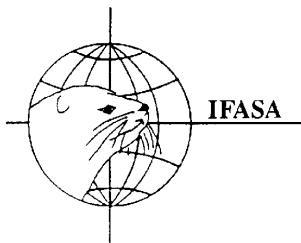
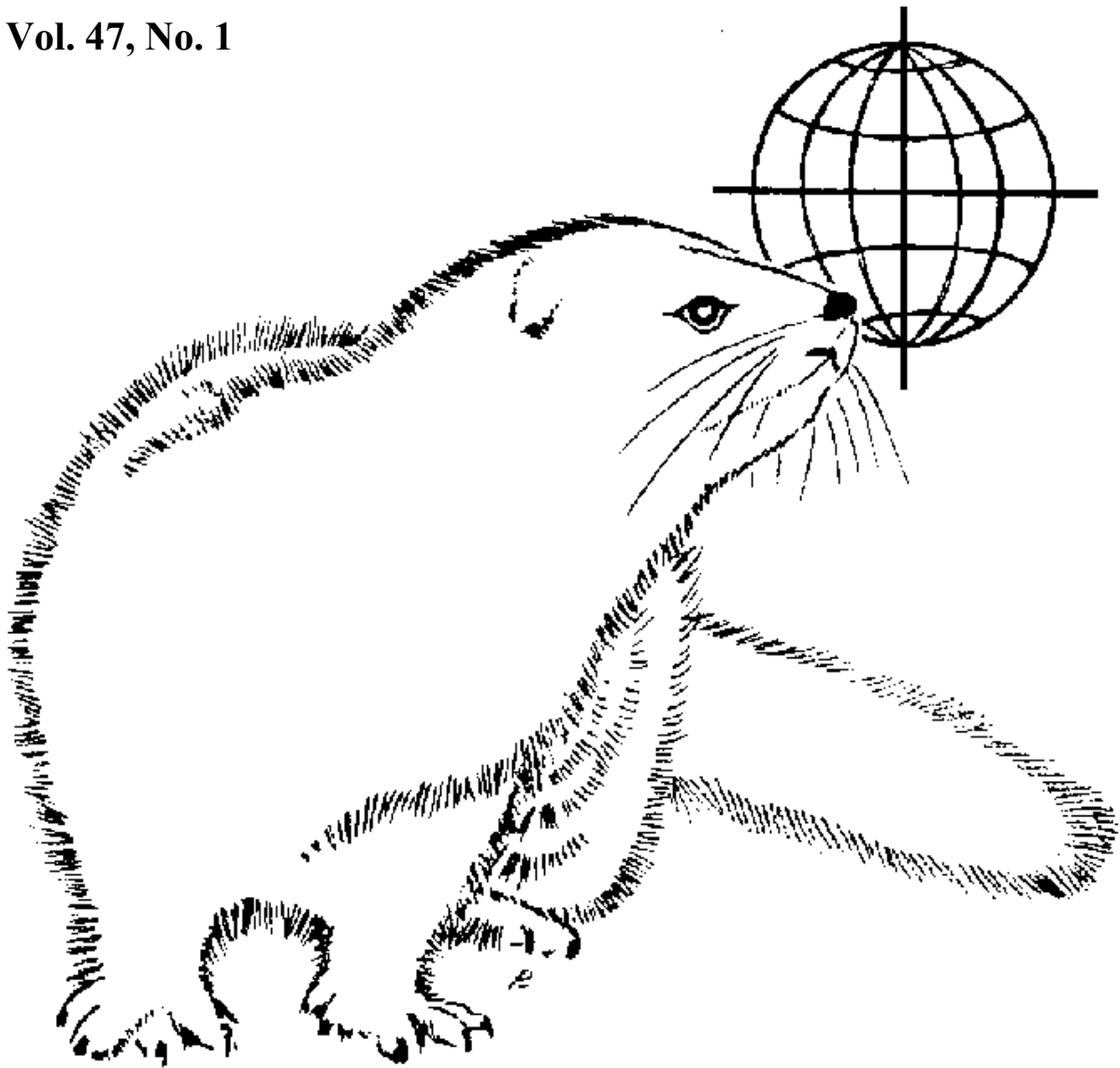


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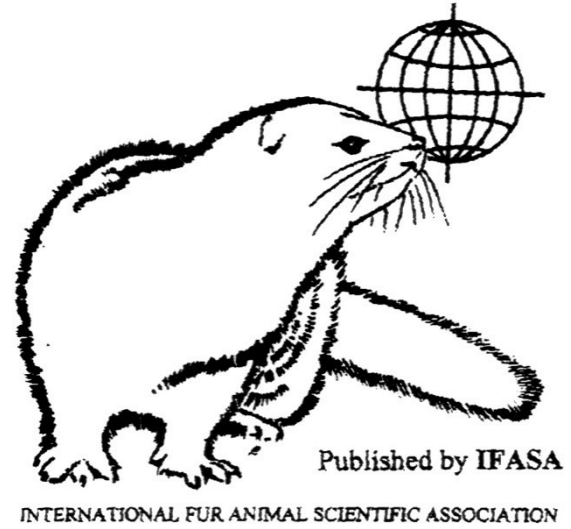
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Notes from the Editor

Genetics and breeding are powerful tools as they can ensure continuous improvement of the selected traits, given the same environmental circumstances. Abstracts in this issue of *Scientifur*, show the potential of applying both the more conventional quantitative genetics and the now well-established genomic techniques in breeding.

A study shows high genetic correlations between skin size and body weight and body length, which is promising for including these traits into a breeding program. In the same way, a positive genetic correlation has been shown between Aleutian disease tests and production traits, so it will be possible to select for Aleutian disease-resistant mink. Another study reports a new, high-quality chromosome-level genome assembly for American mink. Other genomic studies have identified chromosomal regions for skin quality traits and fur colors as well as the genomic basis for semi-aquatic adaptation in mink. Also new

to mink farming, is the use of machine learning algorithms to classify mink infected with Aleutian disease.

