origin, year of isolation or pathogenicity. The isolates that were identified on mink farms around the world originated from North America because American mink were introduced to Europe and Asia for breeding purposes and to restock natural populations.

*Animals (Basel)*. 2021 Oct 15; 11 (10): 2975.  
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### **Assessment of Aleutian mink disease virus (AMDV) prevalence in feral American mink in Iceland. Case study of a pending epizootiological concern in Europe**

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### **Background**

Recurring escapes or deliberate releases and subsequent infiltration or establishment of feral populations by individuals from fur farms have been commonly noted since the beginning of fur industry expansion. Once animals have invaded ecosystems adjacent to source farms escapees can change the demography of the feral populations through hybridization, outbreeding depression, competition and spreading of various pathogens which can decimate wild populations. In our study, we aimed to assess spread of Aleutian mink disease virus (AMDV) in the feral population of American mink (*Neovison vison*) in Iceland. The additional objective was to elucidate whether basic morpho-anatomical parameters (*i.e.*, Fulton's condition factor or spleen to body weight ratio) might be used as a preliminary indicator of AMDV infection.

### **Methods**

American mink ( $n = 164$ ) were captured by professional hunters in 8 regions of Iceland. The detection of AMDV in the spleen of male and female individuals was based on PCR amplification of an NS1 gene fragment.

### **Results**

We confirmed AMDV presence in 23.8% ( $n = 39$ ) of collected samples with no significant difference in infection rate between males and females. Additionally, we revealed that the prevalence of virus in the feral population was higher closer to fur farms. However, the countrywide prevalence and direction of AMDV distribution needs to be further investigated. Comparison of condition indices in non-infected and infected animals showed significant deterioration of body and spleen parameters in the latter group. Therefore, the application of basic measurements of the American mink may be used to evaluate the health status of individuals in terms of pathogen infection.

### **Conclusions**

The study shed a new light on prevalence and distribution of AMDV in the feral population of American mink in Iceland and the results might be successfully applied to develop models to infer dynamics of various pathogens, even those latently transmitted by disease-free animals.

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### **Diversity and transmission of Aleutian mink disease virus in feral and farmed American mink and native mustelids**

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Aleutian mink disease virus (AMDV), which causes Aleutian disease, is widely spread both in farmed mink and wild mustelids. However, only limited data are available on the role of wild animals in AMDV transmission and spread. Our aim was to shed light on AMDV transmission among wild mustelids and estimate the effect of intense farming practices on the virus circulation by studying AMDV prevalence and genetic diversity among wild mustelids in Poland. We compared AMDV seroprevalence and proportion of PCR-positive individuals in American mink, polecats, otters, stone martens, and pine martens and used the phylogenetic analysis of the NS1 region to study transmission. In addition, we used a metagenomic approach to sequence complete AMDV genomes from tissue samples. In eastern Poland, AMDV seroprevalence in wild mustelids varied from 22 per cent in otters to 62 per cent and 64 per cent in stone martens and feral mink, respectively. All studied antibody-positive mink were also PCR positive, whereas only 10, 15, and 18 per cent of antibody-positive polecats, pine martens, and stone martens, respectively, were PCR positive, suggesting lower virus persistence among these animal species as compared to feral mink. In phylogenetic analysis, most sequences from feral mink formed region-specific clusters that have most likely emerged through multiple introductions of AMDV to feral mink population over decades. However, virus spread between regions was also observed. Virus sequences derived from farmed and

wild animals formed separate subclusters in the phylogenetic tree, and no signs of recent virus transmission between farmed and wild animals were observed despite the frequent inflow of farmed mink escapees to wild populations. These results provide new information about the role of different mustelid species in AMDV transmission and about virus circulation among the wild mustelids. In addition, we pinpoint gaps of knowledge, where more studies are needed to achieve a comprehensive picture of AMDV transmission.

*Virus Evol. 2021 Aug 28; 7 (2): veab075.*  
*Doi: 10.1093/ve/veab075. eCollection 2021.*

### **Impact of viral features, host jumps and phylogeography on the rapid evolution of Aleutian mink disease virus (AMDV)**

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Aleutian mink disease virus (AMDV) is one the most relevant pathogens of domestic mink, where it can cause significant economic losses, and wild species, which are considered a threat to mink farms. Despite their relevance, many aspects of the origin, evolution, and geographic and host spreading patterns of AMDV have never been investigated on a global scale using a comprehensive biostatistical approach. The present study, benefitting from a large dataset of sequences collected worldwide and several phylogenetic-based approaches, demonstrates the ancient origin of AMDV and its broad, unconstrained circulation from the initial intercontinental spread to the massive among-country circulation, especially within Europe, combined with local persistence and evolution. Clear expansion of the viral population size occurred over time until more effective control measures started to be applied. The role of frequent changes in epidemiological niches, including different hosts, in driving the high nucleotide and amino acid evolutionary rates was also explored by comparing the strengths of selective pressures acting on different populations. The obtained results suggest that



the viral passage among locations and between wild and domesticated animals poses a double threat to farm profitability and animal welfare and health, which is particularly relevant for endangered species. Therefore, further efforts must be made to limit viral circulation and to refine our knowledge of factors enhancing AMDV spread, particularly at the wild-domestic interface.

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### RT-qPCR assay for detection of mink astrovirus in outbreaks of diarrhea on Danish mink farms

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Diarrhea in mink kits is a major cause of disease and mortality in the mink production. The etiology remains unknown in most outbreaks due to a lack of diagnostic assays. In the current study we present an RT-qPCR method to detect mink astrovirus in fecal samples from mink kits with diarrhea. All sampled animals were classified based on age and pathological evaluation as having pre-weaning diarrhea, diarrhea in the growth period or as having no macroscopic signs of diarrhea. Fecal samples were analyzed for MiAstV with RT-qPCR, next generation sequencing and electron microscopy in parallel. Mink astrovirus was detected with RT-qPCR in 92 out of 203 samples. This detection was confirmed by next generation sequencing in a high proportion of samples (22/27), and by visualization of astrovirus particles with EM in some of the samples. Mink astrovirus was highly prevalent (68%) among kits in the outbreaks of pre-weaning diarrhea, in particular outbreaks from May, while less prevalent in outbreaks in June. Mink astrovirus was detected in outbreaks of diarrhea in the growth period, though in a much lesser extent than in the pre-weaning period. The role of

mink astrovirus in the diarrhea disease complex of mink remain to be investigated, and for that purpose this sensitive and robust RT-qPCR can be a valuable tool in the future.

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### Detection of mink astrovirus in Poland and further phylogenetic comparison with other European and Canadian astroviruses

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Mink astrovirus infection remains a poorly understood disease entity, and the aetiological agent itself causes disease with a heterogeneous course, including gastrointestinal and neurological symptoms. This paper presents cases of astrovirus infection in mink from continental Europe. RNA was isolated from the brains and intestines of animals showing symptoms typical of shaking mink syndrome (n = 6). RT-PCR was used to amplify astrovirus genetic material, and the reaction products were separated on a 1% agarose gel. The specificity of the reaction was confirmed by sequencing fragment coding RdRP protein (length of sequencing product 170 bp) from all samples. The presence of astrovirus RNA was detected in each of the samples tested. Sequencing and bioinformatic analysis indicated the presence of the same variant of the virus in all samples. Comparison of the variant with the sequences available in bioinformatics databases confirmed that the Polish isolates form a separate clade, closely related to Danish isolates. The dissimilarity of the Polish variant to those isolated in other countries ranged from 2.4% (in relation to Danish isolates) to 7.1% (in relation to Canadian isolates). Phylogenetic relationships between variants appear to be associated with the geographic distances

between them. To our knowledge, this work describes the first results on the molecular epidemiology of MAstV in continental Europe. The detection of MAstV in Central Europe indicates the need for further research to broaden our understanding of the molecular epidemiology of MAstV in Europe.

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### **Molecular survey of Bartonella spp. and haemoplasmas in American minks (*Neovison vison*)**

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The aim of this study was to perform a molecular survey and characterize Bartonella spp. and haemotropic Mycoplasma (haemoplasmas) in invasive American minks (*Neovison vison*) from Southern Chile. Additionally, we addressed risk factors for positivity in both groups of agents. Blood and/or tissue samples from 246 minks were analysed by qPCR targeting the nuoG gene for Bartonella spp. and conventional (c)PCR for 16S rRNA for haemotropic Mycoplasma spp. nuoG qPCR-positive Bartonella spp. samples were submitted to cPCR assays (ITS, ribC, gltA, rpoB, pap-31 and ftsZ genes) to perform phylogenetic inferences. Haemotropic Mycoplasma spp. 16S-positive samples were further amplified by cPCR targeting RNaseP gene (160-210 bp) and by two overlapping 16S rRNA cPCR assays to amplify a larger portion of the gene (1,200bp) for phylogenetics. Bartonella DNA was detected in 8.9% of minks (22/246). Out of 22 nuoG qPCR-positive samples, one and two showed positive results in cPCR assays based on ITS and ribC, respectively. Consistent se-

quencing results were obtained for only one ITS sample (464 bp sequence), which shared 99.6% identity with *B. clarridgeiae*. Two per cent of minks (5/246) were positive for 16S rRNA haemotropic Mycoplasma-cPCR assay. Two concatenated sequences of 16S rRNA (1,176 and 1,230 bp) were obtained: one sample shared 97.87% identity with haemotropic Mycoplasma sp. from a wild rodent, and the other 96.49% identity with 'Candidatus Mycoplasma haematoparvum' from a dog. All BLAST results were supported by phylogenetic analysis. One haemoplasma RNase P sequence shared 94.86% identity with Mycoplasma haemofelis from a cat. No risk factors for PCR positivity were identified. In a nutshell, Bartonella clarridgeiae and a potentially novel haemoplasma closely related to haemoplasmas previously reported in rodents, dogs, domestic and wild cats were described for the first time in American minks.

*Transbound Emerg Dis*. 2021 Jul; 68 (4): 2094-2110.

Doi: 10.1111/tbed.13857. Epub 2020 Oct 16.

