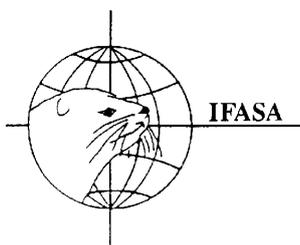
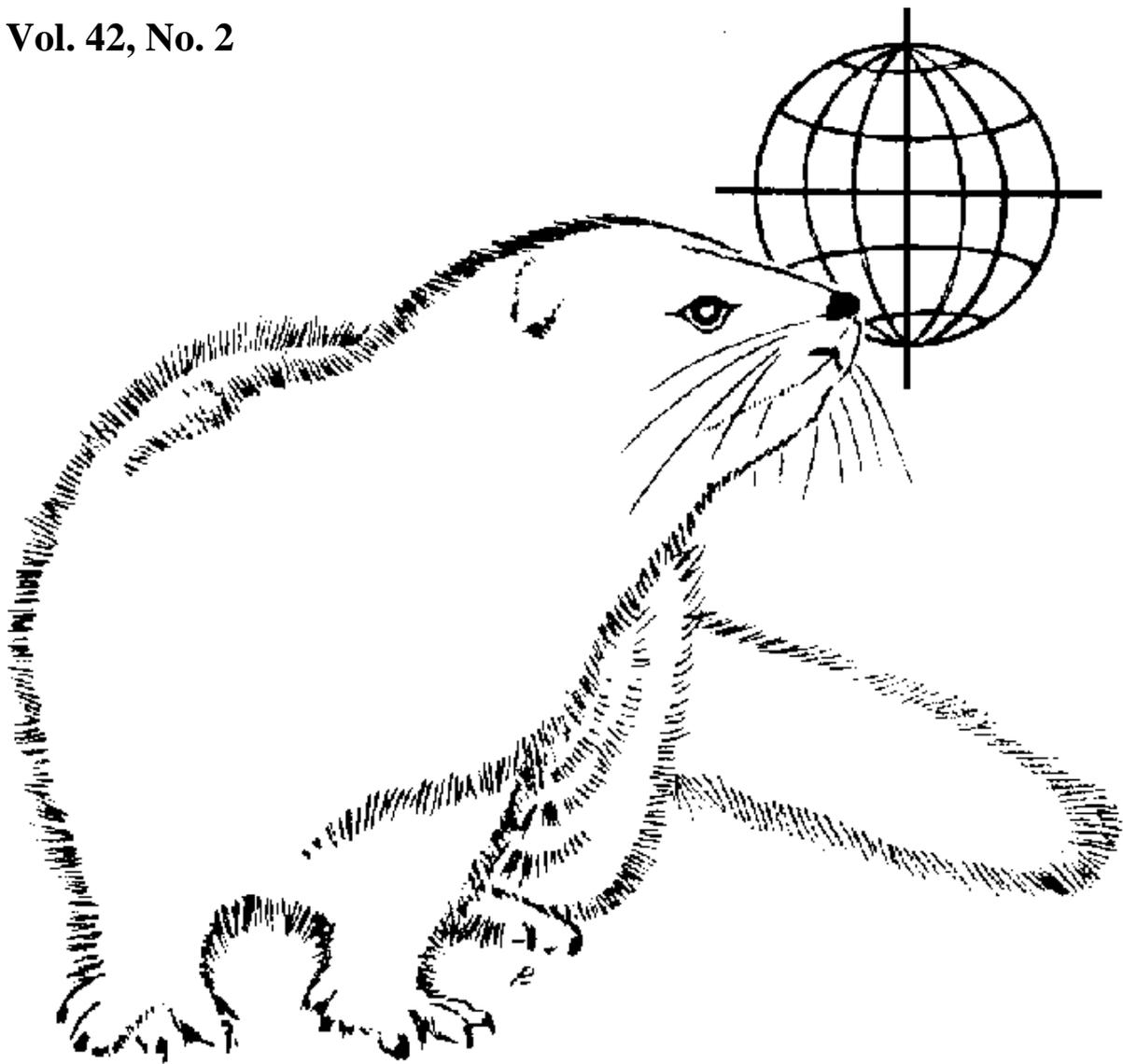