Investigations into SARS-CoV-2 still give rise to numerous research projects. A study shows that mink can serve as a model for studies of SARS-CoV-2 infections, as mink develop clinical features characteristic of severe COVID-19 in humans. Mink is therefore suitable for testing viral countermeasures. As Covid-19 is a zoonosis, investigations also continue into which animals - including production animals, animals in zoos and pets - can become infected with SARS-CoV-2 and also infect others. Among others, it has been shown that ferrets and raccoons can be experimentally infected.

Vivi Hunnicke Nielsen

Editor *Scientifur*

BREEDING, GENETICS AND REPRODUCTION**Genetic and Phenotypic Parameters for Pelt Quality and Body Length and Weight Traits in American Mink**

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Understanding the genetics of fur characteristics and skin size is important for developing effective breeding programs in the mink industry. Therefore, the objectives of this study were to estimate the genetic and phenotypic parameters for pelt quality traits including live grading overall quality (LQU), live grading nap size (LNAP), dried pelt size (DPS), dried pelt nap size (DNAP) and overall quality of dried pelt (DQU), and body length and weight traits, including November body weight (Nov_BW), November body length (Nov_BL), harvest weight (HW) and harvest length (HL) in American mink. Dried pelt quality traits on 1195 mink and pelt quality traits on live animals on 1680 were collected from mink raised at two farms, in Nova Scotia and Ontario. A series of univariate analyses were implemented in ASReml 4.1 software to identify the significance ($p < 0.05$) of random effects (maternal genetic effects, and common litter effects) and fixed effects (farm, sex, color type, year, and age) for each trait. Subsequently, bivariate models were used to estimate the genetic and phenotypic parameters using ASReml 4.1. Heritability (\pm SE) estimates were 0.41 ± 0.06 for DPS, 0.23 ± 0.10 for DNAP, 0.12 ± 0.04 for DQU, 0.28 ± 0.06 for LQU, 0.44 ± 0.07 for LNAP, 0.29 ± 0.10 for Nov_BW, 0.28 ± 0.09 for Nov_BL, 0.41 ± 0.07 for HW and 0.31 ± 0.06 for HL. DPS had high positive genetic correlations (\pm SE) with Nov_BW (0.89 ± 0.10), Nov_BL (0.81 ± 0.07), HW (0.85 ± 0.05) and HL (0.85 ± 0.06). These results suggested that body weight and length measured on live animals in November of the first year were reliable indicators of dried pelt size. DQU had favorable genetic correlations with

Nov_BL (0.55 ± 0.24) and HL (0.46 ± 0.20), and non-significant genetic correlations with DNAP (0.13 ± 0.25), Nov_BW (0.25 ± 0.25) and HW (0.06 ± 0.20), which made body length traits an appealing trait for selection for increased pelt size. High positive genetic correlation (\pm SE) was observed between LNAP and DNAP (0.82 ± 0.22), which revealed that nap size measurement on live animals is a reliable indicator trait for dried pelt nap size. However, nonsignificant ($p > 0.05$) low genetic correlation (\pm SE) was obtained between LQU and DQU (0.08 ± 0.45), showing that indirect selection based on live grading might not lead to the satisfactory improvement of dried pelt overall quality. The estimated genetic parameters for live grading, dried pelt quality, and body weight and body length traits may be incorporated into breeding programs to improve fur characteristics in Canadian mink populations.

Animals (Basel). 2022 Nov 17; 12 (22): 3184.

Doi: 10.3390/ani12223184.

Genetic and phenotypic correlations between Aleutian disease tests with body weight, growth, and feed efficiency traits in mink

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The ineffectiveness of vaccination, medicine, and culling strategy leads mink farmers to control Aleutian disease (AD) by selecting AD-resilient mink based on AD tests. However, the genetic background of AD tests and their correlations with economically important or AD-resilient traits are limited. This study estimated the genetic and phenotypic correlations between four AD tests and seven body weight

(BW) traits, six growth parameters from the Richards growth model, and eight feed-related traits. Univariate models were used to test the significance ($P < 0.05$) of fixed effects (sex, color type, AD test year, birth year, and row-by-year), random effects (additive genetic, maternal genetic, and permanent environmental), and a covariate of age using ASReml 4.1. Likewise, pairwise bivariate analyses were conducted to estimate the phenotypic and genetic correlations among the studied traits. Both antigen- and virus capsid protein-based enzyme-linked immunosorbent assay tests (ELISA-G and ELISA-P) showed significant ($P < 0.05$) moderate positive genetic correlations (\pm SE) with maturation rate (from 0.36 ± 0.18 to 0.38 ± 0.19). ELISA-G showed a significant negative genetic correlation (\pm SE) with average daily gain (ADG, -0.37 ± 0.16). ELISA-P showed a significant positive moderate genetic correlation (\pm SE) with off-feed days (DOF, 0.42 ± 0.17). These findings indicated that selection for low ELISA scores would reduce the maturation rate, increase ADG (by ELISA-G), and minimize DOF (by ELISA-P). The iodine agglutination test (IAT) showed significant genetic correlations with DOF (0.73 ± 0.16), BW at 16 weeks of age (BW16, 0.45 ± 0.23), and BW at harvest (HW, -0.47 ± 0.20), indicating that selection for lower IAT scores would lead to lower DOF and BW16, and higher HW. These estimated genetic correlations suggested that the selection of AD tests would not cause adverse effects on the growth, feed efficiency, and feed intake of mink. The estimates from this study might strengthen the previous finding that ELISA-G could be applied as a reliable and practical indicator trait in the genetic selection of AD-resilient mink in AD-positive farms.

J Anim Sci. 2022 Dec 1; 100 (12): skac346.
Doi: 10.1093/jas/skac346.

