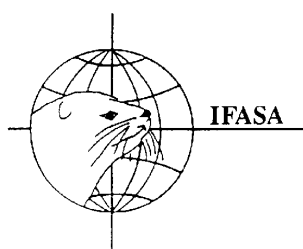
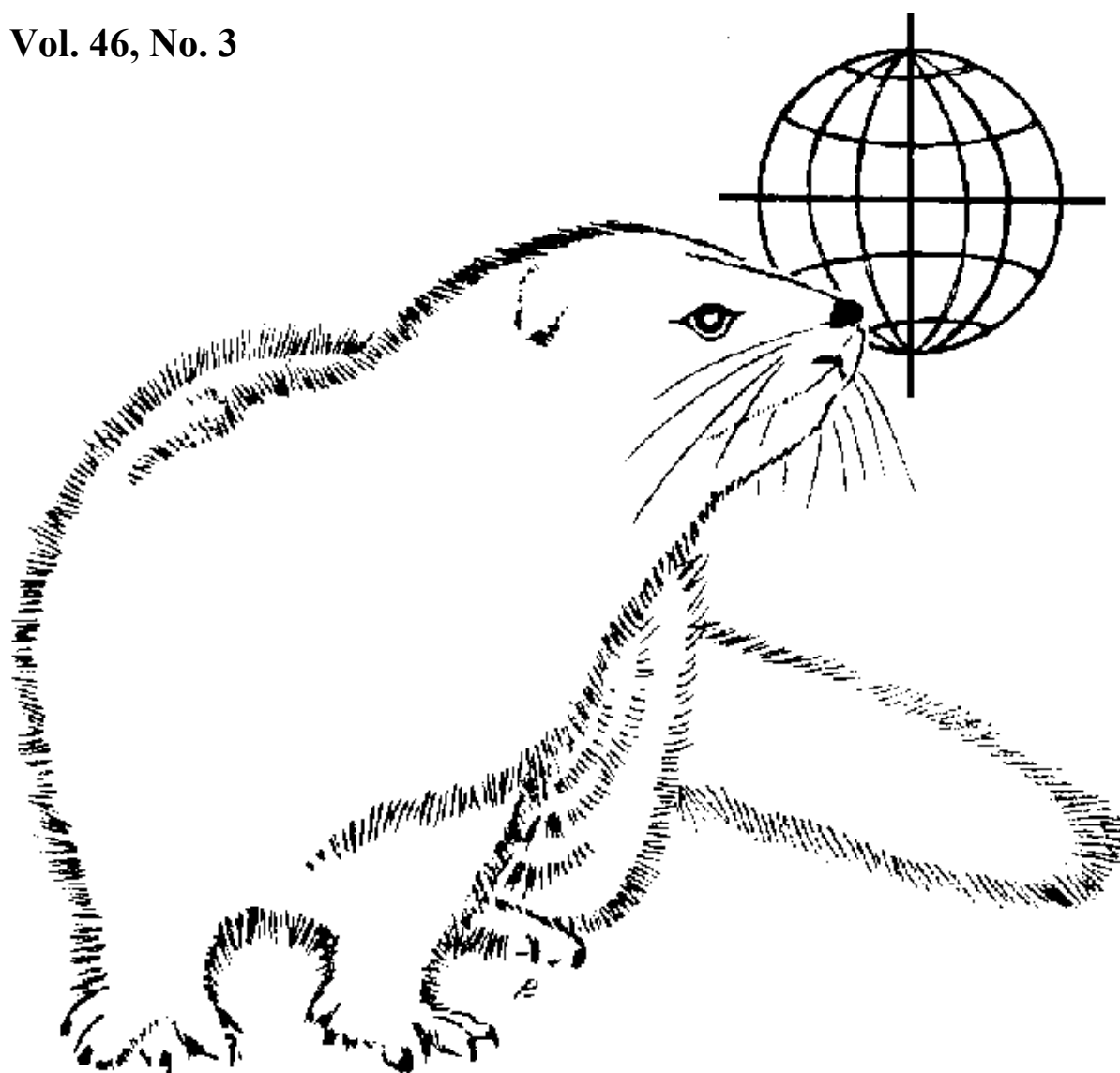


SCIENTIFUR

SCIENTIFIC INFORMATION IN FUR ANIMAL PRODUCTION

Vol. 46, No. 3



INTERNATIONAL FUR ANIMAL SCIENTIFIC ASSOCIATION

SCIENTIFUR scientific information for those involved in fur animal production is published by the International Fur Animal Scientific Association (IFASA).