### **A highly virulent canine distemper virus strain isolated from vaccinated mink in China**

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An outbreak of canine distemper in 2017 in mink breeding farms (Shandong province, China) caused severe pneumonia, hardened footpads, and death in more than 5000 vaccinated animals. Sequencing of the hemagglutinin and fusion protein genes from the WH2 canine distemper virus (CDV) strain we isolated from the infected minks were clustered into the recently isolated CDV Asia-1 genotype group. The WH2 strain was distinct from the current vaccine

strains, containing a novel potential N-glycosylation site in its hemagglutinin protein. It also contained amino acid mutations in the fusion protein gene (I87N, T110P and L386I), and the T110P mutation results in N-glycosylation site silencing. WH2 was highly virulent in both unvaccinated and vaccinated animals in our pathogenesis experiments. Immunohistochemistry results revealed positive staining of different organs in unvaccinated and vaccinated animals. The serum in vitro neutralizing antibody titers for the vaccinated mink group and a dog were higher for the WH2 strain than those of the HNly150520B strain (isolated from a dog). These findings indicate that the current commercial vaccines provide incomplete protection against WH2 challenge infections. Thus, a new vaccine strain is urgently needed to protect against variant CDV strains.

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### Detection of Extended-Spectrum Beta-Lactamase-Producing and Carbapenem-Resistant Bacteria from Mink Feces and Feed in the United States

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Antibiotic-resistant infections caused by extended-spectrum  $\beta$ -lactamases (ESBLs) and carbapenemases are increasing worldwide. Bacteria resistant to extended-spectrum cephalosporins and last resort carbapenems have been reported from food animals and their environments. Other concentrated nonfood-producing animals such as mink farming can be a reservoir of bacteria resistant to these critically important antibiotics. The objective of this study was to determine the prevalence of ESBL-producing bacteria and carbapenem-resistant (CR) bacteria from mink fecal ( $n = 42$ ) and feed ( $n = 8$ ) samples obtained from a commercial mink farm in the United States. The most prevalent ESBL-producing bacteria identified from the fecal samples were *Escherichia coli* (93%), *Klebsiella pneumoniae* (76%), and *Proteus* species

(88%). *E. coli* (100%) and *K. pneumoniae* (75%) were also the most prevalent ESBL-producing bacteria identified from feed samples. All ESBL *E. coli* isolates were resistant to penicillin and most cephem beta-lactam antibiotics. Among the ESBL *E. coli* isolates, co-resistance was observed to ciprofloxacin (33%) and gentamicin (28%) indicating multidrug resistance. ESBL *E. coli* isolates predominantly carried *bla*<sub>CTX-M-14</sub> and *bla*<sub>CTX-M-15</sub> genes. Although all feed *K. pneumoniae* isolates carried *bla*<sub>CTX-M-9</sub>, all fecal *K. pneumoniae* isolates carried *bla*<sub>SHV</sub>. CR *Pseudomonas* species (7%), *Hafnia alvei* (24%), and *Myroides odoratimimus* (9.5%) were detected from fecal samples. *H. alvei* (37.5%) was the only CR bacteria detected from the feed samples. All CR isolates were polymerase chain reaction negative for the tested carbapenemases that are commonly reported, which may indicate intrinsic rather than acquired resistance. This study indicates that mink production can be a reservoir for bacteria resistant to the highest priority critically important antibiotics for human health.

*Foodborne Pathog Dis.* 2021 Jul; 18 (7): 497-505.  
Doi: 10.1089/fpd.2020.2898. Epub 2021 May 11.

### An outbreak of SARS-CoV-2 with high mortality in mink (Neovison vison) on multiple Utah farms

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The breadth of animal hosts that are susceptible to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and may serve as reservoirs for continued viral transmission are not known entirely. In August 2020, an outbreak of SARS-CoV-2 occurred on five mink farms in Utah and was associated with high mink mortality (35-55% of adult mink) and rapid viral transmission between animals. The premise and clinical disease information, pathology, molecular characterization, and tissue distribution of vi-

rus within infected mink during the early phase of the outbreak are provided. Infection spread rapidly between independently housed animals and farms, and caused severe respiratory disease and death. Disease indicators were most notably sudden death, anorexia, and increased respiratory effort. Gross pathology examination revealed severe pulmonary congestion and edema. Microscopically there was pulmonary edema with moderate vasculitis, perivascularitis, and fibrinous interstitial pneumonia. Reverse transcriptase polymerase chain reaction (RT-PCR) of tissues collected at necropsy demonstrated the presence of SARS-CoV-2 viral RNA in multiple organs including nasal turbinates, lung, tracheobronchial lymph node, epithelial surfaces, and others. Localization of viral RNA by in situ hybridization revealed a more localized infection, particularly of the upper respiratory tract. Whole genome sequencing from multiple mink was consistent with published SARS-CoV-2 genomes with few polymorphisms. The Utah mink SARS-CoV-2 strains fell into Clade GH, which is unique among mink and other animal strains sequenced to date. While sharing the N501T mutation which is common in mink, the Utah strains did not share other spike RBD mutations Y453F and F486L found in nearly all mink from the United States. Mink in the outbreak reported herein had high levels of SARS-CoV-2 in the upper respiratory tract associated with symptomatic respiratory disease and death.

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**Severe Acute Respiratory Syndrome Coronavirus 2 in Farmed Mink (*Neovison vison*), Poland**  
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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiologic agent of coronavirus disease and has been spreading worldwide since December 2019. The virus can infect different animal species under experimental conditions, and mink on fur farms in Europe and other areas are susceptible to SARS-CoV-2 infection. We investigated SARS-CoV-2 infection in 91 mink from a farm in northern

Poland. Using reverse transcription PCR, antigen detection, and next-generation sequencing, we confirmed that 15 animals were positive for SARS-CoV-2. We verified this finding by sequencing full viral genomes and confirmed a virus variant that has sporadic mutations through the full genome sequence in the spike protein (G75V and C1247F). We were unable to find other SARS-CoV-2 sequences simultaneously containing these 2 mutations. Country-scale monitoring by veterinary inspection should be implemented to detect SARS-CoV-2 in other mink farms.

*Emerging Infectious Diseases* 2021 Sep; 27 (9): 2333-2339.  
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**SARS-CoV-2 mutations acquired in mink reduce antibody-mediated neutralization**

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### **Infection, recovery and re-infection of farmed mink with SARS-CoV-2**

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Mink, on a farm with about 15,000 animals, became infected with SARS-CoV-2. Over 75% of tested animals were positive for SARS-CoV-2 RNA in throat swabs and 100% of tested animals were seropositive. The virus responsible had a deletion of nucleotides encoding residues H69 and V70 within the spike protein gene as well as the A22920T mutation, resulting in the Y453F substitution within this protein, seen previously in mink. The infected mink recovered and after free-testing of 300 mink (a level giving 93% confidence of detecting a 1% prevalence), the animals remained seropositive. During further follow-up studies, after a period of more than 2 months without any virus detection, over 75% of tested animals again scored positive for SARS-CoV-2 RNA. Whole genome sequencing showed that the viruses circulating during this re-infection were most closely related to those identified in the first outbreak on this farm but additional sequence changes had occurred. Animals had much higher levels of anti-SARS-CoV-2 antibodies in serum samples after the second round of infection than at free-testing or during recovery from

initial infection, consistent with a boosted immune response. Thus, it was concluded that following recovery from an initial infection, seropositive mink were readily re-infected by SARS-CoV-2.

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### **Review: SARS-CoV-2 infection in farmed minks - an overview of current knowledge on occurrence, disease and epidemiology**

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### **Outbreaks of SARS-CoV-2 in naturally infected mink farms: Impact, transmission dynamics, genetic patterns, and environmental contamination**

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SARS-CoV-2 infection outbreaks in minks have serious implications associated with animal health and welfare, and public health. In two naturally infected mink farms (A and B) located in Greece, we investigated the outbreaks and assessed parameters associated with virus transmission, immunity, pathology, and environmental contamination. Symptoms ranged from anorexia and mild depression to respiratory signs of varying intensity. Although the farms were at different breeding stages, mortality was similarly high (8.4% and 10.0%). The viral strains belonged to lineages B.1.1.218 and B.1.1.305, possessing the mink-specific S-Y453F substitution. Lung histopathology identified necrosis of smooth muscle and connective tissue elements of vascular walls, and vasculitis as the main early key events of the acute SARS-CoV-2-induced broncho-interstitial pneumonia. Molecular investigation in two dead minks indicated a consistently higher (0.3-1.3 log<sub>10</sub> RNA copies/g) viral load in organs of the male mink compared to the female. In farm A, the infected farmers were responsible for the significant initial infection of 229 out of 1,000 handled minks, suggesting a very efficient human-to-mink transmission. Subsequent infections across the sheds wherein animals were being housed occurred due to airborne transmission. Based on a R<sub>0</sub> of 2.90 and a growth rate equal to 0.293, the generation time was estimated to be 3.6 days, indicative of the massive SARS-CoV-2 dispersal among minks. After the end of the outbreaks, a similar percentage of animals were immune in the two farms (93.0% and 93.3%), preventing further virus transmission whereas, viral RNA was detected in samples collected from shed surfaces and air. Consequently, strict biosecurity is imperative during the occurrence of clinical signs. Environmental viral load monitoring, in conjunction with NGS should be adopted in mink farm surveillance. The minimum proportion of minks that need to be immunized to avoid outbreaks

in farms was calculated at 65.5%, which is important for future vaccination campaigns.

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### **Wild American mink (*Neovison vison*) may pose a COVID-19 threat**

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Over the past year, farmed American mink (*Neovison vison*) have gained notoriety due to their unfortunate susceptibility to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19 in humans. SARS-CoV-2-infected mink have been reported on fur farms across Europe and North America (OIE 2021), and whole-genome sequencing of the virus isolated from mink on farms in the Netherlands has provided evidence of both human-to-mink and mink-to-human transmission of the virus (Munnink *et al.* 2021). Further sequencing of samples from humans infected with mink-related SARS-CoV-2 in Denmark has revealed that the virus had accumulated mutations with potentially adverse consequences for human health (Larsen *et al.* 2021). In response, animal welfare organizations and others have called for the closure of mink farms on public-health grounds in Europe (FOUR PAWS in Europe 2020) and elsewhere (Xia *et al.* 2020).

But it's not just on farms that American mink pose a potential disease threat. Mink farms are porous, and feral populations exist in almost all countries where mink are currently farmed or have previously been farmed (Bouros *et al.* 2016). American mink are native to North America but now occur in the wild across most of Europe and Russia, and as far afield as Argentina (Figure 1).



**Fig. 1**

Global distribution of wild American mink (*Neovison vison*), showing their native North American range and their introduced range, where they are considered an invasive species, in Eurasia and southern South America. Note that although American mink were eradicated from the Estonian island of Hiiumaa, they are present on the mainland; for further country-specific details, see CABI (2019). Image credits: CABI 2019 (map); © A Harrington (photo).