Serum Analytes of American Mink (*Neovison vison*) Challenged with Aleutian Mink Disease Virus

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Black American mink (*Neovison vison*), which had been selected for tolerance to Aleutian mink disease virus (AMDV) for more than 20 years (TG100) or

were from herds that have been free of AMDV (TG0), along with their progeny and crosses with 50% and 75% tolerance ancestry, were inoculated with a local isolate of AMDV. Blood samples were collected from 493 mink between 120 and 1211 days post-inoculation, and concentrations of 14 serum analytes were measured. Distributions of all analytes significantly deviated from normality, and data were analyzed after Box-Cox power transformation. Significant differences were observed among tolerant groups in the concentrations of globulin (GLO), total protein (TP), alkaline phosphatase, urea nitrogen, and calcium. Concentrations of GLO and TP linearly and significantly decreased with an increasing percentage of tolerance ancestry. Eleven analytes had the smallest values in the tolerant groups (TG100 or TG75), and eight analytes had the greatest values in the non-selected groups (TG0 or TG50). Antibody titer had the greatest correlation coefficients with GLO (0.62), TP (0.53), and creatinine (0.36). It was concluded that selection for tolerance decreased the concentrations of most serum analytes, and TP and GLO were the most accurate biomarkers of tolerance to AMDV infection. Males had significantly greater values than females for phosphorus and total bilirubin concentrations, but females had significantly greater amylase, cholesterol, and BUN concentrations than males.

Animals (Basel) 2022 Oct 11; 12 (20): 2725.
Doi: 10.3390/ani12202725.

Long-term antibody production and viremia in American mink (*Neovison vison*) challenged with Aleutian mink disease virus

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Background

Selecting American mink (*Neovison vison*) for tolerance to Aleutian mink disease virus (AMDV) has

gained popularity in recent years, but data on the outcomes of this activity are scant. The objectives of this study were to determine the long-term changes in viremia, seroconversion and survival in infected mink. Mink were inoculated intranasally with a local isolate of Aleutian mink disease virus (AMDV) over 4 years ($n = 1742$). The animals had been selected for tolerance to AMDV for more than 20 years (TG100) or were from herds free of AMDV (TG0). The progenies of TG100 and TG0, and their crosses with 25, 50 and 75% tolerance ancestry were also used. Blood samples were collected from each mink up to 14 times until 1211 days post-inoculation (dpi) and were tested for viremia by PCR and for anti-AMDV antibodies by counter-immunoelectrophoresis (CIEP). Viremia and CIEP status were not considered when selecting replacements. Low-performing animals were pelted and the presence of antibodies in their blood and antibody titer were measured by CIEP, and viremia and viral DNA in seven organs ($n = 936$) were tested by PCR.

Results

The peak incidences of viremia (66.7%) and seropositivity (93.5%) were at 35 dpi. The incidence of viremia decreased over time while the incidence of seroconversion increased. The least-squares means of the incidence of PCR positive of lymph node (0.743) and spleen (0.656) were significantly greater than those of bone marrow, liver, kidneys, lungs and small intestine (0.194 to 0.342). Differences in tolerant ancestry were significant for every trait measured. Incidences of viremia over time, terminal viremia, seropositivity over time, AMDV DNA in organs and antibody titer were highest in the susceptible groups (TG0 or TG25) and lowest in the tolerant groups (TG100 or TG75).

Conclusion

Previous history of selection for tolerance resulted in mink with reduced viral replication and antibody titer. Viremia had a negative effect and antibody production had a positive effect on survival and productivity.

BMC Vet Res. 2022 Oct 3; 18 (1): 364.
Doi: 10.1186/s12917-022-03462-7.

Genome-Wide Detection of Selection Signatures for Pelt Quality Traits and Coat Color Using Whole-Genome Sequencing Data in American Mink

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Domestication and selection are the major driving forces responsible for creating genetic variability in farmed species. American mink has been under selection for more than 100 years for improved body size and pelt quality. This study aimed to identify the genomic regions subjected to selection for pelt quality traits, and coat color using the whole genome sequences of 100 mink raised in the Canadian Centre for Fur Animal Research (CCFAR) at Dalhousie Agriculture Campus (Truro, NS, Canada), and Millbank fur farm (Rockwood, ON, Canada). Measurements of three dried pelt characteristics (including pelt size ($n = 35$), overall quality of fur ($n = 27$), and nap size ($n = 29$)), and three coat color of Black, Stardust, and Pastel (Stardust_Black ($n = 38$), and Pastel_Black ($n = 41$)) were used to assign animals to pairwise groups. Signatures of selection were detected using integrated measurement of fixation index (Fst), extended haplotype homozygosity (XP-EHH), and nucleotide diversity ($\theta\pi$) tests. In total, overlapping top 1% of Fst and XP-EHH harbored 376 genes for pelt quality traits (110 for nap size, 163 for overall quality of fur, and 98 pelt size), and 194 genes for coat color (123 for Pastel_Black and 71 for Stardust_Black) were detected in different groups. Integrating results of Fst, and XP-EHH with the $\theta\pi$ test supported 19 strongly selected regions on chromosomes 3, 4, 5, 6, 7, 8, 9, and 10 that contained 33 candidate genes related to fur quality, hair follicle function, and pelt size traits. Gene ontology revealed numerous genes related to the hair cycle process and molting cycle process, epidermis development, Wnt signaling pathway and muscle development. This study provided the first map of putative selection signals related to pelt quality and coat color in American mink, which

could be used as a reference for future studies attempting to identify genes associated with economically important traits in mink.

Genes (Basel). 2022 Oct 25; 13 (11): 1939.
Doi: 10.3390/genes13111939.

A chromosome-level genome assembly reveals genomic characteristics of the American mink (*Neogale vison*)

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Availability of a contiguous chromosome-level genome assembly is the foundational step to develop genome-based studies in American mink (*Neogale vison*). The main objective of this study was to provide a high quality chromosome-level genome assembly for American mink. An initial draft of the genome assembly was generated using 2,884,047 PacBio long reads. Integration of Hi-C data into the initial draft led to an assembly with 183 scaffolds and scaffold N50 of 220 Mb. This gap-free genome assembly of American mink (ASM_NN_V1) had a length of 2.68 Gb in which about 98.6% of the whole genome was covered by 15 chromosomes. In total, 25,377 genes were predicted across the American mink genome using the NCBI Eukaryotic Genome Annotation Pipeline. In addition, gene orthology, demographic history, synteny blocks, and phylogenetic relationships were studied in connection with the genomes of other related Carnivora. Furthermore, population-based statistics of 100 sequenced mink were presented using the newly assembled genome. Remarkable improvements were observed in genome

contiguity, the number of scaffolds, and annotation compared to the first draft of mink genome assembly (NNQGG.v01). This high-quality genome assembly will support the development of efficient breeding strategies as well as conservation programs for American mink.