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SCIENTIFUR is published as four issues per year (one volume).

SCIENTIFIC ARTICLES. Papers forwarded can be published in Scientifur. The scientific content of the article is the sole responsibility of the author(s)

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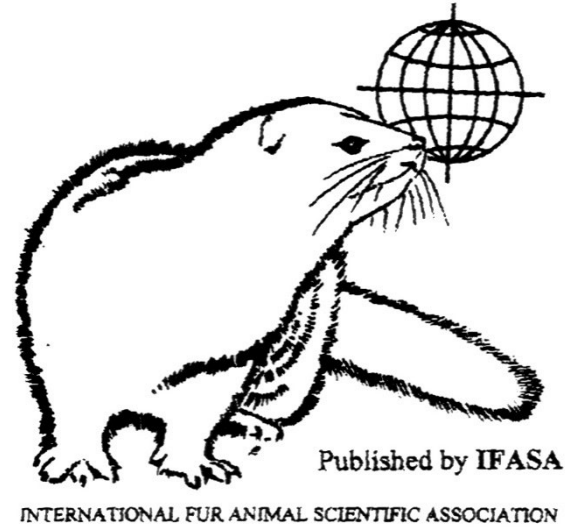
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ISSN: 2445-6292

SCIENTIFUR
ISSN 0105-2403
Vol. 46, No. 3



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Notes from the Editor

Fur farming in Europe is debated. An information note on European fur farming from the Dutch and Austrian delegations, supported by the Belgian, German, Luxembourg and Slovak delegations, was presented at a meeting in the Council of the European Union (Agriculture and Fisheries) in 2021. The note deals with considerations on animal welfare, ethics and public health, focusing on SARS-CoV-2 in relation to fur production and a call on the European Commission to examine the possibilities for a permanent ban on fur farming in the European Union and to present a legislative proposal to achieve this goal.

SARS-CoV-2, which causes COVID-19, naturally continues to have a strong research focus. This issue of *Scientifur* brings new abstracts from research into coronavirus infection across species with a focus on fur animals and humans. The results point to the need for continued surveillance of viral spread in non-human hosts including fur animals.

Aleutian Disease has severe consequences for mink production. Breeding for tolerance to Aleutian Disease was shown in a study to be a tool to control the disease on farms. Mink selected for tolerance against Aleutian Disease were mostly free of clinical Aleutian Disease, had normal pelt quality and litter size, and had low virus copy numbers in tissues and low antibody titers in ELISA.

Circularity is key in any livestock production. Mink manure contributes to highly nitrogenous wastes with potential nitrogen mineralization during composting, which then results in low resource reutilization. However, inoculation with functional microbial agents was found to elevate the bioavailability of organic nitrogen during composting.

Vivi Hunnicke Nielsen

Editor *Scientifur*

BREEDING, GENETICS AND REPRODUCTION**Shadow coat colour in American mink associated with a missense mutation in the KIT gene**

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Anim Genet. 2022 Apr 28.

Doi: 10.1111/age.13202. Online ahead of print.

Identification of mutant gene for Black crystal coat and non-allelic gene interactions in Neogale vison

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Sable (*Martes zibellina*) and American mink (*Neogale vison*) are valuable species characterized by a variety of coat colour produced on fur farms. Black crystal fur phenotype is Mendelian codominant trait: heterozygous animals (C^r/+) have white guard hairs scattered predominantly on the spine and the head, while homozygous (C^r/C^r) minks have coats resembling the Himalayan (c^h/c^h) or white Hedlund (h/h) types. It is one of the most recent of more than 35 currently known phenotypic traits of fur colour in American mink. Black crystal fur phenotype was first described in 1984 in the Russian population of mink, which had undergone selection for domestic defensive response to humans. Here, we performed whole-genome sequencing of American mink with C^r/C^r phenotype. We identified a missense mutation in the gene encoding the α -COP subunit of the COPI complex (COPA). The COPI complex mediates retrograde trafficking from the Golgi system to the endoplasmic reticulum and sorting of transmembrane proteins. We observed an interaction between a newly identified mutation in the COPA gene and a mutation in the microphthalmia-associated transcription factor (MITF), the latter mutation led to the formation of the white Hedlund (h/h) phenotype. Double heterozygotes for these mutations have an entirely white coat and a black-eyed phenotype similar to the phenotype of C^r/C^r or h/h minks. Our data could be useful for tracking economically valuable fur traits in mink breeding programs to contribute to global fur production.