# SCIENTIFUR

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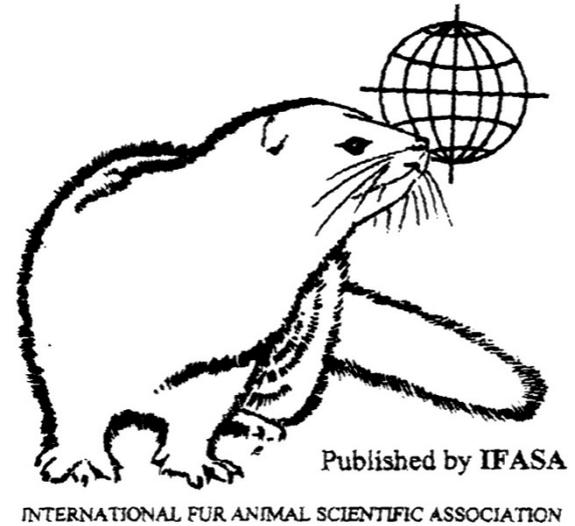
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## Notes from the Editor

Size, fertility and fur quality are goals for improvement in breeding, but selection for larger size often results in poorer fur quality and reduced fertility. The results from a Polish study published in this issue of *Scientifur* comparing groups of Pearl male mink with different body condition show that males with an ideal weight have the largest success rate in mating compared to very thin and obese males.

While extensive research has been performed for fur animals like mink and foxes, chinchillas are only poorly investigated. However, a recent study was set up to characterize the reproductive biology of female chinchillas for improved breeding of farmed animals and conservation effort of wild chinchillas.

Mink and other fur animals respond differently to environmental stimuli, which is used with great advantage in the Welfur project. Thus, each individual mink expresses a personality. A new study shows that the number of neurons in the amygdala region is associated with aspects of the mink personality.

This issue of *Scientifur* publishes abstracts from the Annual Report 2017 from Copenhagen Fur Research. Several abstracts deal with adequate feeding. It is shown that both the content of methionine, minerals and vitamins in the feed can be reduced without affecting skin size or quality. Another focus in the abstracts is the increasing antimicrobial resistance, which also is increasing in mink production.

Vivi Hunnicke Nielsen

Editor *Scientifur*



**BREEDING, GENETICS AND REPRODUCTION****Activation of the IGF1 receptor stimulates glyco-gen synthesis by mink uterine epithelial cells**Dean M.<sup>1,2</sup>, Rose J.<sup>2</sup><sup>1</sup>Department of Medicinal Chemistry and Pharmacognosy, Center for Biomolecular Sciences, College of Pharmacy, University of Illinois at Chicago.<sup>2</sup>Department of Biological Sciences, College of Science and Engineering, Idaho State University, Pocatello.*Mol Reprod Dev.* 2018 Mar 24.

Doi: 10.1002/mrd.22981. Epub ahead of print.