Commun Biol. 2022 Dec 16; 5 (1): 1381.
Doi: 10.1038/s42003-022-04341-5.

Comparative Genome Analysis Reveals the Genomic Basis of Semi-Aquatic Adaptation in American Mink (*Neovison vison*)

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Although the American mink is extremely dependent on water and has evolved a range of aquatic characteristics, its structural adaptation to water is still less obvious than that of other typical semi-aquatic mammals, such as otters. Therefore, many scholars consider it not to be a semi-aquatic mammal. In order to make the point that minks are semi-aquatic mammals more convincing, we provide evidence at the micro (genome)-level. In particular, we used the genomes of the American mink and 13 mammalian species to reconstruct their evolutionary history, identified genes that affect aquatic adaptation, and examined the evolution of aquatic adaptation. By analyzing unique gene families, the expansion and contraction of gene families, and positive selection genes, we found that the American mink genome has evolved specifically for aquatic adaptation. In particular, we found that the main adaptive characteristics of the American mink include the external structural characteristics of bone and hair development, as well as the internal physiological characteristics of immunity, olfaction, coagulation, lipid metabolism, energy metabolism, and nitrogen metabolism. We also observed that the genomic characteristics of the American mink are similar to those of other aquatic and semi-aquatic mammals. This not only provides solid genomic evidence for the idea that minks are semi-aquatic mammals, but also leads to a clearer understanding of semi-aquatic species. At the same time,

this study also provides a reference for the protection and utilization of the American mink.

Animals (Basel). 2022 Sep 13; 12 (18): 2385.
Doi: 10.3390/ani12182385.

The Mechanisms of Fur Development and Color Formation in American Mink Revealed Using Comparative Transcriptomics

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American mink fur is an important economic product, but the molecular mechanisms underlying its color formation and fur development remain unclear. We used RNA-seq to analyze the skin transcriptomes of young and adult mink with two different hair colors. The mink comprised black adults (AB), white adults (AW), black juveniles (TB), and white juveniles (TW) (three each). Through pair comparison and cross-screening among different subgroups, we found that 13 *KRTAP* genes and five signaling pathways (the JAK-STAT signaling pathway (cfa04630), signaling pathways regulating pluripotency of stem cells (cfa04550), ECM-receptor interaction (cfa04512), focal adhesion (cfa04510), and the Ras signaling pathway (cfa04014)) were related to mink fur development. We also found that members of a tyrosinase family (*TYR*, *TYRP1*, and *TYRP2*) are involved in mink hair color formation. The expression levels of *TYR* were higher in young black mink than in young white mink, but this phenomenon was not observed in adult mink. Our study found significant differences in adult and juvenile mink skin transcriptomes, which may shed light on the mechanisms of mink fur development. At the same time, the skin transcriptomes of black and white mink also showed differences, with the results varying by age, suggesting that the genes regulating hair color are active in early development rather than in adulthood. The results of this study provide molecular support in breeding for mink coat color and improving fur quality.

Animals (Basel). 2022 Nov 9; 12 (22): 3088.

Doi: 10.3390/ani12223088.

Involvement of testicular N-glycoproteome heterogeneity in seasonal spermatogenesis of the American mink (*Neovison vison*)

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Spermatogenesis in the American mink is characterized by an annual cycle of transition involving completely inactive and fully activated stages. N-glycosylation of proteins has emerged as an important regulator as it affects protein folding, secretion, degradation, and activity. However, the function of protein N-glycosylation in seasonal spermatogenesis of the American mink remains unclear. In the present study, we established a proteome-wide stoichiometry of N-glycosylation in mink testes at various phases of spermatogenesis using N-linked glycosylated-peptide enrichment in combination with liquid chromatography-tandem mass spectrometry analysis. A total of 532 N-glycosylated sites matching the canonical Asn-X-Ser/Thr motif were identified in 357 testicular proteins. Both the number of glycoproteins and the sites of N-glycosylated proteins in mink testes were highly dynamic at different stages. Functional analyses showed that testicular proteins with different N-glycosylation might play a vital role in spermatogenesis by affecting their folding, distribution, stability, and activity. Overall, our data suggest that the dynamics of N-glycosylation of testicular proteins are involved in seasonal spermatogenesis in the American mink.

Front Vet Sci. 2022 Nov 4; 9: 1001431.

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Factors influencing the timing and frequency of litters in captive fennec foxes (*Vulpes zerda*)

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NUTRITION, FEEDING AND MANAGEMENT

Requirements and Metabolism for Calcium, Phosphorus and Vitamin D₃ in the Growing-Furring Blue Foxes

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A 3 × 3 factorial experiment was conducted to investigate the influence of dietary calcium, phosphorus, and vitamin D₃ (VD₃) supplement levels on the growth performance, nutrient digestibility, and serum biochemical indices of growing-furring blue foxes. One hundred and thirty-five 120-day-old male blue foxes were randomly allocated into nine groups. The nine treatment diets were supplemented with 0%, 0.4%, or 0.8% Ca, and 1000, 2000, or 4000 IU·kg⁻¹ VD₃. The base diet contained 0.8% Ca and 327 IU·kg⁻¹ VD₃. The dietary calcium level had a significant effect on the average daily gain (ADG) of blue foxes at 121 to 135 days of age and 136 to 150 days ($p < 0.05$). The ADG of blue foxes at 121 to 135 days of age was significantly decreased by VD₃ level ($p < 0.05$). The Ca dosage decreased the nutrient digestibility ($p < 0.05$). The Ca dosage increased the fecal Ca and P and decreased the P digestibility ($p < 0.05$). Interactions were found between the Ca and VD₃ levels, which affected the digestibility of Ca and P ($p < 0.05$). In conclusion, this research determined the suitable doses of Ca and VD₃ for growing-furring blue foxes.