Sci Rep. 2022 Jun 21; 12 (1): 10483.

Doi: 10.1038/s41598-022-14079-z.

NUTRITION, FEEDING AND MANAGEMENT**Resource utilization of mink manure: Functional microbial inoculation to elevate the bioavailability of organic nitrogen during composting**

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Bioresour Technol. 2022 Jun; 353: 127149.

Doi: 10.1016/j.biortech.2022.127149.

Epub 2022 Apr 12.

Are fur farms a potential source of persistent organic pollutants or mercury to nearby freshwater ecosystems?

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Sci Total Environ. 2022 Aug 10; 833: 155100.

Doi: 10.1016/j.scitotenv.2022.155100.

Epub 2022 Apr 6.

Mandible shape variation and feeding biomechanics in minks

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European and American minks are very similar in ecology, behavior and morphology. Both species hunt terrestrial vertebrates and aquatic prey, but the American mink is a more generalist predator, which, among other factors, allows it to outcompete the European mink in areas where it has been introduced. We used 3D geometric morphometrics and estimates of muscle mechanical advantage to assess the degree of variation in

mandibular morphology, and to determine whether such variation reflects dietary differences between the two species. The three main axes of variation represented interspecific differences, a common allometric trajectory between species and sexes, and the interspecific effect of sexual size dimorphism, with males having overall stronger bites than females. Differences in mandible shape and biomechanical parameters suggest that American minks are better equipped for preying on terrestrial vertebrates, while the features seen in European mink could be related to tougher prey, fish capture, or both. Additionally, within each species, the larger specimens of each sex present indicators of a higher percentage of terrestrial prey in their diet. These results indicate a low potential dietary overlap between both species, suggesting that factors other than prey competition may have a role in the decline of the European mink.

Sci Rep. 2022 Mar 23; 12 (1): 4997.

Doi: 10.1038/s41598-022-08754-4.

BEHAVIOUR AND WELFARE

Practice does not make perfect: Juvenile object play does not improve the predation skills of adult mink in "simulated prey" tests

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Dev Psychobiol. 2022 May; 64 (4): e22268.

Doi: 10.1002/dev.22268.

HEALTH AND DISEASE

Mechanisms behind the varying severity of Aleutian mink disease virus: Comparison of three farms with a different disease status

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Aleutian mink disease virus (AMDV) is distributed widely among mink farms and wild mustelids despite ongoing attempts to stop the spread. The severity of Aleutian disease (AD) varies from subclinical to fatal but the reasons for its varying severity are complex and unclear. Recently, breeding of tolerant mink has drawn attention as the possible solution to reduce the effects of AD in farms. The aim of this study was to gather information on the effects of breeding based on overall health, production traits, and antibody titer on AD severity by comparing a positive farm (farm 1) that has been breeding for tolerance in mink to an infected farm without tolerance selection, and an AMDV-free farm. During the 2.5-year follow-up, the mink in farm 1 remained mostly free of clinical AD, had normal pelt quality and litter size, and had low virus copy numbers in tissues and low antibody titers in ELISA. In histopathological studies, most of the farm 1 mink had no/mild lesions in their kidneys. 29-43% of the mink were ELISA negative but PCR positive throughout the follow-up and frequent changes in virus strains and coinfections were observed. Several differences in gene expression between animals from different farms were also detected. These results indicate that the disease burden of AMDV can be reduced, with seemingly normal health and production rates, despite continual circulation of ADMV in cases where eradication attempts are unsuccessful.

Vet Microbiol. 2022 Jul; 270: 109452.

Doi: 10.1016/j.vetmic.2022.109452.

Epub 2022 May 12.