Outside their natural range, American mink are invasive, and often have negative impacts on native taxa (eg Macdonald and Harrington 2003), including prey species and other predators that they outcompete and sometimes kill (eg the critically endangered European mink [*Mustela lutreola*]; Maran *et al.* 2016). Under the current global COVID-19 pandemic, the susceptibility of American mink to the virus (Molenaar *et al.* 2020; Oreshkova *et al.* 2020; Sharun *et al.* 2021), their widespread presence in semi-aquatic habitats (Macdonald and Harrington 2003), and siting of fur farms in rural locations (Bouros *et al.* 2016) suggest that American mink may now pose a new threat to both humans and native wildlife. Although mink are a solitary species, which might minimize virus transmission and persistence (Delahay *et al.* 2021), the home ranges of individuals overlap (Macdonald *et al.* 2015) and like other mustelids they deposit feces at prominent marking spots that are investigated by neighbors (Hutchings and White 2000); such behaviors could facilitate viral transmission. In addition, during the mating season males will visit multiple females (Macdonald *et al.* 2015), and there is widespread and sometimes extensive movement of both males and females during the autumn when the young-of-the-year disperse from their natal territory (eg Oliver *et al.* 2016); both of these behaviors would also potentially facilitate viral spread if movements

involve infected individuals. In Canada, there is evidence that the Aleutian Mink Disease virus – a parvovirus that causes spontaneous abortions in and mortality of mink and other mustelids – may be transmitted from farmed to feral mink (Nituch *et al.* 2011). Although human-to-human transmission of SARS-CoV-2 is currently the dominant mechanism for the spread of COVID-19, the establishment of a wildlife reservoir could undermine efforts to combat the virus, by providing a source for re-emergence at any time and promoting conditions for the emergence and potential spread of new variants (Delahay *et al.* 2021; Sharun *et al.* 2021). Amongst wildlife, a disease outbreak could be devastating for the last remaining European mink populations.

Reports of escaped SARS-CoV-2-infected mink in Oregon (ODA 2020) and an apparently wild mink in Utah that may or may not have been a fur farm escapee (OIE 2021) have already made the news. To date, known escapees have apparently been recaptured but there is no guarantee that future escapees will not evade capture, if they have not already. Countries without mink farms (eg the UK) are not without risk either, given that feral mink could potentially be exposed to SARS-CoV-2 through sewage released into rivers (Franklin and Bevins 2020) intentionally or unintentionally (eg during flooding). Banning mink farming provides only a partial solution: it

does not remove the risk of the establishment of a viral reservoir in wildlife.

In Europe, there are policies in place for the management of invasive species; for instance, under EU Regulation (1143/2014) on Invasive Alien Species (IAS), member states are obliged to implement “effective management measures” for species included on a list of Union Concern. In March 2018, a risk assessment proposing the addition of American mink to this list (Bouros *et al.* 2016) was submitted to the European Commission (EC). However, despite approval by the EC’s Scientific Forum on IAS, mink were excluded from the EC’s proposed list and from discussion in the voting process. Denmark led the opposition to the listing of mink in order to protect its fur farming industry, which, ironically, the pandemic has now destroyed (at least temporarily), as Danish fur farmers were compelled to slaughter millions of farmed mink in an attempt to contain the disease following reports of SARS-CoV-2-infected mink at more than 200 farms worldwide (WHO 2020). Mink farming has now been banned in Denmark until 2022.

Formal listing of American mink as a species of Union Concern would probably not have affected the fate of the Danish farmers or their mink in the current global pandemic; nevertheless, beyond the captive farmed environment, we argue that it is time to reconsider the place of invasive mink in the countryside. We urge vigilance and action in all European countries, and elsewhere, around mink farms. Testing for exposure to SARS-CoV-2 in wild American mink is required, which would, in countries where they have been introduced, be most efficiently achieved in combination with removal trapping. This approach would have considerable additional biodiversity benefits but only if carried out at a large scale, with coordinated action among neighboring countries. Europe has recorded some of the highest COVID-19-associated human mortality rates in the world; it would be a great pity if the EU failed to take the actions necessary to substantially reduce the risk of a virus reservoir becoming established in feral mink. For the benefit of conservation and human health, we believe that the landscape-scale management of invasive mink that many scientists have long advocated is warranted.

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### First Description of SARS-CoV-2 Infection in Two Feral American Mink (*Neovison vison*) Caught in the Wild

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causal agent of COVID-19, is considered a pathogen of animal origin that is mainly transmitted from human to human. Several animal species can be naturally or experimentally infected by SARS-CoV-2, with compelling evidence that mink is highly susceptible to SARS-CoV-2 infection. Human-to-mink infection cases have been reported and there are also suggestions that mink-to-human infection occurs. Mink infections have been reported to date only on fur farms, except for one infected free-ranging wild mink near a Utah (USA) fur farm, which suggests a transmission pathway from farms to wild mink. We now report the detection of SARS-CoV-2 in 2 of 13 feral dark brown American mink (*Neovison vison*) trapped in the Valencian Community (Eastern Spain), during an invasive species trapping campaign. They were trapped in riverbeds in sparsely inhabited rural areas known to harbor self-sustained feral mink populations. The closest fur farm is about 20 km away. SARS-CoV-2 RNA was detected by two-step RT-PCR in these animals' mesenteric lymph nodes and was confirmed by sequencing a 397-nucleotide amplified region of the *S* gene, yielding identical sequences in both animals. A molecular phylogenetic analysis was run on this sequence, which was found to correspond to the consensus SARS-CoV-2 sequence from Wuhan. Our findings appear to represent the first example of SARS-CoV-2 acquired in the wild by feral mink in self-sustained populations.



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### **SARS-CoV-2 Seroprevalence in Household Domestic Ferrets (*Mustela putorius furo*)**

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Animal infections with SARS-CoV-2 have been reported in different countries and several animal species have been proven to be susceptible to infection with SARS-CoV-2 both naturally and by experimental infection. Moreover, infections under natural

conditions in more than 20 mink farms have been reported where humans could have been the source of infection for minks. However, little information is available about the susceptibility of pet animals under natural conditions and currently there is no SARS-CoV-2 epidemiological assessment occurrence in household ferrets. In this study, the presence of SARS-CoV-2 antibodies was evaluated in serum samples obtained from 127 household ferrets (*Mustela putorius furo*) in the Province of Valencia (Spain). Two ferrets tested positive to SARS-CoV-2 (1.57%) by in-house enzyme-linked immunosorbent assay based on receptor binding domain (RBD) of Spike antigen. Furthermore, anti-RBD SARS-CoV-2 antibodies persisted at detectable levels in a seropositive SARS-CoV-2 domestic ferret beyond 129 days since the first time antibodies were detected. This study reports for the first time the evidence of household pet ferrets exposure to SARS-CoV-2 in Spain to date.

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### **SARS-CoV and SARS-CoV-2 are transmitted through the air between ferrets over more than one meter distance**

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SARS-CoV-2 emerged in late 2019 and caused a pandemic, whereas the closely related SARS-CoV was contained rapidly in 2003. Here, an experimental setup is used to study transmission of SARS-CoV and SARS-CoV-2 through the air between ferrets over more than a meter distance. Both viruses cause a robust productive respiratory tract infection resulting in transmission of SARS-CoV-2 to two of four indirect recipient ferrets and SARS-CoV to all four. A control pandemic A/H1N1 influenza virus also transmits efficiently. Serological assays confirm all virus transmission events. Although the experiments do not discriminate between transmission via small aerosols, large droplets and fomites, these results demonstrate

that SARS-CoV and SARS-CoV-2 can remain infectious while traveling through the air. Efficient virus transmission between ferrets is in agreement with frequent SARS-CoV-2 outbreaks in mink farms. Although the evidence for virus transmission via the air between humans under natural conditions is absent or weak for SARS-CoV and SARS-CoV-2, ferrets may represent a sensitive model to study interventions aimed at preventing virus transmission.

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### Surveillance and control of SARS-CoV-2 in mustelids: An evolutionary perspective

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The relevance of mustelids in SARS-CoV-2 transmission has become increasingly evident. Alongside experimental demonstration of airborne transmission among ferrets, the major animal model for human respiratory diseases, transmission of SARS-CoV-2 within- and/or between-commercial mink farms has occurred and continues to occur. The number of mink reared for the luxury fur trade is approximately 60.5 million, across 36 mustelid-farming countries. By July 2021, SARS-CoV-2 outbreaks have been reported in 12 of these countries, at 412 European and 20 North American mink farms. Reverse zoonotic transmission events (from humans to mink) have introduced the virus to farms with subsequent extensive mink-to-mink transmission as well as further zoonotic (mink-to-human) transmission events generating cases among both farm workers and the broader community. Overcrowded housing conditions inherent within intensive mink farms, often combined with poor sanitation and welfare, both guarantee spread of SARS-CoV-2 and facilitate opportunities for viral variants, thereby effectively representing biotic hubs for viral transmission and evolution of virulence. Adequate preventative, surveillance and control measures within the mink industry are imperative both for the control of the current global pandemic and to mitigate against future outbreaks.

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### SARS-CoV-2 in animals: potential for unknown reservoir hosts and public health implications

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously 2019-nCoV) is suspected of having originated in 2019 in China from a coronavirus infected bat of the genus *Rhinolophus*. Following the initial emergence, possibly facilitated by a mammalian bridge host, SARS-CoV-2 is currently transmitted across the globe via efficient human-to-human transmission. Results obtained from experimental studies indicate that animal species such as cats, ferrets, raccoon dogs, cynomolgus macaques, rhesus macaques, white-tailed deer, rabbits, Egyptian fruit bats, and Syrian hamsters are susceptible to SARS-CoV-2 infection, and that cat-to-cat and ferret-to-ferret transmission can take place via contact and air. However, natural infections of SARS-CoV-2 have been reported only in pet dogs and cats, tigers, lions, snow leopards, pumas, and gorillas at zoos, and farmed mink and ferrets. Even though human-to-animal spillover has been reported at several instances, SARS-CoV-2 transmission from animals-to-humans has only been reported from mink-to-humans in mink farms. Following the rapid transmission of SARS-CoV-2 within the mink population, a new mink-associated SARS-CoV-2 variant emerged that was identified in both humans and mink. The increasing reports of SARS-CoV-2 in carnivores indicate the higher susceptibility of animal species belonging to this order. The sporadic reports of SARS-CoV-2 infection in domestic and wild animal species require further investigation to determine if SARS-CoV-2 or related Betacoronaviruses can get established in kept, feral or wild animal populations, which may eventually act as viral reservoirs. This review analyzes the current evidence of SARS-CoV-2 natural infection in domestic and wild animal species and their possible implications on public health.