Animals (Basel). 2022 Oct 14; 12 (20): 2776.

Doi: 10.3390/ani12202776.

Effect of n-6/n-3 PUFA ratio on body fat deposition, tissues fatty acid composition and key genes expression of liver lipid metabolism in silver foxes (*Vulpes vulpes fulva*) during the winter fur-growth period

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Objective

The proportion of n-6/n-3 polyunsaturated fatty acid (PUFA) plays an important role in regulating lipid metabolism. This study aimed to investigate the effects of dietary n-6/n-3 PUFA ratios on body fat deposition, tissues fatty acid composition, and gene expression of liver lipid metabolism of silver foxes during the winter fur growth period.

Methods

Forty-eight age-matched male silver foxes with similar body weights were randomly divided into four dietary groups for 47 days, which were fed n-6/n-3 PUFA ratio with 3, 18, 41, and 136 experimental diets, respectively.

Results

Dietary n-6/n-3 PUFA ratio did not significantly influence fat deposition parameters except for hepatic fat content. The variation trend of the fatty acid composition of liver, intramuscular fat, and subcutaneous fat in silver fox was directly related to dietary fatty acid content ($p < 0.05$). With the dietary n-6/n-3 PUFA ratio increasing, the expression of liver fatty acid synthase (FAS) mRNA and peroxisome proliferator-activated receptor (PPAR) mRNA exhibited the trend of first decreasing and then increasing ($p < 0.05$), whereas L-fatty acid binding protein (L-FABP) mRNA expression showed a gradual increasing trend ($p < 0.05$).

Conclusion

In summary, silver foxes fed an n-6/n-3 PUFA ratio 18:1 diet (supplementing with 9.38% corn oil and 4.62% soybean oil) was more conducive to lipid decomposition, PUFA transport, and utilization of tis-

sues, thereby meeting it for supplying energy and withstanding the cold.

Front Vet Sci. 2022 Oct 13; 9: 986388.
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Applying Machine Learning Algorithms for the Classification of Mink Infected with Aleutian Disease Using Different Data Sources

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American mink (*Neogale vison*) is one of the major sources of fur for the fur industries worldwide, whereas Aleutian disease (AD) is causing severe financial losses to the mink industry. A counterimmunoelectrophoresis (CIEP) method is commonly employed in a test-and-remove strategy and has been considered a gold standard for AD tests. Although machine learning is widely used in livestock species, little has been implemented in the mink industry. Therefore, predicting AD without using CIEP records will be important for controlling AD in mink farms. This research presented the assessments of the CIEP classification using machine learning algorithms. The Aleutian disease was tested on 1157 individuals using CIEP in an AD-positive mink farm (Nova Scotia, Canada). The comprehensive data collection of 33 different features was used for the classification of AD-infected mink. The specificity, sensitivity, accuracy, and F1 measure of nine machine learning algorithms were evaluated for the classification of AD-infected mink. The nine models were artificial neural networks, decision tree, extreme gradient boosting, gradient boosting method, K-nearest neighbors, linear discriminant analysis, support vector machines, naive bayes, and random forest. Among the 33 tested features, the Aleutian mink disease virus capsid protein-based enzyme-linked immunosorbent assay was found to be the most important feature for classifying AD-infected mink. Overall, random forest was the best-performing algorithm for the current dataset with a mean sensitivity of 0.938 ± 0.003 , specificity of 0.986 ± 0.005 , accuracy of 0.962 ± 0.002 , and F1 value of 0.961 ± 0.088 , and across tenfold of the cross-validation. Our work

demonstrated that it is possible to use the random forest algorithm to classify AD-infected mink accurately. It is recommended that further model tests in other farms need to be performed and the genomic information needs to be used to optimize the model for implementing machine learning methods for AD detection.

Animals (Basel). 2022 Sep 13; 12 (18): 2386.
Doi: 10.3390/ani12182386.

GPS Tracking of Free-Roaming Cats (*Felis catus*) on SARS-CoV-2-Infected Mink Farms in Utah

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Zoonotic transmission of SARS-CoV-2 from infected humans to other animals has been documented around the world, most notably in mink farming operations in Europe and the United States. Outbreaks of SARS-CoV-2 on Utah mink farms began in late July 2020 and resulted in high mink mortality. An investigation of these outbreaks revealed active and past SARS-CoV-2 infections in free-roaming and in feral cats living on or near several mink farms. Cats were captured using live traps, were sampled, fitted with GPS collars, and released on the farms. GPS tracking of these cats show they made frequent visits to mink sheds, moved freely around the affected farms, and visited surrounding residential properties and neighborhoods on multiple occasions, making them potential low risk vectors of additional SARS-CoV-2 spread in local communities.

Viruses. 2022 Sep 27; 14 (10): 2131.
Doi: 10.3390/v14102131.

The genetic diversity and structure in the European polecat were not affected by the introduction of the American mink in Poland

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The introduction and expansion of an invasive non-native species could have important consequences for the genetic patterns and processes of native species, moreover if the new arrival competes strongly for resources and space. This may result in the demographic decline of the native species. Knowing the effects on the levels of genetic diversity and structure in native species is key in terms of their conservation. We analysed temporal (over 50 years) genetic variation of the population of the European polecat (*Mustela putorius*), a species under threat in several European countries, in the Białowieża Primeval Forest (BPF), Poland, before and after the invasion of the American mink (*Neovison vison*). Using 11 microsatellite loci and a fragment of the mitochondrial control region we show that levels of diversity changed in the polecat population over 53 generations (over the period 1959-2012) and after the invasion of mink. When compared with other threatened European polecat populations, high levels of diversity are observed in the population in BPF in both periods, as well as in other areas in Poland. Our data shows that genetic structure was not present either before or after the mink invasion in BPF. This would suggest that the polecat population in Poland was not affected by invasive species and other negative factors and would be a potential good source of individuals for captive breeding or genetic rescue conservation management actions in areas where such actions are needed, for example the UK.