Phagocytic Activity, Oxygen Metabolism and Serum Amyloid a Concentration in Peripheral Blood of Mink with Subclinical Aleutian Virus Infection

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Aleutian disease (AD) is a chronic disease of mink caused by the Aleutian Mink Disease Virus (AMDV) that results in dysfunction of the immune system. The prevalence of asymptomatic AMDV infections suggests a necessity to explore their effects on the cellular mechanisms of non-specific immunity in farmed mink. The study evaluated the phagocytic activity and oxygen metabolism of peripheral blood granulocytes and monocytes in mink with chronic subclinical AMDV infection. Moreover, the intensity of inflammatory processes was assessed based on the serum amyloid A (SAA) concentration. The analyses involved 24 brown mink females aged 12-24 months. The experimental group (group I) consisted of mink with chronic subclinical AMDV infections, and the control group (group II) included healthy animals. The statistical analysis was performed using the Mann-Whitney U rank test. Phagocytic activity of granulocytes and monocytes was carried out using flow cytometry, and SAA concentration was determined with enzyme-linked immunosorbent assay (ELISA). Compared with the control group, there was a significant decrease in the phagocytic activity and oxygen metabolism of granulocytes and monocytes in the AMDV-infected mink ($p < 0.05$). Additionally, it was found that the mean SAA value was significantly higher in the group infected with AMDV than in the control group ($p < 0.05$). The obtained data indicate that monitoring the serum SAA levels in mink with asymptomatic inflammation may help assess the health of mink and detect asymptomatic inflammation caused by AMDV infection.

Animals (Basel). 2022 Apr 11; 12 (8): 987.

Doi: 10.3390/ani12080987.

The basis of mink susceptibility to SARS-CoV-2 infection

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Of all known airborne diseases in the twenty-first century, coronavirus disease 19 (COVID-19) has the highest infection and death rate. Over the past few decades, animal origin viral diseases, notably those of bat-linked, have increased many folds in humans with cross-species transmissions noted and the ongoing COVID-19 pandemic has emphasized the importance of understanding the evolution of natural hosts in response to viral pathogens. Cross-species transmissions are possible due to the possession of the angiotensin-converting enzyme 2 (ACE2) receptor in animals. ACE2 recognition by SARS-CoV-2 is a critical determinant of the host range, interspecies transmission, and viral pathogenesis. Thus, the phenomenon of breaking the cross-species barrier is mainly associated with mutations in the receptor-binding domain (RBD) of the spike (S) protein that interacts with ACE2. In this review, we raise the issue of cross-species transmission based on sequence alignment of S protein. Based on previous reports and our observations, we can conclude that the occurrence of one of two mutations D614G or Y453F is sufficient for infection of minks by SARS-CoV-2 from humans. Unfortunately, D614G is observed in the world's most common line of virus B.1.1.7 and the latest SARS-CoV-2 variants B.1.617.1, B.1.617.2, and B.1.617.3 too.

J Appl Genet. 2022 Apr 9; 1-13.

Doi: 10.1007/s13353-022-00689-w.

Online ahead of print.

Experimental Infection of Mink with SARS-COV-2 Omicron Variant and Subsequent Clinical Disease

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We report an experimental infection of American mink with SARS-CoV-2 Omicron variant and show that mink remain positive for viral RNA for days, experience clinical signs and histopathologic changes, and transmit the virus to uninfected recipients. Preparedness is crucial to avoid spread among mink and spillover to human populations.

Emerg Infect Dis. 2022 Jun; 28 (6): 1286–1288.

Doi: 10.3201/eid2806.220328

Transmission of SARS-CoV-2 from humans to animals and potential host adaptation

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SARS-CoV-2, the causative agent of the COVID-19 pandemic, can infect a wide range of mammals. Since its spread in humans, secondary host jumps of SARS-

CoV-2 from humans to multiple domestic and wild populations of mammals have been documented. Understanding the extent of adaptation to these animal hosts is critical for assessing the threat that the spillback of animal-adapted SARS-CoV-2 into humans poses. We compare the genomic landscapes of SARS-CoV-2 isolated from animal species to that in humans, profiling the mutational biases indicative of potentially different selective pressures in animals. We focus on viral genomes isolated from mink (*Neovison vison*) and white-tailed deer (*Odocoileus virginianus*) for which multiple independent outbreaks driven by onward animal-to-animal transmission have been reported. We identify five candidate mutations for animal-specific adaptation in mink (NSP9_G37E, Spike_F486L, Spike_N501T, Spike_Y453F, ORF3a_L219V), and one in deer (NSP3a_L1035F), though they appear to confer a minimal advantage for human-to-human transmission. No considerable changes to the mutation rate or evolutionary trajectory of SARS-CoV-2 has resulted from circulation in mink and deer thus far. Our findings suggest that minimal adaptation was required for onward transmission in mink and deer following human-to-animal spillover, highlighting the 'generalist' nature of SARS-CoV-2 as a mammalian pathogen.