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### **SARS-CoV-2 infection in cats and dogs in infected mink farms**

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Animals like mink, cats and dogs are susceptible to SARS-CoV-2 infection. In the Netherlands, 69 out of 127 mink farms were infected with SARS-CoV-2 between April and November 2020 and all mink on infected farms were culled after SARS-CoV-2 infection to prevent further spread of the virus. On some farms, (feral) cats and dogs were present. This study provides insight into the prevalence of SARS-CoV-2-positive cats and dogs in 10 infected mink farms and their possible role in transmission of the virus. Throat and rectal swabs of 101 cats (12 domestic and 89 feral cats) and 13 dogs of 10 farms were tested for SARS-CoV-2 using PCR. Serological assays were performed on serum samples from 62 adult cats and all 13 dogs. Whole Genome Sequencing was performed on one cat sample. Cat-to-mink transmission parameters were estimated using data from all 10 farms. This study shows evidence of SARS-CoV-2 infection in 12 feral cats and 2 dogs. Eleven cats (18%) and two dogs (15%) tested serologically positive. Three feral cats (3%) and one dog (8%) tested PCR-positive. The sequence generated from the cat throat swab clustered with mink sequences from the same farm. The calculated rate of mink-to-cat transmission showed that cats on average had a chance of 12% (95%CI 10%-18%) of becoming infected by mink, assuming no cat-to-cat transmission. As only feral cats were infected it is most likely that infections in cats were initiated by mink, not by humans. Whether both dogs were infected by mink or humans

remains inconclusive. This study presents one of the first reports of interspecies transmission of SARS-CoV-2 that does not involve humans, namely mink-to-cat transmission, which should also be considered as a potential risk for spread of SARS-CoV-2.

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### **Spatial epidemiology and genetic diversity of SARS-CoV-2 and related coronaviruses in domestic and wild animals**

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The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) showed susceptibility to diverse animal species. We conducted this study to understand the spatial epidemiology, genetic diversity, and statistically significant genetic similarity along with per-gene recombination events of SARS-CoV-2 and related viruses (SC2r-CoVs) in animals globally. We collected a number of different animal species infected with SARS-CoV-2 and its related viruses. Then, we retrieved genome sequences of SARS-CoV-2 and SC2r-CoVs from GISAID and NCBI GenBank for genomic and mutational analysis. Although the evolutionary origin of SARS-CoV-2 remains elusive, the diverse SC2r-CoV have been detected in multiple *Rhinolophus* bat species and in Malayan pangolin. To date, human-to-animal spillover events have been reported in cat, dog, tiger, lion, gorilla, leopard, ferret, puma, cougar, otter, and mink in 25 countries. Phylogeny and genetic recombination events of SC2r-CoVs showed higher similarity to the bat coronavirus RaTG13 and BANAL-103 for most

of the genes and to some Malayan pangolin coronavirus (CoV) strains for the N protein from bats and pangolin showed close resemblance to SARS-CoV-2. The clustering of animal and human strains from the same geographical area has proved human-to-animal transmission of the virus. The Alpha, Delta and Mu-variant of SARS-CoV-2 was detected in dog, gorilla, lion, tiger, otter, and cat in the USA, India, Czech Republic, Belgium, and France with momentous genetic similarity with human SARS-CoV-2 sequences. The mink variant mutation (spike\_Y453F) was detected in both humans and domestic cats. Moreover, the dog was affected mostly by clade O (66.7%), whereas cat and American mink were affected by clade GR (31.6 and 49.7%, respectively). The  $\alpha$ -variant was detected as 2.6% in cat, 4.8% in dog, 14.3% in tiger, 66.7% in gorilla, and 77.3% in lion. The highest mutations observed in mink where the substitution of D614G in spike (95.2%) and P323L in NSP12 (95.2%) protein. In dog, cat, gorilla, lion, and tiger, Y505H and Y453F were the common mutations followed by Y145del, Y144del, and V70I in S protein. We recommend vaccine provision for pet and zoo animals to reduce the chance of transmission in animals. Besides, continuous epidemiological and genomic surveillance of coronaviruses in animal host is crucial to find out the immediate ancestor of SARS-CoV-2 and to prevent future CoVs threats to humans.

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### **Serological Detection of SARS-CoV-2 Antibodies in Naturally-Infected Mink and Other Experimentally-Infected Animals**

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The recent emergence of SARS-CoV-2 in humans from a yet unidentified animal reservoir and the capacity of the virus to naturally infect pets, farmed animals and potentially wild animals has highlighted the need for serological surveillance tools. In this study, the luciferase immunoprecipitation systems (LIPS), employing the spike (S) and nucleocapsid proteins (N) of SARS-CoV-2, was used to examine the suitability of the assay for antibody detection in different animal species. Sera from SARS-CoV-2 naturally-infected mink ( $n = 77$ ), SARS-CoV-2 experimentally-infected ferrets, fruit bats and hamsters and a rabbit vaccinated with a purified spike protein were examined for antibodies using the SARS-CoV-2 N and/or S proteins. From comparison with the known neutralization status of the serum samples, statistical analyses including calculation of the Spearman rank-order-correlation coefficient and Cohen's kappa agreement were used to interpret the antibody results and diagnostic performance. The LIPS immunoassay robustly detected the presence of viral antibodies in naturally infected SARS-CoV-2 mink, experimentally infected ferrets, fruit bats and hamsters as well as in an immunized rabbit. For the SARS-CoV-2-LIPS-S assay, there was a good level of discrimination between the positive and negative samples for each of the five species tested with 100% agreement with the virus neutralization results. In contrast, the SARS-CoV-2-LIPS-N assay did not consistently differentiate between SARS-CoV-2 positive and negative sera. This study demonstrates the suitability of the SARS-CoV-2-LIPS-S assay for the sero-surveillance of SARS-CoV-2 infection in a range of animal species.

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Doi: 10.3390/v13081649.

### **An Opportunistic Survey Reveals an Unexpected Coronavirus Diversity Hotspot in North America**

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In summer 2020, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was detected on mink farms in Utah. An interagency One Health response was initiated to assess the extent of the outbreak and included sampling animals from on or near affected mink farms and testing them for SARS-CoV-2 and non-SARS coronaviruses. Among the 365 animals sampled, including domestic cats, mink, rodents, raccoons, and skunks, 261 (72%) of the animals harbored at least one coronavirus. Among the samples that could be further characterized, 127 alphacoronaviruses and 88 betacoronaviruses (including 74 detections of SARS-CoV-2 in mink) were identified. Moreover, at least 10% ( $n = 27$ ) of the coronavirus-positive animals were found to be co-infected with more than one coronavirus. Our findings indicate an unexpectedly high prevalence of coronavirus among the domestic and wild free-roaming animals tested on mink farms. These results raise the possibility that mink farms could be potential hot spots for future trans-species viral spillover and the emergence of new pandemic coronaviruses.

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Doi: 10.3390/v13102016.

### ***In vitro* Characterization of Fitness and Convalescent Antibody Neutralization of SARS-CoV-2 Cluster 5 Variant Emerging in Mink at Danish Farms**

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In addition to humans, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can transmit to animals that include hamsters, cats, dogs, mink, ferrets, tigers, lions, cynomolgus macaques, rhesus macaques, and treeshrew. Among these, mink are particularly susceptible. Indeed, 10 countries in Europe and North America reported SARS-CoV-2 infection among mink on fur farms. In Denmark, SARS-CoV-2 spread rapidly among mink farms and spilled-over back into humans, acquiring mutations/deletions with unknown consequences for virulence and antigenicity. Here we describe a mink-associated SARS-CoV-2 variant (Cluster 5) characterized by 11 amino acid substitutions and four amino acid deletions relative to Wuhan-Hu-1. Temporal virus titration, together with genomic and subgenomic viral RNA quantitation, demonstrated a modest *in vitro* fitness attenuation of the Cluster 5 virus in the Vero-E6 cell line. Potential alterations in antigenicity conferred by amino acid

changes in the spike protein that include three substitutions (Y453F, I692V, and M1229I) and a loss of two amino acid residues 69 and 70 ( $\Delta$ H69/V70), were evaluated in a virus microneutralization assay. Compared to a reference strain, the Cluster 5 variant showed reduced neutralization in a proportion of convalescent human COVID-19 samples. The findings underscore the need for active surveillance SARS-CoV-2 infection and virus evolution in susceptible animal hosts.

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### **Mink, SARS-CoV-2, and the Human-Animal Interface**

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Mink are small carnivores of the Mustelidae family. The American mink is the most common and was imported to Europe, Asia, and Latin America for breeding, as its fur is very popular. Denmark, the Netherlands, and China are the biggest producers of mink. Mink farms with a high population density in very small areas and a low level of genetic heterogeneity are places conducive to contagion. The mink's receptor for SARS-CoV-2 is very similar to that of humans. Experimental models have shown the susceptibility of the ferret, another mustelid, to become infected with SARS-CoV-2 and to transmit it to other ferrets. On April 23, 2020, for the first time, an outbreak of SARS-CoV-2 in a mink farm was reported in the Netherlands. Since then, COVID-19 has reached numerous mink farms in the Netherlands, Denmark, United States, France, Greece, Italy, Spain, Sweden, Poland, Lithuania, and Canada. Not only do mink become infected from each other, but also they are capable of infecting humans, including with virus variants that have mutated in mink. Human infection with variant mink viruses with spike mutations led to

the culling in Denmark of all mink in the country. Several animals can be infected with SARS-CoV-2. However, anthro-zoonotic outbreaks have only been reported in mink farms. The rapid spread of SARS-CoV-2 in mink farms raises questions regarding their potential role at the onset of the pandemic and the impact of mutants on viral fitness, contagiousness, pathogenicity, re-infections with different mutants, immunotherapy, and vaccine efficacy.

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### **Mink SARS-CoV-2 Infection in Poland - Short Communication**

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### **Introduction**

Since April 2020, when the first SARS-CoV-2 infection was reported in mink and subsequently in mink farm workers in the Netherlands, it has been confirmed that human-to-mink and mink-to-human transmission can occur. Later, SARS-CoV-2 infections in mink were reported in many European and North American countries.

### **Material and methods**

Samples from 590 mink from a total of 28 farms were tested by real-time RT-PCR. Whole genome sequences from one positive farm were generated and genetic relatedness was established.