PLoS One. 2022 Sep 28; 17(9): e0266161.
Doi: 10.1371/journal.pone.0266161.
eCollection 2022.

BEHAVIOUR AND WELFARE

A mink (*Neovison vison*) model of self-injury: Effects of CBP-CREB axis on neuronal damage and behavior

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Objective

Self-injurious behavior (SIB) is a clinically challenging problem in the general population and several clinical disorders. However, the precise molecular mechanism of SIB is still not clear. In this paper, the systematic investigation of the genesis and development of SIB is conducted based on behavioral and pathophysiology studies in mink (*Neovison vison*) models.

Method

The night-vision video was used to observe the mink behavior, and the duration was a month. HE stain was performed to characterize the pathology change in the brain of a mink. IHC assay was performed to conduct the protein level detection of Iba-1, p-CREB, CBP, and p300 in the brain tissues. Elisa assay was used to examine the levels of NfL and NfH in serum and CSF of mink. The qRT-PCR assay was used to detect the expression of *Bcl-2*, *NOR1*, *FoxO4*, *c-FOS*, *CBP*, and *p300* in brain tissues. Western blot was used to detect the protein levels of p-CREB, CBP, and p300 in brain tissues. We also used Evans Blue as a tracer to detect whether the blood-brain barrier was impaired in the brain of mink.

Result

The behavioral test, histopathological and molecular biology experiments were combined in this paper, and the results showed that CBP was related to SIB. Mechanism analysis showed that the dysregulation of CBP in brain-activated CREB signaling will result in nerve damage of the brain and SIB symptoms in minks. More importantly, the CBP-CREB interaction inhibitor might help relieve SIB and nerve damage in brain tissues.

Conclusion

Our results illustrate that the induction of CBP and the activation of CREB are novel mechanisms in the genesis of SIB. This finding indicates that the CBP-CREB axis is critical for SIB and demonstrates the

efficacy of the CBP-CREB interaction inhibitor in treating these behaviors.

Front Vet Sci. 2022 Nov 10; 9: 975112.

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HEALTH AND DISEASE

Antiviral activity of mink interferon alpha expressed in the yeast *Pichia pastoris*

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Many viruses can cause infections in mink, including canine distemper virus, mink enteritis virus, and Aleutian disease virus. Current treatments are ineffective, and these infections are often fatal, causing severe economic losses. As antiviral drugs may effectively prevent and control these infections, recent research has increasingly focused on antiviral interferons. Herein, the gene encoding a mature mink interferon alpha (MiIFN- α) was synthesized according to the *P. pastoris* preference of codon usage and a recombinant plasmid, pPICZ α A-MiIFN- α , was constructed. pPICZ α A-MiIFN- α was linearized and transformed into the *P. pastoris* X33 strain, and zeocin-resistant transformants were selected. Protein expression was induced by methanol. SDS-PAGE and western blot analyses showed that a 25-kDa fusion protein was expressed in the culture supernatant. Antiviral activity of the expressed protein was determined using cytopathic effect inhibition (CPEI). The purified MiIFN- α significantly inhibited the cytopathic effect of vesicular stomatitis virus with a green fluorescent protein (VSV-GFP) in F81 feline kidney cells, with an antiviral activity of 6.4×10^7 IU/mL; it also significantly inhibited MEV replication in F81 cells. MiIFN- α antiviral activity against VSV-GFP was significantly reduced on treatment with pH 4 and pH 10 conditions for 24 h ($p < 0.01$). Serum MiIFN- α concentrations in rat were measured using enzyme-linked immune-sorbent assay; MiIFN- α concentra-

tions in rat serum peaked at ~36 h after injection. A high dose of MiIFN- α was safe for use. There were no significant differences in body temperature, tissue changes, and lymphocyte, total white blood cell, and central granulocyte counts between the injected and control groups ($p > 0.05$). These findings lay a foundation for the large-scale production of recombinant MiIFNs.

Front Vet Sci. 2022 Sep 14; 9: 976347.

Doi: 10.3389/fvets.2022.976347. eCollection 2022.

Capsid Structure of Aleutian Mink Disease Virus and Human Parvovirus 4: New Faces in the Parvovirus Family Portrait

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Parvoviruses are small, single-stranded DNA viruses with non-enveloped capsids. Determining the capsid structures provides a framework for annotating regions important to the viral life cycle. Aleutian mink disease virus (AMDV), a pathogen in minks, and human parvovirus 4 (PARV4), infecting humans, are parvoviruses belonging to the genera *Amdoparvovirus* and *Tetraparvovirus*, respectively. While Aleutian mink disease caused by AMDV is a major threat to mink farming, no clear clinical manifestations have been established following infection with PARV4 in humans. Here, the capsid structures of AMDV and PARV4 were determined via cryo-electron microscopy at 2.37 and 3.12 Å resolutions, respectively. Despite low amino acid sequence identities (10-30%) both viruses share the icosahedral nature of parvovirus capsids, with 60 viral proteins (VPs) assembling the capsid via two-, three-, and

five-fold symmetry VP-related interactions, but display major structural variabilities in the surface loops when the capsid structures are superposed onto other parvoviruses. The capsid structures of AMDV and PARV4 will add to current knowledge of the structural platform for parvoviruses and permit future functional annotation of these viruses, which will help in understanding their infection mechanisms at a molecular level for the development of diagnostics and therapeutics.

Viruses. 2022 Oct 9; 14 (10): 2219.
Doi: 10.3390/v14102219.