Nat Commun. 2022 May 27; 13 (1): 2988.
Doi: 10.1038/s41467-022-30698-6.

Geographical distribution of SARS-CoV-2 amino acids mutations and the concomitant evolution of seven distinct clades in non-human hosts

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Zoonoses Public Health. 2022 May 25.
Doi: 10.1111/zph.12971. Online ahead of print.

Molecular phylogeny of coronaviruses and host receptors among domestic and close-contact animals reveals subgenome-level conservation, crossover, and divergence

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Background

Coronaviruses have the potential to cross species barriers. To learn the molecular intersections among the most common coronaviruses of domestic and close-contact animals, we analyzed representative coronavirus genera infecting mouse, rat, rabbit, dog, cat, cattle, white-tailed deer, swine, ferret, mink, alpaca, Rhinolophus bat, dolphin, whale, chicken, duck and turkey hosts; reference or complete genome sequences were available for most of these coronavirus genera. Protein sequence alignments and phylogenetic trees were built for the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. The host receptors and enzymes aminopeptidase N (APN), angiotensin converting enzyme 2 (ACE2), sialic acid synthase (SAS), transmembrane serine protease 2 (TMPRSS2), dipeptidyl peptidase 4 (DPP4), cathepsin L (and its analogs) and furin were also compared.

Results

Overall, the S, E, M, and N proteins segregated according to their viral genera (α , β , or γ), but the S proteins of alphacoronaviruses lacked conservation of phylogeny. Interestingly, the unique polybasic furin cleavage motif found in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) but not in severe acute respiratory syndrome coronavirus (SARS-CoV) or Middle East respiratory syndrome coronavirus (MERS-CoV) exists in several β -coronaviruses and a few α - or γ -coronaviruses. Receptors and enzymes retained host species-dependent relationships with one another. Among the hosts, critical ACE2 residues essential for SARS-CoV-2 spike protein binding were most conserved in white-tailed deer and cattle.

Conclusion

The polybasic furin cleavage motif found in several β - and other coronaviruses of animals points to the existence of an intermediate host for SARS-CoV-2, and it also offers a counternarrative to the theory of a laboratory-engineered virus. Generally, the S proteins of coronaviruses show crossovers of phylogenies indica-

tive of recombination events. Additionally, the consistency in the segregation of viral proteins of the MERS-like coronavirus (NC_034440.1) from pipistrelle bat supports its classification as a β -coronavirus. Finally, similarities in host enzymes and receptors did not always explain natural cross-infections. More studies are therefore needed to identify factors that determine the cross-species infectivity of coronaviruses.

BMC Vet Res. 2022 Apr 1; 18 (1): 124.

Doi: 10.1186/s12917-022-03217-4.

The Finding of the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) in a Wild Eurasian River Otter (*Lutra lutra*) Highlights the Need for Viral Surveillance in Wild Mustelids

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Animals have been involved in the three known outbreaks of severe respiratory syndromes due to coronaviruses (years 2005, 2012, and 2019). The pandemic nature of the SARS-CoV-2 outbreak increases the likelihood of infection from humans of susceptible animal species that, thus, could become secondary viral hosts and even disease reservoirs. We present evidence of spillover infection of wild mustelids by reporting the presence of SARS-CoV-2 in a Eurasian river otter found near a water reservoir in the Valencian Community (Spain). We detected the virus using two different commercial RTqPCR assays on RNA extracted from the nasopharynx (swabbing) and from lung tissue and