### **Results**

SARS-CoV-2 RNA was detected on a breeder farm with stock of 5,850 mink. Active viraemia was confirmed in individually tested samples with Ct values respectively between 19.4 and 29.6 for E and N gene fragments. Further testing of samples from culled animals revealed 70% positivity in throat swabs and 30% seropositivity in blood samples. Phylogenetic analysis of full-length nucleotide sequences of two SARS-CoV-2 isolates revealed that they belong to

the 20B Nextstrain clade. Several nucleotide mutations were found in analysed samples compared to the reference Wuhan HU-1 strain and some of them were nonsynonymous.

### **Conclusion**

We report the infection of mink with SARS-CoV-2 on one farm in Poland and the results of subsequent analysis of virus sequences from two isolates. These data can be useful for assessment of the epidemiological situation of SARS-CoV-2 in Poland and how it endangers public health.

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### **SARS-CoV-2 outbreaks on Danish mink farms and mitigating public health interventions**

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### **Background**

First severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections on Danish mink farms were reported in June 2020 and thereupon spread geographically. We provide population-level evidence on excess human incidence rates in Danish municipalities affected by disease outbreaks on mink farms and evaluate the effectiveness of two non-pharmaceutical interventions, i.e. culling of infected mink and local lockdowns.

### **Methods**

We use information on SARS-CoV-2 outbreaks on mink farms in 94 Danish municipalities together with data on human SARS-CoV-2 cases and tested persons in weeks 24 to 51 of 2020. Difference-in-difference estimations and panel event studies for weekly human incidence rates are applied to 1) identify epidemiological trends of human SARS-CoV-2 infections associated disease outbreaks on mink farms, and 2) quantify the mitigating effects from the two non-pharmaceutical interventions.

### **Results**

SARS-CoV-2 outbreaks on mink farms in a municipi-

pality associate with increase in weekly human incidence rates by about 75%; spatial spillover effects to neighbouring municipalities are also observed. Local lockdowns reduce human incidence rates, while culling of mink appears to be more effective in combination with a lockdown. The temporal lag between an outbreak on a mink farm and a significant increase in human incidence rates is estimated to be 1-3 weeks; lockdowns and culling of mink neutralizes this effect 4-8 weeks after the initial outbreak.

### Conclusion

SARS-CoV-2 infections among farmed mink in Denmark significantly link to local human infection trends. Strict animal and human disease surveillance in regions with mink farming should be pursued internationally to mitigate future epidemic developments.

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### Spread of Mink SARS-CoV-2 Variants in Humans: A Model of Sarbecovirus Interspecies Evolution

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The rapid spread of SARS-CoV-2 variants has quickly spanned doubts and the fear about their ability escape vaccine protection. Some of these variants initially identified in caged were also found in humans. The claim that these variants exhibited lower susceptibility to antibody neutralization led to the slaughter of 17 million minks in Denmark. SARS-CoV-2 prevalence tests led to the discovery of infected farmed minks worldwide. In this study, we revisit the issue of the circulation of SARS-CoV-2 variants in minks as a model of sarbecovirus interspecies evolution by: (1) comparing human and mink angiotensin I converting enzyme 2 (ACE2) and neuropilin 1 (NRP-1) receptors; (2) comparing SARS-CoV-2 sequences from humans and minks; (3) analyzing the

impact of mutations on the 3D structure of the spike protein; and (4) predicting linear epitope targets for immune response. Mink-selected SARS-CoV-2 variants carrying the Y453F/D614G mutations display an increased affinity for human ACE2 and can escape neutralization by one monoclonal antibody. However, they are unlikely to lose most of the major epitopes predicted to be targets for neutralizing antibodies. We discuss the consequences of these results for the rational use of SARS-CoV-2 vaccines.

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### Mutation Y453F in the spike protein of SARS-CoV-2 enhances interaction with the mink ACE2 receptor for host adaption

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COVID-19 patients transmitted SARS-CoV-2 to minks in the Netherlands in April 2020. Subsequently, the mink-associated virus (miSARS-CoV-2) spilled back over into humans. Genetic sequences of the miSARS-CoV-2 identified a new genetic variant known as "Cluster 5" that contained mutations in the



spike protein. However, the functional properties of these "Cluster 5" mutations have not been well established. In this study, we found that the Y453F mutation located in the RBD domain of miSARS-CoV-2 is an adaptive mutation that enhances binding to mink ACE2 and other orthologs of *Mustela* species without compromising, and even enhancing, its ability to utilize human ACE2 as a receptor for entry. Structural analysis suggested that despite the similarity in the overall binding mode of SARS-CoV-2 RBD to human and mink ACE2, Y34 of mink ACE2 was better suited to interact with a Phe rather than a Tyr at position 453 of the viral RBD due to less steric clash and tighter hydrophobic-driven interaction. Additionally, the Y453F spike exhibited resistance to convalescent serum, posing a risk for vaccine development. Thus, our study suggests that since the initial transmission from humans, SARS-CoV-2 evolved to adapt to the mink host, leading to widespread circulation among minks while still retaining its ability to efficiently utilize human ACE2 for entry, thus allowing for transmission of the miSARS-CoV-2 back into humans. These findings underscore the importance of active surveillance of SARS-CoV-2 evolution in *Mustela* species and other susceptible hosts in order to prevent future outbreaks.

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eCollection 2021 Nov.

### **SARS-CoV-2 mutations among minks show reduced lethality and infectivity to humans**

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SARS-CoV-2 infection in minks has become a serious problem, as the virus may mutate and reinfect humans; some countries have decided to cull minks. Here, the virus sequencing data in minks were analysed and compared to those of human-virus. Although the mink-virus maintained the characteristics of human-virus, some variants rapidly mutated, adapting to minks. Some mink-derived variants infected humans, which accounted for 40% of the total SARS-CoV-2 cases in the Netherlands. These variants appear to be less lethal and infective compared to those in humans. Variants that have mutated further among minks were not found in humans. Such mink-viruses might be suitable for vaccination for

humans, such as in the case of the smallpox virus, which is less infective and toxic to humans.

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### **SARS-CoV2 spike protein gene variants with N501T and G142D mutation-dominated infections in mink in the United States**

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Large numbers of mink have been infected with SARS-CoV2 containing the spike protein Y453F mutation in Europe, causing zoonosis concerns. To evaluate the genetic characteristics of the U.S. and Canadian mink-derived SARS-CoV2 sequences, we analyzed all animal-derived (977) and all Canadian (19,529) and U.S. (173,277) SARS-CoV2 sequences deposited in GISAID from December 2019 to March 12, 2021, and identified 2 dominant novel variants, the N501T-G142D variant and N501T-G142D-F486L variant, in the U.S. mink-derived SARS-CoV2 sequences. These variants were not found in mink from Canada or other countries. The Y453F mutation was not identified in the mink-derived sequences in the United States and Canada. The N501T mutation occurred 2 mo earlier in humans than in mink in the United States, and the novel N501T-G142D and N501T-G142D-F486L variants were found in humans prior to mink. Our results suggest that the novel SARS-CoV2 variants may have evolved during human infection and were then transmitted to mink populations in the United States.

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### **SARS-CoV-2 Infections in Animals: Reservoirs for Reverse Zoonosis and Models for Study**

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The recent SARS-CoV-2 pandemic has brought many questions over the origin of the virus, the threat it poses to animals both in the wild and captivity, and the risks of a permanent viral reservoir developing in animals. Animal experiments have shown that a variety of animals can become infected with the virus. While coronaviruses have been known to infect animals for decades, the true intermediate host of the virus has not been identified, with no cases of SARS-CoV-2 in wild animals. The screening of wild, farmed, and domesticated animals is necessary to help us understand the virus and its origins and prevent future outbreaks of both COVID-19 and other diseases. There is intriguing evidence that farmed mink infections (acquired from humans) have led to infection of other farm workers in turn, with a recent outbreak of a mink variant in humans in Denmark. A thorough examination of the current knowledge and evidence of the ability of SARS-CoV-2 to infect different animal species is therefore vital to evaluate the threat of animal to human transmission and reverse zoonosis.

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Doi: 10.3390/v13030494.

### Occupational and environmental exposure to SARS-CoV-2 in and around infected mink farms

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### Objective

Unprecedented SARS-CoV-2 infections in farmed minks raised immediate concerns regarding transmission to humans and initiated intensive environmental investigations to assess occupational and environmental exposure.

### Methods

Air sampling was performed at infected Dutch mink farms, at farm premises and at nearby residential sites. A range of other environmental samples were collected from minks' housing units, including bedding materials. SARS-CoV-2 RNA was analysed in all samples by quantitative PCR.

### Results

Inside the farms, considerable levels of SARS-CoV-2 RNA were found in airborne dust, especially in personal inhalable dust samples (approximately 1000-10 000 copies/m<sup>3</sup>). Most of the settling dust samples tested positive for SARS-CoV-2 RNA (82%, 75 of 92). SARS-CoV-2 RNA was not detected in outdoor air samples, except for those collected near the entrance of the most recently infected farm. Many samples of minks' housing units and surfaces contained SARS-CoV-2 RNA.

### Conclusions

Infected mink farms can be highly contaminated with SARS-CoV-2 RNA. This warns of occupational exposure, which was substantiated by considerable SARS-CoV-2 RNA concentrations in personal air samples. Dispersion of SARS-CoV-2 to outdoor air was found to be limited and SARS-CoV-2 RNA was not detected in air samples collected beyond farm premises, implying a negligible risk of environmental exposure to nearby communities. Our occupational and environmental risk assessment is in line with whole genome sequencing analyses showing mink-

to-human transmission among farm workers, but no indications of direct zoonotic transmission events to nearby communities.

*Occup Environ Med.* 2021 Dec; 78 (12): 893-899.  
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### Host Diversity and Potential Transmission Pathways of SARS-CoV-2 at the Human-Animal Interface

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Emerging infectious diseases present great risks to public health. The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), has become an urgent public health issue of global concern. It is speculated that the virus first emerged through a zoonotic spillover. Basic research studies have suggested that bats are likely the ancestral reservoir host. Nonetheless, the evolutionary history and host susceptibility of SARS-CoV-2 remains unclear as a multitude of animals has been proposed as potential intermediate or dead-end hosts. SARS-CoV-2 has been isolated from domestic animals, both companion and livestock, as well as in captive wildlife that were in close contact with human COVID-19 cases. Currently, domestic mink is the only known animal that is susceptible to a natural infection, develop severe illness, and can also transmit SARS-CoV-2 to other minks and humans. To improve foundational knowledge of

SARS-CoV-2, we are conducting a synthesis review of its host diversity and transmission pathways. To mitigate this COVID-19 pandemic, we strongly advocate for a systems-oriented scientific approach that comprehensively evaluates the transmission of SARS-CoV-2 at the human and animal interface.