First Identification and Phylogenetic Analysis of Porcine Circovirus Type 4 in Fur Animals in Hebei, China

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A novel circovirus called porcine circovirus type 4 (PCV4) was recently detected in pigs suffering from severe clinical diseases in Hunan province, China. There are few reports on the origin and evolution of PCV4, although some researchers have conducted epidemiological investigations of PCV4 and found that PCV4 is widespread in pigs. Based on the previous study, we detected PCV2 in farmed foxes and raccoon dogs with reproductive failure. To explore whether the PCV4 genome also exists in fur animals, we detected 137 cases admitted from fur animal farms in Hebei China between 2015 and 2020, which were characterized by inappetence, lethargy, depression, abortion, and sterility. The overall infection rate of PCV4 was 23.36% (32/137), including 20.37% (22/108) for raccoon dogs, 18.75% (3/16) for foxes, and 53.85% (7/13) for minks. Finally, five raccoon dog-origin PCV4 strains and one fox-origin PCV4 strain were sequenced in our study, whose nucleotide identities with other representative PCV4 strains varied from 96.5% to 100%. Phylogenetic analysis based on the complete genomes of PCV4 strains indicated a close relationship with those of PCV4

strains identified from pigs. To our knowledge, this is the first study to detect PCV4 in fur animals. Interestingly, we also identified PCV4 in a mixed farm (feeding pigs and raccoon dogs at the same time). In summary, our findings extend the understanding of the molecular epidemiology of PCV4 and provide new evidence for its cross-species transmission.

Animals (Basel). 2022 Nov 28; 12 (23): 3325.
Doi: 10.3390/ani12233325.

Severe acute respiratory disease in American mink experimentally infected with SARS-CoV-2

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An animal model that fully recapitulates severe COVID-19 presentation in humans has been a top priority since the discovery of SARS-CoV-2 in 2019. Although multiple animal models are available for

mild to moderate clinical disease, models that develop severe disease are still needed. Mink experimentally infected with SARS-CoV-2 developed severe acute respiratory disease, as evident by clinical respiratory disease, radiological, and histological changes. Virus was detected in nasal, oral, rectal, and fur swabs. Deep sequencing of SARS-CoV-2 from oral swabs and lung tissue samples showed repeated enrichment for a mutation in the gene encoding non-structural protein 6 in open reading frame 1ab. Together, these data indicate that American mink develop clinical features characteristic of severe COVID-19 and, as such, are uniquely suited to test viral countermeasures.

JCI Insight. 2022 Nov 22; 7 (22): e159573.
Doi: 10.1172/jci.insight.159573.

Limited permissibility of ENL-R and Mv-1-Lu mink cell lines to SARS-CoV-2

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The SARS-CoV-2 pandemic started in the end of 2019 in Wuhan, China, which highlighted the scenario of frequent cross-species transmission events. From the outbreak possibly initiated by viral spill-over into humans from an animal reservoir, now we face the human host moving globally while interacting with domesticated and peridomestic animals. The emergence of a new virus into the ecosystem leads to selecting forces and species-specific adaptations. The

adaptation of SARS-CoV-2 to other animals represents a risk to controlling the dissemination of this coronavirus and the emergence of new variants. Since 2020, several mink farms in Europe and the United States have had SARS-CoV-2 outbreaks with human-mink and mink-human transmission, where the mink-selected variants possibly hold evolutionary concerning advantages. Here we investigated the permissibility of mink lung-derived cells using two cell lines, Mv-1-Lu and ENL-R, against several lineages of SARS-CoV-2, including some classified as variants of concern. The viral release rate and the infectious titers indicate that these cells support infections by different SARS-CoV-2 lineages. The viral production occurs in the first few days after infection with the low viral release by these mink cells, which is often absent for the omicron variant for lung cells. The electron microscopy reveals that during the viral replication cycle, the endomembrane system of the mink-host cell undergoes typical changes while the viral particles are produced, especially in the first days of infection. Therefore, even if limited, mink lung cells may represent a selecting source for SARS-CoV-2 variants, impacting their transmissibility and pathogenicity and making it difficult to control this new coronavirus.

Front Microbiol. 2022 Oct 12; 13: 1003824.
Doi: 10.3389/fmicb.2022.1003824.
eCollection 2022.

A Deletion Encompassing the Furin Cleavage Site in the Spike Encoding Gene Does Not Alter SARS-CoV-2 Replication in Lung Tissues of Mink and Neutralization by Convalescent Human Serum Samples

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SARS-CoV-2 has been shown to lose the furin polybasic cleavage site (FCS) following adaptation on cell culture. Deletion occurring in this region, which may include also the FCS flanking regions, seem not to affect virus replication *in vitro*; however, a chimeric SARS-CoV-2 virus without the sole FCS motif has been associated with lower virulence in mice and lower neutralization values. Moreover, SARS-CoV-2 virus lacking the FCS was shed to lower titers from experimentally infected ferrets and was not transmitted to cohoused sentinel animals, unlike wild-type virus. In this study, we investigated the replication kinetics and cellular tropism of a SARS-CoV-2 isolate carrying a 10-amino acid deletion in the spike protein spanning the FCS in lung *ex vivo* organ cultures of mink. Furthermore, we tested the neutralization capabilities of human convalescent SARS-CoV-2 positive serum samples against this virus. We showed that this deletion did not significantly hamper neither *ex vivo* replication nor neutralization activity by convalescent serum samples. This study highlights the importance of the preliminary phenotypic characterization of emerging viruses in *ex vivo* models and demonstrates that mink lung tissues are permissive to the replication of a mutant form of SARS-CoV-2 showing a deletion spanning the FCS. Notably, we also highlight the need for sequencing viral stocks before any infection study as large deletions may occur leading to the misinterpretation of results.

Pathogens. 2022 Oct 6; 11 (10): 1152.
Doi: 10.3390/pathogens11101152.

SARS CoV-2 infections in animals, two years into the pandemic

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In December 2019, several cases of pneumonia caused by a novel coronavirus, later identified as SARS-CoV-2, were detected in the Chinese city of Wuhan. Due to its rapid worldwide spread, on 11 March 2020 the World Health Organization declared a pandemic state. Since this new virus is genetically

similar to the coronaviruses of bats, SARS-CoV-2 was hypothesized to have a zoonotic origin. Within a year of the appearance of SARS-CoV-2, several cases of infection were also reported in animals, suggesting human-to-animal and animal-to-animal transmission among mammals. Natural infection has been found in companion animals as well as captive animals such as lions, tigers, and gorillas. Among farm animals, so far, minks have been found to be susceptible to SARS-CoV-2 infection, whereas not all the relevant studies agree on the susceptibility of pigs. Experimental infections have documented the susceptibility to SARS-CoV-2 of further animal species, including mice, hamsters, cats, dogs, ferrets, raccoon dogs, cattle, and non-human primates. Experimental infections have proven crucial for clarifying the role of animals in transmission and developing models for viral pathogenesis and immunotherapy. On the whole, this review aims to update and critically revise the current information on natural and experimental SARS-CoV-2 infections in animals.