mediastinal lymph node homogenates. The corresponding samples from two additional otters from distant sites tested negative in identical assays. The diagnosis in the positive otter was confirmed by two-tube RT-PCR assay in which RNA was first retrotranscribed, and then specific regions of the spike (*S*), nucleocapsid (*N*), and *ORF10* genes were separately amplified from the produced cDNA, followed by electrophoretic visualization and Sanger sequencing. The sequences of the amplified products revealed some non-synonymous changes in the *N* and *ORF10* partial sequences, relative to the consensus sequence. These changes, identified already in human patient samples, point to human origin of the virus, although their specific combination was unique. These findings, together with our previous report of SARS-CoV-2 infection of feral American mink, highlight the need for SARS-CoV-2 surveillance of wild or feral mustelids to evaluate the risk that these animals could become SARS-CoV-2 reservoirs.

Front Vet Sci. 2022 Mar 31; 9: 826991.

Doi: 10.3389/fvets.2022.826991. eCollection 2022.

Adaptive Evolution of the Fox Coronavirus Based on Genome-Wide Sequence Analysis

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Purpose

To report the first complete fox coronavirus (CoV) genome sequence obtained through genome-wide amplifications and to understand the adaptive evolution of fox CoV.

Methods

Anal swab samples were collected from 35 foxes to detect the presence of CoV and obtain the virus sequence. Phylogenetic analysis was conducted using MrBayes. The possibility of recombination within these sequences was assessed using GARD. Analysis of the levels of selection pressure experienced by these sequences was assessed using methods on both the PAML and Data Monkey platforms.

Results

Of the 35 samples, two were positive, and complete genome sequences for the viruses were obtained. Phylogenetic analysis, using Bayesian methods, of these sequences, together with other CoV sequences, revealed that the fox CoV sequences clustered with canine coronavirus (CCoV) sequences, with sequences from other carnivores more distantly related. In contrast to the feline, ferret and mink CoV sequences that clustered into species-specific clades, the fox CoV fell within the CCoV clade. Minimal evidence for recombination was found among the sequences. A total of 7, 3, 14, and 2 positively selected sites were identified in the M, N, S, and 7B genes, respectively, with 99, 111, and 581 negatively selected sites identified in M, N, and S genes, respectively.

Conclusion

The complete genome sequence of fox CoV has been obtained for the first time. The results suggest that the genome sequence of fox CoV may have experienced adaptive evolution in the genes replication, entry, and virulence. The number of sites in each gene that experienced negative selection is far greater than the number that underwent positive selection, suggesting that most of the sequence is highly conserved and important for viral survive. However, positive selection at a few sites likely aided these viruses to adapt to new environments.

Biomed Res Int. 2022 Apr 13; 2022: 9627961.
Doi: 10.1155/2022/9627961. eCollection 2022.

Integrated Analysis of miRNA-mRNA Expression in Mink Lung Epithelial Cells Infected With Canine Distemper Virus

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Canine distemper (CD) caused by canine distemper virus (CDV) is one of the major infectious diseases in minks, bringing serious economic losses to the mink breeding industry. By an integrated analysis of microRNA (miRNA)-messenger RNA (mRNA), the present study analyzed the changes in the mink transcriptome upon CDV infection in mink lung epithelial cells (Mv. 1. Lu cells) for the first time. A total of 4,734 differentially expressed mRNAs (2,691 upregulated and 2,043 downregulated) with $|\log_2(\text{FoldChange})| > 1$ and $P\text{-adj} < 0.05$ and 181 differentially expressed miRNAs (152 upregulated and 29 downregulated) with $|\log_2(\text{FoldChange})| > 2$ and $P\text{-adj} < 0.05$ were identified. Gene Ontology (GO) enrichment indicated that differentially expressed genes (DEGs) were associated with various biological processes and molecular function, such as response to stimulus, cell communication, signaling, cytokine activity, transmembrane signaling receptor activity and signaling receptor activity. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the combination of miRNA and mRNA was done for immune and inflammatory responses, such as Janus kinase (JAK)-signal transducer and activator (STAT) signaling pathway and nuclear factor (NF)-kappa B signaling pathway. The enrichment analysis of target mRNA of differentially expressed miRNA revealed that mir-140-5p and mir-378-12 targeted corresponding genes to regulate NF-kappa B signaling pathway. JAK-STAT signaling pathway could be modulated by mir-425-2, mir-139-4, mir-140-6, mir-145-3, mir-140-5p and mir-204-2. This study compared the influence of miRNA-mRNA expression in Mv. 1. Lu cells before and after CDV infection by integrated analysis of miRNA-mRNA and analyzed the complex network interaction between virus and host cells. The results can help understand the molecular mechanism of the natural immune response induced by CDV infection in host cells.