**Effects of halogenated contaminants on reproductive development in wild mink (*Neovison vison*) from locations in Canada**Elliott J.E.<sup>1</sup>, Kirk D.A.<sup>2</sup>, Martin P.A.<sup>3</sup>, Wilson L.K.<sup>4</sup>, Kardosi G.<sup>4</sup>, Lee S.<sup>5</sup>, McDaniel T.<sup>3</sup>, Hughes K.D.<sup>6</sup>, Smith B.D.<sup>4</sup>, Idrissi A.M.<sup>7</sup><sup>1</sup>Environment and Climate Change Canada, Ecotoxicology and Wildlife Health Division, Delta, BC, Canada.<sup>2</sup>Aquila Conservation & Environment Consulting, 75 Albert Street, Ottawa, ON, Canada.<sup>3</sup>Environment and Climate Change Canada, Ecotoxicology and Wildlife Health Division, Burlington, ON, Canada.<sup>4</sup>Environment and Climate Change Canada, Canadian Wildlife Service, Delta, BC, Canada.<sup>5</sup>Environment and Climate Change Canada, Ecotoxicology and Wildlife Health Division, Delta, BC, Canada.<sup>6</sup>Broadwing Biological Consulting, 1944 Parkside Drive, Pickering, ON, Canada.<sup>7</sup>Environment and Climate Change Canada, Ecotoxicology and Wildlife Health Division, Ottawa, ON, Canada.

The concept of the Anthropocene, that humans are now re-engineering global ecosystems, is in part evidenced by the pervasive pollution by persistent organic pollutants (POPs). Certain POPs are hormone mimics and can disrupt endocrine and hence reproductive processes, shown mainly by laboratory studies with model species. There are, in contrast, fewer confirmations of such disruption from eco-epidemiological studies of wild mammals. Here we used the

American mink (*Neovison vison*) as a sentinel species for such a study. Over the period 1998-2006, 161 mink carcasses were obtained from commercial trappers in the Canadian provinces of British Columbia and Ontario. Mink were aged, sexed, measured, and body condition assessed. Livers were analyzed either individually or pooled for organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), and subsets for polybrominated diphenyl ethers (PBDEs). We primarily addressed whether contaminants affected male reproductive development by measuring baculum size and assessing the influences of age and body condition. We also considered the influence of spatial variation on relative exposure and size of baculum. Statistical models separated by age class revealed that significant relationships between baculum length or mass and juvenile mink were mostly positive, whereas for adults and first year mink they were mostly negative. A significant negative relationship for adult mink was determined between DDE and both baculum length and mass. For juvenile mink we found significant positive relationships between  $\sum$ PCBs, DDE and  $\sum$ PBDEs with baculum length. Our results provide some indication of negative effects of halogenated contaminants on male reproductive development in wild mink, and the most likely candidate chemical is the confirmed anti-androgenic compound, DDE, rather than PCBs or other compounds.

*Ecotoxicology.* 2018 Apr 6.

Doi: 10.1007/s10646-018-1926-4.

Epub ahead of print.

**Body condition of male farm mink (*Neovison vison*) during a mating season**Felska-Blaszczyk L.<sup>1</sup>, Dziadosz-Stys M.<sup>1</sup>, Lawrow N.<sup>1</sup><sup>1</sup>Laboratory of Animal Anatomy, West Pomeranian University of Technology, Szczecin, Poland.

The study was aimed to evaluate the relationship between the body condition of males of farmed mink (*Neovison vison*) and their behaviour as well as mating performance. The observations involved 170 males of Pearl mink. Before the breeding season, males were assigned to three groups according to their condition: Group I – very thin, Group II – thin and ideal weight, Group III – heavy and obese. On the completion of the mating season, the following reproductive indices were analysed: number of days in mating, number of matings per day and during the

entire mating season, fraction of males that failed to mate, incidence of aggressive behaviours. Statistical analysis did not reveal significant differences in the mean number of days in mating, total number of matings or daily number of matings. Male condition score has a significant effect on the behaviour of the animals. The highest rate of aggressive behaviours, 16%, was observed in groups I and II, i.e. in very thin, thin and ideal weight animals. Males of group III, i.e. overweight and obese, showed one half of aggressive behaviours, about 8%. A different pattern was seen in fur chewing, since none of thin or ideal-weight males (Group II) did chew its fur. Only very thin (Group I, 2%) and obese males (Group III, 4.17%) chewed their fur. Group I comprised the lowest fraction of males that failed to mate, about 6%, compared to approx. 10% in Groups I and III each.

*Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech.* 2017, 334(42)2, 29–34  
Doi: 10.21005/AAPZ2017.42.2.03

### **Oviduct morphology and estrogen receptors ER $\alpha$ and ER $\beta$ expression in captive *Chinchilla lanigera* (*Hystricomorpha: Chinchillidae*)**

Machado-Neves M.<sup>1</sup>, Assis W.A.<sup>2</sup>, Gomes M.G.<sup>3</sup>, Oliveira C.A.<sup>2</sup>

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*Chinchilla lanigera* is a hystricomorph rodent from South America whose reproductive biology presents particular characteristics that distinguishes it from other Rodentia species, such as low reproductive rate, seasonal breeding pattern, and long estrous cycle. Nevertheless, reproductive features in female chinchillas are still poorly investigated, with a scarce knowledge concerning the estrous cycle and the histology of reproductive organs. In this study, we investigate the morphology, histomorphometry, secretory activity, and immunolocalization of estrogen receptors ER $\alpha$  and ER $\beta$  in oviducts of nulliparous chinchillas, euthanized at fall season in Brazil. Follicular phase of estrous cycle of all studied animals was

characterized by ovary and uterine morphology inspection, as well as vaginal cytology. Similar to other mammals, the oviduct wall of infundibulum, ampulla and isthmus was composed of mucosa, muscle, and serosa layers. Morphometric data of oviduct layers were used for identifying each oviduct segment. In the follicular phase, the oviduct was characterized by intense secretory activity, mainly in the ampulla, and expression of ER $\alpha$  and ER $\beta$  throughout the oviduct epithelium. Both ER $\alpha$  and ER $\beta$  were also detected in the connective tissue and smooth muscle cells. Our findings point out to the important role of estrogen in this female organ. Similar wide distribution of both ER proteins has been described for human Fallopian tube. Taken together, our data add to the understanding of the reproductive biology of female chinchillas, and may assist in the intensive breeding of this species and any eventual endeavor for conservation of chinchillas in the wild.

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Pii: S0016-6480(18)30028-5.  
Doi: 10.1016/j.ygcen.2018.03.023.  
Epub ahead of print.

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

#### **Effects of sources and concentrations of zinc on growth performance, nutrient digestibility, and fur quality of growing-furring female mink (*Mustela vison*)**

Cui H.<sup>1,2</sup>, Zhang T.T.<sup>3</sup>, Nie H.<sup>1</sup>, Wang Z.C.<sup>1</sup>, Zhang X.L.<sup>3</sup>, Shi B.<sup>1</sup>, Yang F.H.<sup>3</sup>, Gao X.H.<sup>2</sup>

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A completely randomized 3 × 3 + 1 factorial experiment was conducted to evaluate the effects of sources and concentrations of Zn on growth performance, nutrient digestibility, serum biochemical endpoints, and fur quality in growing-furring female black mink. One hundred fifty healthy 15-wk-old female mink were randomly allocated to 10 dietary treatments (= 15/group) for a 60-d trial. Animals in the control

group were fed a basal diet, which consisted of mainly corn, soybean oil, meat and bone meal, and fish meal, with no Zn supplementation. Mink in the other 9 treatments were fed the basal diet supplemented with Zn from either zinc sulfate (ZnSO), zinc glycinate (ZnGly), or Zn pectin oligosaccharides (ZnPOS) at concentrations of either 100, 300, or 900 mg Zn/kg DM. The results showed that mink in the ZnPOS groups had higher ADG than those in the ZnSO groups (main effect,  $< 0.05$ ). The addition of Zn reduced the G:F ( $< 0.05$ ). In addition, CP and crude fat digestibility were linearly increased with Zn supplementation ( $< 0.05$ ) and N retention tended to increase with Zn addition ( $= 0.08$ ). Dietary Zn supplementation increased the concentration of serum albumin and activity of alkaline phosphatase ( $< 0.05$ ). There was a linear effect of dietary Zn on the concentration of tibia Zn and pancreatic Zn ( $< 0.05$ ). For fur quality characteristics, the fur density and hair color of mink were improved by dietary Zn concentration ( $< 0.05$ ). Compared with ZnSO (100%), relative bioavailability values of ZnGly were 115 and 118%, based on tibia and pancreatic Zn, respectively, and relative bioavailability values of ZnPOS were 152 and 142%, respectively. In conclusion, this study demonstrates that Zn supplementation can promote growth and increase nutrient digestibility and fur quality and that ZnPOS is more bioavailable than ZnSO and ZnGly in growing-furring female mink.