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SARS-CoV-2 as a Zoonanthropotic Infection: Spillbacks, Secondary Spillovers, and Their Importance

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In the midst of a persistent pandemic of a probable zoonotic origin, one needs to constantly evaluate the interplay of SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus-2) with animal populations. Animals can get infected from humans, and certain species, including mink and white-tailed deer,

exhibit considerable animal-to-animal transmission resulting in potential endemicity, mutation pressure, and possible secondary spillover to humans. We attempt a comprehensive review of the available data on animal species infected by SARS-CoV-2, as presented in the scientific literature and official reports of relevant organizations. We further evaluate the lessons humans should learn from mink outbreaks, white-tailed deer endemicity, zoo outbreaks, the threat for certain species conservation, the possible implication of rodents in the evolution of novel variants such as Omicron, and the potential role of pets as animal reservoirs of the virus. Finally, we outline the need for a broader approach to the pandemic and epidemics, in general, incorporating the principles of One Health and Planetary Health.

Microorganisms. 2022 Oct 31; 10 (11): 2166.
Doi: 10.3390/microorganisms10112166.

Susceptibility of SARS Coronavirus-2 infection in domestic and wild animals: a systematic review

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3 Biotech. 2023 Jan; 13 (1): 5.
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Transmission dynamics and susceptibility patterns of SARS-CoV-2 in domestic, farmed and wild animals: Sustainable One Health surveillance for conservation and public health to prevent future epidemics and pandemics

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Transbound Emerg Dis. 2022 Sep; 69 (5): 2523-2543.
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Host adaptation of codon usage in SARS-CoV-2 from mammals indicates potential natural selection and viral fitness

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Inhibition of transcription of VP2 by mutations in the DNA binding domains of mink enteritis virus NS1 protein

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The NS1 protein of mink enteritis virus (MEV) is a multidomain and multifunctional protein that plays a critical role in viral replication, with predicted nuclease, helicase and transactivation activities. The nuclease and helicase domains of NS1 protein are involved in interaction with viral DNA. Herein, potential amino acids critical for DNA binding in the MEV NS1 were mutated, all of which resulted in a termination of viral production from an infectious MEV clone. Although E121, H129/131, Y212 and K470/472 mutants retained their P38 and 5'UTR transactivation activity, K196/197 and K406 mutations eliminated this. Interestingly, VP2 protein was produced following transfection of F81 cells with pMEV-NS1-196K2G (K196G and K197G) and pMEV-NS1-K406G when pNS1 was co-transfected in trans, indicating that the substitutions did not affect the integrity of the DNA sequence that bound to NS1 protein but inhibited the biological properties of NS1 protein itself. The ability of NS1 protein to interact with SP1 was inhibited by both 196K2G and K406G substitutions, while 196K2G resulted in failure to bind to the DNA-binding sites in the P38 promoter, and the oligomerization of K406G was inhibited. All of these could explain the transcriptional repression.

Virus Res. 2022 Oct 17; 323: 198972.

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Transcriptional activation of Mink enteritis virus VP2 by the C-terminal of its NS1 protein

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Virus Genes. 2022 Oct 22.

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The Prevalence of *Salmonella* spp. in Two Arctic Fox (*Alopex lagopus*) Farms in Poland

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The objective of the study was to determine the occurrence of *Salmonella* spp. infections in two Arctic fox (*Alopex lagopus*) farms in Poland, and to analyse the correlations between animals that tested positive for *Salmonella* spp and breeding results. Faecal samples were taken from 1094 clinically healthy blue foxes from the basic stock of farms A and B. *Salmonella* spp. were detected in 18.06% (56/310) of the samples collected in farm A and in 15.94% (125/784) of the samples collected in farm B. All isolated strains belonged to *S. enterica* subsp. *enterica* serotypes *Salmonella* Saintpaul (*S. Saintpaul*), *Salmonella* Reading (*S. Reading*), and *Salmonella* Heidelberg (*S. Heidelberg*). All three serotypes are typically isolated from commercial poultry flocks. *Salmonella* spp. infections significantly increased the risk of female infertility, but further research is needed to confirm the results. This is the first report on the prevalence of *S. Heidelberg*, *S. Saintpaul*, and *S. Reading* in faecal samples collected from Arctic fox (*Alopex lagopus*) farms in Poland.

Animals (Basel). 2020 Sep 18; 10 (9): 1688.
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Novel study on the prevalence of *Trichinella spiralis* in farmed American minks (*Neovison vison*) associated with exposure to wild rats (*Rattus norvegicus*) in China

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Zoonoses Public Health. 2022 Dec; 69 (8): 938-943.
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Urolithiasis in foxes: Assessment of 65 urolith submissions to the MINNESOTA Urolith Center from 1981 to 2021

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This article summarizes urinary stone submissions from foxes in human care to the Minnesota Urolith Center over 40 years. A previous report documented the analysis of uroliths from foxes that were submitted between 1981 and 2007.¹³ New data compiled from 2008 to 2021 included an additional 38 stones submitted from foxes, totaling 65 fox urolith submissions from 1981 to 2021. Struvite and cystine uroliths were most common, with the remainder comprised of

calcium phosphate, calcium oxalate, compound, mixed, or miscellaneous material. Most stones were submitted from male foxes. Seventy-two percent of the stones were urocystoliths, and from 2010 to 2021, most stones were diagnosed antemortem and removed surgically. More than half of the stones were submitted from red foxes (*Vulpes vulpes*) and fennec foxes (*Vulpes zerda*). Urolithiasis in foxes may be an underrecognized condition, and data from this study suggest that clinicians should consider routine urinalysis and diagnostic imaging as part of the preventive medicine program for fox species, especially red foxes and fennec foxes.

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

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