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### Assessing the extent of community spread caused by mink-derived SARS-CoV-2 variants

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SARS-CoV-2 has recently been found to have spread from humans to minks and then to have transmitted back to humans. However, it is unknown to what extent the human-to-human transmission caused by the variant has reached. Here, we used publicly available SARS-CoV-2 genomic sequences from both humans and minks collected in Denmark and the Netherlands, and combined phylogenetic analysis with Bayesian inference under an epidemiological model, to trace the possibility of person-to-person transmission. The results showed that at least 12.5% of all people being infected with dominated mink-derived SARS-CoV-2 variants in Denmark and the Netherlands were caused by human-to-human transmission, indicating that this "back-to-human" SARS-CoV-2 variant has already caused human-to-human transmission. Our study also indicated the need for monitoring this mink-derived and other animal source "back-to-human" SARS-CoV-2 in future and that prevention and control measures should be tailored to avoid large-scale community transmission caused by the virus jumping between animals and humans.

*Innovation (N Y).* 2021 Aug 28; 2 (3): 100128.  
Doi: 10.1016/j.xinn.2021.100128. Epub 2021 Jun 7.

## Adaptation, spread and transmission of SARS-CoV-2 in farmed minks and associated humans in the Netherlands

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In the first wave of the COVID-19 pandemic (April 2020), SARS-CoV-2 was detected in farmed minks and genomic sequencing was performed on mink farms and farm personnel. Here, we describe the outbreak and use sequence data with Bayesian phylogenetic methods to explore SARS-CoV-2 transmission in minks and humans on farms. High number of farm infections (68/126) in minks and farm workers (>50% of farms) were detected, with limited community spread. Three of five initial introductions of SARS-CoV-2 led to subsequent spread between mink

farms until November 2020. Viruses belonging to the largest cluster acquired an amino acid substitution in the receptor binding domain of the Spike protein (position 486), evolved faster and spread longer and more widely. Movement of people and distance between farms were statistically significant predictors of virus dispersal between farms. Our study provides novel insights into SARS-CoV-2 transmission between mink farms and highlights the importance of combining genetic information with epidemiological information when investigating outbreaks at the animal-human interface.

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## Paired SARS-CoV-2 spike protein mutations observed during ongoing SARS-CoV-2 viral transfer from humans to minks and back to humans

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A mutation analysis of SARS-CoV-2 genomes collected around the world sorted by sequence, date, geographic location, and species has revealed a large number of variants from the initial reference sequence in Wuhan. This analysis also reveals that humans infected with SARS-CoV-2 have infected mink populations in the Netherlands, Denmark, United States, and Canada. In these animals, a small set of

mutations in the spike protein receptor binding domain (RBD), often occurring in specific combinations, has transferred back into humans. The viral genomic mutations in minks observed in the Netherlands and Denmark show the potential for new mutations on the SARS-CoV-2 spike protein RBD to be introduced into humans by zoonotic transfer. Our data suggests that close attention to viral transfer from humans to farm animals and pets will be required to prevent build-up of a viral reservoir for potential future zoonotic transfer.

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**Receptor-binding domain-based immunoassays for serosurveillance differentiate efficiently between SARS-CoV2-exposed and non-exposed farmed mink**

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During the COVID-19 pandemic, infection of farmed mink has become not only an economic issue but also a widespread public health concern. International agencies have advised the use of strict molecular and serosurveillance methods for monitoring the SARS-CoV2 status on mink farms. We developed 2 ELISAs and a duplex protein microarray immunoassay (MI), all in a double-recognition format (DR), to detect SARS-CoV2 antibodies specific to the receptor-binding domain (RBD) of the spike protein and to the full-length nucleoprotein (N) in mink sera. We collected 264 mink serum samples and 126 oropharyngeal samples from 5 Spanish mink farms. In both of the ELISAs and the MI, RBD performed better than N protein for serologic differentiation of mink from SARS-CoV2-positive and -negative farms. Therefore, RBD was the optimal antigenic target for serosurveillance of mink farms.

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**Mathematical modelling of SARS-CoV-2 variant outbreaks reveals their probability of extinction**

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When a virus spreads, it may mutate into, e.g., vaccine resistant or fast spreading lineages, as was the case for the Danish Cluster-5 mink variant (belonging to the B.1.1.298 lineage), the British B.1.1.7 lineage, and the South African B.1.351 lineage of the SARS-CoV-2 virus. A way to handle such spreads is through a containment strategy, where the population in the affected area is isolated until the spread has been stopped. Under such circumstances, it is important to monitor whether the mutated virus is extinct via massive testing for the virus sub-type. If successful, the strategy will lead to lower and lower numbers of the sub-type, and it will eventually die out. An important question is, for how long time one should wait to be sure the sub-type is extinct? We use a hidden Markov model for infection spread and an approximation of a two stage sampling scheme to infer the probability of extinction. The potential of the method is illustrated via a simulation study. Finally, the model is used to assess the Danish containment strategy when SARS-CoV-2 spread from mink to man during the summer of 2020, including the Cluster-5 sub-type. In order to avoid further spread and mink being a large animal virus reservoir, this situation led to the isolation of seven municipalities in the Northern part of the country, the culling of the entire Danish 17 million large mink population, and a bill to interim ban Danish mink production until the end of 2021.

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### Survival of SARS-CoV-2 on Clothing Materials

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In order to plan and execute proper preventative measures against COVID-19, we need to understand how SARS-CoV-2 is transmitted. It has been shown to remain infectious on surfaces from hours to days depending on surface type and environmental factors. The possibility of transmission through fur animals and contaminated pelts, along with the safety of those working with them, is a major concern. SARS-CoV-2 can infect minks and raccoon dogs and has spread to mink farms in numerous countries. Here, we studied the stability of SARS-CoV-2 on blue fox, Finn raccoon, and American mink pelt, fake fur, cotton, plastic, faux leather, and polyester and tested its inactivation by UV light and heat treatment. We detected infectious virus up to 5 days on plastic, up to 1 day on fake fur, less than a day on cotton, polyester, and faux leather, and even 10 days on mink fur. UV light failed to inactivate SARS-CoV-2 on pelts, most likely due to the mechanical protection by the fur. Hence, it should not be used to inactivate the virus on fur products, and its use for other surfaces should also be considered carefully. Heat treatment at 60°C for 1 h inactivated the virus on all surfaces and is a promising method to be applied in practice. This study helps prevent further spread of COVID-19 by increasing our understanding about risks of SARS-CoV-2 spread through contaminated clothing materials and giving important information needed to improve safety of those working in the production line as well as people using the products.

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### The Mink Circovirus Capsid Subunit Expressed by Recombinant Baculovirus Protects Minks against Refractory Diarrhea in Field

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Mink refractory diarrhea is a seasonal disease that occurs in many mink farms in China. Mink circovirus (MiCV) has been recognized as the causative agent of the disease. The aim of the study was to develop a subunit vaccine against mink refractory diarrhea. A recombinant baculovirus strain expressing the capsid protein was constructed using the baculovirus expression vector system (BEVS). A subunit vaccine was developed based on the capsid protein with appropriate adjuvant. Then, a field trial was carried out in two districts in order to evaluate the efficiency of the subunit vaccine. The field trial indicated that in total, only 1.8% of the minks developed typical diarrhea in the vaccinated group compared with 74.5% in the control group. The vaccination could significantly reduce the infection rate of MiCV among the mink herds and could restrain the virus' shedding from feces. Furthermore, the vaccinated group had a higher average litter size in the following year compared to the control group. Collectively, the results indicated that the subunit vaccine based on the capsid protein can provide reliable protection against MiCV infection.

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### Pathogenicity comparison of the SMPV-11 and attenuated mink enteritis virus F61 in mink

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**Molecular detection and genotypes of *Enterocytozoon bieneusi* in farmed mink (*Neovison vison*), blue foxes (*Alopex lagopus*), and raccoon dogs (*Nyctereutes procyonoides*) in Xinjiang, China**

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*Enterocytozoon bieneusi* is a zoonotic pathogen that infects a variety of hosts including humans, livestock, wildlife, companion animals, and birds, as well as being abundant in the environment. Humans and non-human animals could be infected with *E. bieneusi* via consumption of food or water that contains zoonotic and host-adapted genotypes. In this study, 288 fecal

specimens were collected from farmed minks, blue foxes, and raccoon dogs, in Xinjiang, China. *Enterocytozoon bieneusi* was examined by PCR amplification based on sequence analysis of the internal transcribed spacer (ITS) region. The overall infection rate of *E. bieneusi* was 4.9% (14/288), with mink samples showing the highest infection rate (5.6%, 12/214), followed by blue foxes (2.9%, 1/35), and then raccoon dogs (2.6%, 1/39). Six *E. bieneusi* genotypes were identified, including D (n = 5), PigEBITS7 (n = 4), EbpA (n = 2), CAM5 (n = 1), WildBoar3 (n = 1), and a novel genotype XJMI-1 (n = 1). Phylogenetic analysis showed that all *E. bieneusi* genotypes belonged to group 1, which composed of over 300 genotypes and most of them have been identified in human and variety of animals, suggesting a risk of zoonotic transmission from farmed wildlife to humans.

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**Pandemic lineage 2009 H1N1 influenza A virus infection in farmed mink in Utah**

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Mink are susceptible to infection with influenza A virus (IAV) of swine and human origin. In 2019, a Utah mink farm had an outbreak of respiratory disease in kits caused by infection with the pandemic influenza A(H1N1)2009 virus [A(H1N1)pdm09]. In 3 wk, ~325, 1-2-wk-old kits died (10% mortality in kits). All deaths occurred in a single barn that housed 640 breeding females. No clinical signs or deaths occurred among adult mink. Five dead kits and 3 euthanized female mink were autopsied. All kits had moderate-to-severe neutrophilic and lymphohistiocytic interstitial pneumonia; adult mink had minimal-to-moderate lymphohistiocytic bronchointerstitial pneumonia. Immunohistochemistry and real-time PCR targeting the matrix gene detected IAV in lung

of kits and adults. Virus isolation and genetic analysis identified the A(H1N1)pdm09 virus. The source of the virus was not determined but is thought to be the result of reverse zoonosis. Our case emphasizes the need for close monitoring on mink farms for interspecies transmission of IAV and for safe work practices on farms and in diagnostic laboratories. Additionally, a pandemic virus may continue to circulate at low levels long after the global event is declared over.