Front Vet Sci. 2022 May 31; 9: 897740.
Doi: 10.3389/fvets.2022.897740. eCollection 2022.

Development of an Immunochromatographic Strip for Rapid Detection of Mink Enteritis Virus

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Although mink enteritis virus (MEV) is an acute, virulent, and highly contagious pathogen in minks, there is currently a lack of a quick diagnostic method. By conjugating colloidal gold nanoparticles with the MEV-specific monoclonal antibody, monoclonal antibody (MAb) 14, we developed a single-step competitive immunochromatographic strip (ICS) assay for simple determination of MEV. The optimal concentrations of the colloidal gold-coupled MAb 14 (coating antibody), the capture protein (MEV VP2 protein), and the goat anti-mouse antibody were 1.0, 0.8, and 1.0 mg/ml, respectively. The limit of detection was approximately 512 hemagglutination units/100 µl of MEV B strain. Other common viruses of mink were tested to evaluate the specificity of the ICS, and the results showed no cross-reactivity for other pathogens. In comparison with the Anigen Rapid canine parvovirus (CPV) Ag Test Kit (BioNote, Korea) in testing 289 samples, the percentage of agreement and relative sensitivity and specificity of the MEV ICS assay were 94.1, 93.2, and 97.1%, respectively. The ICS test was found to be a sufficiently sensitive and specific detection method for the convenient and rapid detection of MEV.

Front Microbiol. 2022 Mar 9; 13: 839320.

Doi: 10.3389/fmicb.2022.839320. eCollection 2022.

Tetracycline- and Macrolide-Resistant *Enterococcus* Species Isolated from a Mink Farm in the United States

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Enterococcus species are a normal flora of animals and humans. However, life-threatening opportunistic infections can be caused by antimicrobial resistant strains. Fecal ($n = 42$) and feed ($n = 8$) samples were obtained

from a mink farm and cultured for the enumeration and detection of erythromycin-resistant (a macrolide; ERY^r)- and tetracycline-resistant (TET^r) enterococci. ERY^r and TET^r enterococci were detected from all fecal (mean concentrations = 6 and 7 logs, respectively) and feed (mean concentrations = 5 and 4 logs, respectively) samples. While *Enterococcus faecalis* and *Enterococcus faecium* were detected at equal proportions among the fecal TET^r isolates, *E. faecium* predominated among ERY^r fecal isolates. All ERY^r and 90% of the TET^r isolates ($n = 50$) were multidrug resistant (resistant to three or more antimicrobial classes). Among ERY^r isolates, while 83% of *E. faecalis* ($n = 12$) were positive for *erm*(B), 58% of *E. faecium* ($n = 38$) isolates were positive for *msr*(C). Among ERY^r isolates, *tet*(M) was detected from 92% of *E. faecalis* ($n = 12$) and 97% of *E. faecium* ($n = 38$) isolates. Conversely, however, *erm*(B) was detected in 18% of *E. faecalis* ($n = 22$) and 33% of *E. faecium* ($n = 27$) TET^r isolates. Our study provides a baseline for future efforts to reduce antimicrobial resistance and improve antimicrobial stewardship in commercial mink production facilities.

Microb Drug Resist. 2022 Jun; 28 (6): 734-743.

Doi: 10.1089/mdr.2021.0438. Epub 2022 May 16.

Isolation and pathogenicity analysis of mink orthoreoviruses

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Transbound Emerg Dis. 2022 Mar; 69 (2): 623-631.

Doi: 10.1111/tbed.14028. Epub 2021 Mar 9.

Occurrence of multidrug resistant Gram-negative bacteria and resistance genes in semi-aquatic wildlife - *Trachemys scripta*, *Neovison vison* and *Lutra lutra* - as sentinels of environmental health

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Sci Total Environ. 2022 Jul 15; 830: 154814.

Doi: 10.1016/j.scitotenv.2022.154814.

Epub 2022 Mar 24.

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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.