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### **Mink is a highly susceptible host species to circulating human and avian influenza viruses**

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Pandemic influenza, typically caused by the reassortment of human and avian influenza viruses, can result in severe or fatal infections in humans. Timely identification of potential pandemic viruses must be a priority in influenza virus surveillance. However, the range of host species responsible for the generation of novel pandemic influenza viruses remains unclear. In this study, we conducted serological surveys for avian and human influenza virus infections in farmed mink and determined the susceptibility of mink to prevailing avian and human virus subtypes. The results showed that farmed mink were commonly infected with human (H3N2 and H1N1/pdm) and avian (H7N9, H5N6, and H9N2) influenza A viruses. Cor-

relational analysis indicated that transmission of human influenza viruses occurred from humans to mink, and that feed source was a probable route of avian influenza virus transmission to farmed mink. Animal experiments showed that mink were susceptible and permissive to circulating avian and human influenza viruses, and that human influenza viruses (H3N2 and H1N1/pdm), but not avian viruses, were capable of aerosol transmission among mink. These results indicate that farmed mink could be highly permissive "mixing vessels" for the reassortment of circulating human and avian influenza viruses. Therefore, to reduce the risk of emergence of novel pandemic viruses, feeding mink with raw poultry by-products should not be permitted, and epidemiological surveillance of influenza viruses in mink farms should be urgently implemented.

*Emerg Microbes Infect.* 2021 Dec; 10 (1): 472-480.

Doi: 10.1080/22221751.2021.1899058.

### **Invasive Species as Hosts of Zoonotic Infections: The Case of American Mink (*Neovison vison*) and *Leishmania infantum***

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*Leishmania infantum* produces an endemic disease in the Mediterranean Basin that affects humans and domestic and wild mammals, which can act as reservoir or minor host. In this study, we analyzed the presence of the parasite in wild American minks, an invasive species in Spain. We screened for *L. infantum* DNA by PCR using five primer pairs: Two targeting kinetoplast DNA (kDNA), and the rest targeting the ITS1 region, the small subunit of ribosomal RNA (SSU) and a repetitive sequence (Repeat region). The detection limit was determined for each method using a strain of *L. infantum* and a bone marrow sample from an infected dog. PCR approaches employing the Re-



peat region and kDNA (RV1/RV2 primers) showed higher sensitivity than the other PCR methods when control samples were employed. However, only PCR of the Repeat region and nested PCR of SSU (LnSSU) detected the parasite in the samples, while the other three were unable to do so. The majority of the analyzed animals (90.1%) tested positive. American mink may act as an incidental host of the disease for other mammals and should be further investigated, not only for their negative impact on the local fauna, but also as carriers of zoonotic diseases.

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**Detection of Anti-*Leishmania infantum* antibodies in Wild European and American Mink (*Mustela lutreola* and *Neovison vison*) from Northern Spain, 2014-20**

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The European mink (*Mustela lutreola*) is listed as a critically endangered species because of ongoing

population reduction from habitat degradation and the effects of introduced species, such as American mink (*Neovison vison*). This small, fragmented population becomes vulnerable to many other threats, including diseases. Leishmaniosis is a zoonotic disease caused by the protozoan parasite *Leishmania infantum* found in the Mediterranean area, which affects many mammals, including wild small mammals. Furthermore, clinical disease caused by *L. infantum* has recently been described in other mustelids. To assess the exposure to *Leishmania* sp. infection in mink species in northern Spain, blood samples from 139 feral American mink and 42 native European mink from north Spain were evaluated for *Leishmania* sp. infection using enzyme-linked immunosorbent assays against *Leishmania* spp. antibodies, with 52.4% of American mink and 45.3% of European mink being found seropositive. This finding raises questions regarding how the disease may affect these species and the potential repercussions for conservation efforts. Despite a high seroprevalence being observed in wild mink of both species in this study, association with clinical or pathologic signs of disease has yet to be elucidated.

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**Isolation and pathogenicity analysis of mink orthoreoviruses**

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Mammalian orthoreoviruses (MRVs) can infect many mammals including human, and numerous higher virulent MRVs have been reported in recent years. The first mink orthoreovirus was reported in China in 2011. In the present study, three new strains of mammalian orthoreoviruses were isolated from mink and found to be most closely related to human strain MRV2Tou05 and other human strains. Mink experiments demonstrated that the isolated mink reoviruses did not lead to severe pathogenicity. Viruses were eliminated within 2 weeks after infection, but they may cause viral enteritis disease in puppies.

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### Molecular Survey of Parvoviruses and Mycoplasma spp. in Invasive American Mink (*Neovison vison*) from Southern Chile

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Using PCR, we evaluated the presence of parvoviruses and Mycoplasma spp. in 123 American mink (*Neovison vison*), an introduced invasive carnivore in Chile. Our results showed all analyzed animals were negative for both pathogen groups. We cannot completely dismiss their presence, but if present, their prevalence should be lower than 2%.

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### Molecular Epidemiology of Extraintestinal Pathogenic *Escherichia coli* Causing Hemorrhagic Pneumonia in Mink in Northern China

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The molecular epidemiology and biological characteristics of *Escherichia coli* associated with hemorrhagic pneumonia (HP) mink from five Chinese Provinces were determined. From 2017 to 2019, 85 *E. coli* strains were identified from 115 lung samples of mink suffering from HP. These samples were subjected to serotyping, antimicrobial susceptibility, detection of virulence genes, phylogenetic grouping, whole-genome sequencing, drug resistant gene, multilocus sequence typing (MLST) and biofilm-forming assays. *E. coli* strains were divided into 18 serotypes. Thirty-nine *E. coli* strains belonged to the O11 serotype. Eighty-five *E. coli* strains were classified into seven phylogenetic groups: E (45.9%, 39/85), A (27.1%, 23/85), B1 (14.1%, 12/85), B2 (3.7%, 3/85), D (3.7%, 3/85), F (2.4%, 2/85) and clade I (1.2%, 1/85). MLST showed that the main sequence types (STs) were ST457 (27/66), All *E. coli* strains had  $\geq 4$  virulence genes. The prevalence of virulence was 98.8% for *yjy* and *fimC*, 96.5% for *iucD*, 95.3% for *ompA*, 91.8% for *cnf-I*, 89.4% for *mat*, 82.3% for *hlyF*, and 81.2% for *ibeB*. The prevalence of virulence genes *iss*, *cva/cvi*, *aataA*, *ibeA*, *vat*, *hlyF*, and *STa* was 3.5-57.6%. All *E. coli* strains were sensitive to sulfamethoxazole, but high resistance was shown to tetracycline (76.5%), chloramphenicol (71.8%), ciprofloxacin (63.5%) and florfenicol (52.9%), resistance to other antibiotics was 35.3-16.5%. The types and ratios of drug-resistance genes were *tet(A)*, *strA*, *strB*, *sul2*, *oqxA*, *blaTEM-1B*, *floR*, and *catA1* had the highest frequency from 34%-65%, which were consistent with our drug resistance phenotype tetracycline, florfenicol, quinolones, chloramphenicol, the *bla-NDM-I* and *mcr-I* were presented in ST457 strains. Out of 85 *E. coli* strains, six (7.1%) possessed a strong ability, 12 (14.1%) possessed a moderate ability, and 64 (75.3%) showed a weak ability to form biofilm. Our data will aid understanding of the epidemiological background and provide a

clinical basis for HP treatment in mink caused by *E. coli*.

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### A review of the occurrence and clinical consequences of protozoan infections in carnivorous fur farm animals

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#### Introduction and objective

The aim was to review available data concerning the occurrence of protozoan parasites affecting carnivorous fur farm animals, such as: American mink, blue and silver foxes, and raccoon dogs. Although, many protozoan pathogens have been isolated in wild fur animals, some are still not recognised as relevant to particular fur farm animal species. Protozoans that have been isolated as causative agents of clinical cases and reported in these animals are: *Cryptosporidium* spp., *Eimeria* spp., *Isospora* spp., *Leishmania infantum*, *Neospora caninum*, *Sarcocystis* spp., and *Toxoplasma gondii*. Unfortunately, in most reports, neither clinical symptoms nor detailed pathogenicity and clinical pathology are satisfactory discriminated. This data is essential for preparing treatment and preventive measure protocols. Additionally, attempts to better understand the infections might be useful in the case of outbreaks.

#### Results

The results of the literature research revealed that some detected infections may have zoonotic potential, and are quite likely to be transmitted in both directions. The role of vectors, e.g. small rodents, birds, insects (sand-flies), or pets (cats and dogs), which might be involved in the transmission of various parasitic infections, is also discussed. The summarized list of protozoans involved can be used for further studies on the health and welfare aspects of fur farm animal breeding and public health issues.

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Doi: 10.2644/aaem/120974. Epub 2020 Jun 1.

### Characterization of antimicrobial resistance and virulence genes of *Pseudomonas aeruginosa* isolated from mink in China, 2011-2020

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### Comparative Genomics of 42 *Arcanobacterium phocae* Strains

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For the last 13 years, the fur industry in Europe has suffered from epidemic spouts of a severe necrotizing pyoderma. It affects all species currently farmed for

fur and causes animal welfare problems and significant losses to the farmers. The causative agent of this disease was identified as *Arcanobacterium phocae*. Previously, this bacterium has been isolated from seals and other marine mammals, apparently causing wound and lung infections. Attempts at antibiotic treatment have been unsuccessful and the current advice on preventing the disease is to cull all animals with clinical signs. This poses an urgent question regarding possible vaccine development, as well as the need for further understanding of the pathogenicity of this organism. This study compared the whole genomes of 42 *A. phocae* strains isolated from seals, blue foxes, finnraccoons, mink and otter. The sequences were created using the Illumina technology and annotations were done using the RAST pipeline. A phylogenetic analysis identified a clear separation between the seal strains and the fur-animal-derived isolates, but also indicated that the bacterium readily adapts to new environments and host species with reasonable diversity. A pan- and core-genome was created and analyzed for proteins. A further analysis identified several virulence factors as well as multiple putative and secreted proteins of special interest for vaccine development.

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### Prevalence, Serotypes, and Antimicrobial Resistance of *Salmonella* from Mink Feces and Feed in the United States

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Nontyphoidal *Salmonella* is a leading cause of foodborne illnesses, and concentrated animal production such as commercial mink farming can be a reservoir. The objective of this study was to determine the prevalence, serotypes, antimicrobial resistance, virulence genes and mobile genetic elements, and phylogenetic characterization of *Salmonella* from mink fecal ( $n = 42$ ) and feed ( $n = 8$ ) samples at a commercial mink farm in the United States. *Salmonella* was detected

from 11 (26.2%) fecal and 1 (12.5%) feed samples. Twelve fecal isolates obtained from the positive samples were identified into four serotypes: *Salmonella* Uganda (eight isolates; two isolates were obtained from one sample), *Salmonella* Reading (two isolates), *Salmonella* Heidelberg (one isolate), and *Salmonella* Orion (one isolate). Two isolates from the positive feed sample were identified as *Salmonella* Cerro. Twelve isolates, one isolate per positive sample, were whole genome sequenced for further characterization. The two *Salmonella* Reading isolates were multidrug resistant (MDR) with the classical ASSuT (ampicillin, streptomycin, sulfisoxazole, and tetracycline) phenotype. Genotypically, the isolates were correspondingly positive, both by polymerase chain reaction and by whole-genome sequencing (WGS), for *bla*<sub>TEM-1</sub>, *aph*(3'')-Ib and *aph*(6)-Id, *sul2*, and *tet*(A). WGS additionally identified trimethoprim resistance gene *dfrA8* in *Salmonella* Reading, and fosfomycin resistance gene *fosA7* in *Salmonella* Heidelberg. All isolates carried *Salmonella*-specific multidrug resistance (*mdsA/B*), gold resistance, and intimin-like virulence genes. In addition, *Salmonella* Orion carried copper and silver resistance gene clusters. The two *Salmonella* Reading isolates also carried a cytolethal distension toxin (*cdtB*) gene. *Salmonella* Reading isolates belonged to ST412, a predominant sequence type among turkey and human isolates obtained in relation to recent salmonellosis outbreaks in North America. Phylogenetically, *Salmonella* Uganda, *Salmonella* Heidelberg, and *Salmonella* Reading were mostly associated with historic human/clinical, and poultry and swine source isolates. This study indicates that mink production can be a reservoir for foodborne pathogens such as *Salmonella* with MDR serovars commonly associated with human illnesses.

*Foodborne Pathog Dis*. 2021 Sep 14.  
Doi: 10.1089/fpd.2021.0037. Online ahead of print.

### Invasive American mink (*Neovison vison*) as potential definitive host of *Sarcocystis elongata*, *S. entzerothi*, *S. japonica*, *S. truncata* and *S. silva* using different cervid species as intermediate hosts

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Canids and scavenger birds were shown to act as definitive hosts of numerous *Sarcocystis* species using members of the Cervidae family as an intermediate host, whereas definitive hosts spreading closely related *S. elongata*, *S. entzerothi*, *S. japonica*, *S. mat-suoa*, *S. rangiferi*, *S. truncata*, *S. silva* and *S. tarandi* remain unknown. In the current study, the intestine samples of 40 American minks (*Neovison vison*) were molecularly tested for the presence of the above-mentioned *Sarcocystis* spp. Species-specific PCR of cytochrome c oxidase subunit I (cox1) fragments and subsequent sequencing revealed the presence of sporocysts/oocysts of five species, *S. elongata* (n=2), *S. entzerothi* (n=10), *S. japonica* (n=4), *S. silva* (n=13) and *S. truncata* (n=21) in the analysed samples. *Sarcocystis* infection was confirmed in 32/40 (80%) examined samples. In addition, half of the infected animals (50%) were infected with multiple *Sarcocystis* species suggesting that American minks had access to meat of different deer species, such as roe deer, red deer and sika deer. This causes concern about compliance of hunters and game processing companies with game waste management rules. Further research on the involvement of mustelids in the transmission of various *Sarcocystis* spp. from different geographical locations is needed.

*Parasitol Res* 2021 Jun; 120 (6): 2243-2250.

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### First finding of *Streptococcus phocae* infections in mink (*Neovison vison*)

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### Prevalence of *Toxoplasma gondii* and *Cryptosporidium* in Feral and Farmed American Mink (*Neovison vison*) in Denmark

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#### Purpose

To investigate the prevalence of *Cryptosporidium* spp. infection and *Toxoplasma gondii* antibodies in farmed and feral mink in Denmark.

#### Methods

We examined meat juice from 235 feral mink and 306 farmed mink for *T. gondii* antibodies, and faecal samples from 113 feral mink and 166 farmed mink for *Cryptosporidium* oocyst excretion. Meat juice was analysed using a commercial indirect enzyme-linked immunosorbent assay and oocyst excretion was identified by a modified Ziehl-Neelsen method.

#### Results

All farmed mink tested sero-negative, while 53.6% of feral mink were *T. gondii* sero-positive. The probability of being sero-positive for *T. gondii* was not associated with recent escapes from farms ( $p = 0.468$ ), but was significantly higher for male feral mink (64.2%) than female feral mink (42.5%) ( $p = 0.0008$ ). Only one feral mink and four farmed mink (2.4%) excreted *Cryptosporidium* oocysts.

#### Conclusion

Farmed mink were all *T. gondii* sero-negative, whereas approximately half the feral mink were sero-positive. *Cryptosporidium* prevalence in farmed and feral mink were low. Overall, the public health risk

of transmission of these two parasites via mink in Denmark is low.

*Acta Parasitol 2021 Dec; 66 (4): 1285-1291.*  
*Doi: 10.1007/s11686-021-00409-0. Epub 2021 May 11.*

### **Recent epidemiologic and clinical *Toxoplasma gondii* infections in wild canids and other carnivores: 2009-2020**

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*Vet Parasitol. 2021 Feb; 290: 109337.*  
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*Epub 2020 Dec 17.*

### **Survey of *Trichinella* in American minks (*Neovison vison* Schreber, 1777) and wild rodents (*Muridae* and *Cricetidae*) in Chile**

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Trichinellosis is a zoonosis with worldwide distribution. Several hosts have been recognized around the world; however, there is a lack of knowledge of the

role of feral mammals in Chile in its transmission. Herein, we tested muscle samples from 555 individuals among American minks (*Neovison vison* Schreber, 1777. n = 100) and several myomorph rodent species (*Muridae* and *Cricetidae*. n = 455) from southern Chile by artificial digestion to detect *Trichinella* larvae and identified the larvae at the species level through molecular analyses. Rodents were captured in agricultural and wild protected areas of several administrative regions (hereafter: region), while minks were captured in agricultural areas of the Los Ríos region. *Trichinella spiralis* larvae were detected in a synanthropic black rat (0.24%) of the Ñuble region and in seven minks (7%) trapped in agricultural areas of the Los Ríos region. The present results suggest that *T. spiralis* circulation is restricted to synanthropic rodents and minks living in agricultural areas where the parasite circulates among domesticated pigs. This study represents the first record of *T. spiralis* in a mustelid mammal in South America, increasing the number of feral species that could participate in the reservoir.

*Zoonoses Public Health. 2021 Nov; 68 (7): 842-848.*

*Doi: 10.1111/zph.12845. Epub 2021 May 7.*

### **The effect of antimicrobial treatment on mortality associated with urinary tract disease in mink kits (*Neovison vison*)**

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Mink urinary tract disease (MUTD) often presents as urolithiasis and/or cystitis and is known as an important cause of mortality in mink kits during the early growth season. Antimicrobial flock treatment has been routinely applied as preventive/therapeutic

protocol on Danish mink farms with increased mortality associated with MUTD. The therapeutic effect of this treatment strategy has not previously been investigated. In this study, we applied controlled parallel group treatment trials to assess the effect of sulfadiazine/trimethoprim and amoxicillin treatment on mortality associated with MUTD in mink kits. On farm A, eight mink kits were diagnosed with MUTD post mortem in the treatment group (n = 1920, sulfadiazine/trimethoprim treatment: 30 mg/kg, q 24 h, P.O for 5 days) compared to 16 in the untreated control group (n = 1920). No significant difference in mortality associated with MUTD were found between the treatment and the control group using the Fisher's exact test (P = 0.15). Treatment group 2 (n = 1920, amoxicillin treatment: 14 mg/kg q 24 h, P.O for 5 days) and treatment group 3 (n = 2088, amoxicillin treatment: 7.5 mg/kg q 24 h, P.O for 5 days) were investigated on farm B. Eight and four mink kits were diagnosed with MUTD post mortem in group 2 and 3, respectively. No difference between occurrence of MUTD were found between the control group and treatment group 2 (P = 0.42) or treatment group 3 (P = 0.75). No significant difference between final body weights or weight gain were found between treatment and control weighing groups on farm A or B. In conclusion, antimicrobial treatment administered in the feed showed no significant effect on weight gain or mortality associated with MUTD on the farms included in this study.

*Acta Vet Scand. 2021 Apr 1; 63 (1): 15.*

*Doi: 10.1186/s13028-021-00581-3.*





## Obituary



**Einar Jón Einarsson at a celebration during his years in Foulum**

Dear All,

We are saddened to inform that one of the founders of IFASA, Einar Jón Einarsson, died after a short illness, peacefully at home, surrounded by his family on the 24<sup>th</sup> of November, 2021.

Einar was born on 20<sup>th</sup> of June, 1962 in Reykjavik in Iceland, but lived most of his life in Norway. He was a leading figure in the Nordic and international fur animal research community for many years.

Einar was a true believer in international collaboration between scientists and as a young researcher at the Agricultural University of Norway he joined the Nordic Association of Agricultural Scientists, and became an active member of the Breeding Committee for Fur Animals. In 1986 he was elected to the Board of the Fur Animal Working Group. He, along with Gunnar Jorgensen conceived the idea of the International Fur Animal Scientific Association (IFASA) that was established after the Fourth International Fur Congress in Toronto, Canada in 1988. Einar served as the president of IFASA from 1988 until 2000, when he left the fur animal science.

He became Dr. Agri. in “Selection for Litter Size in Mink” in 1988 and continued as professor in the Agricultural University of Norway. From 1985 to 1987 he was leader of breeding and genetics at the Norwegian Fur Breeders Association.

From 1994 to 1996 Einar was head of the Department for Research in Small Farm Animals (incl. fur animals) at the National Institute of Animal Science in Denmark. We remember Einar as a natural leader, with a rare ability to listen to and understand all opinions, and to develop consensus decisions that suited everyone. This facilitated a very positive and productive working environment at the department.

In addition to his doctorate publications, Einar published several articles on fur animals and was co-writer of the book “Beautiful Fur Animals – and their colour genetics”. Together with Professor Anders Skrede he published the book “Avl og føring av rev” (Breeding and feeding of foxes).

Although Einar’s skills were lost for the fur industry, they were put to use in strengthening and managing the Norwegian book publishing industry. Einar will always be remembered as one of the Grand Old Men and a key figure in the international Fur Animal Science. Our thoughts and sympathy go to Hjördis and the family.

Steen H. Møller, President of IFASA

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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.