*J Anim Sci.* 2017 Dec; 95(12):5420-5429.  
Doi: 10.2527/jas2017.1810.

### **Potential niche expansion of the American mink invading a remote island free of native-predatory mammals**

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The success of an invasive species depends in part on its niche and the new niche opportunities that such

species may find in the invaded habitat. Niche opportunities can be understood as the potential provided by a community to an invasive species to expand its niche by changes in habitat use, behavior, or diet, that favors population growth, reflected in the species occupying more habitat. This may occur under a favorable combination of access to resources that can be further favored by a lack of competitors and a release from natural enemies. The American mink (*Neovison vison*) is a crepuscular/nocturnal and semi-aquatic mustelid native to North America that generally concentrates activities at  $< 100$  m from the water. It has recently established an invasive population on Navarino Island in southern Chile. Here, the mink is now the top terrestrial predator free of predators or competitors. We hypothesized that this lack of potential predators and competitors, together with a more diurnal and terrestrial prey, have resulted in the mink expanding its spatial and temporal niche on Navarino Island as compared to that in its native habitats, expressed in occupancy of sites away from water and diurnal activity. We evaluated this by using 93 randomly-chosen camera-trap stations, occupancy models and mink daily activity patterns. Models showed a dynamic occupancy with the area occupied by mink being highest during summers and lowest in spring with seasonal changes in occupancy related to distance to water sources. Mink occupied and were active at sites up to 880 m from water sources during summers. Occupancy decreased at shorter distances from water during spring, but mink were still active at up to 300 m from water. Mink were active daylong during summers, and nocturnal and crepuscular during winter and spring. These results show that compared to the native and other invaded habitats, on Navarino Island mink use more terrestrial habitats and are more diurnal during summers, suggesting a niche expansion under new niche opportunities that may enhance the negative impacts of this predator on a myriad of small native vertebrates.

*PLoS One.* 2018 Apr 4; 13(4):e0194745.  
Doi: 10.1371/journal.pone.0194745.  
eCollection 2018.

### **BEHAVIOUR AND WELFARE**

#### **The number of neurons in specific amygdala regions is associated with boldness in mink: a study in animal personality**

Wiese A.S.<sup>1,2</sup>, Needham E.K.<sup>3,4</sup>, Noer C.L.<sup>1,5</sup>, Balsby T.J.S.<sup>6</sup>, Dabelsteen T.<sup>1</sup>, Pakkenberg B.<sup>2,7</sup>

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Conspecifics vary consistently in their behavioural responses towards environment stimuli such as exposure to novel objects; ethologists often refer to this variability as animal personality. The neurological mechanisms underlying animal personality traits remain largely unknown, but linking the individual variation in emotional expression to brain structural and neurochemical factors is attracting renewed interest. While considerable research has focused on hormonal and neurotransmitter effects on behavioural responses, less is known about how individual variation in the number of specific neuron populations contributes to individual variation in behaviour. The basolateral amygdala (BLA) and the central nuclei of the amygdala (CeA) mediate emotional processing by regulating behavioural responses of animals in a potentially threatening situation. As such, these structures are good candidates for evaluating the relationship between neuronal populations and behavioural traits. We now show that individual American mink (*Neovison vison*) reacting more boldly towards novelty have more neurons in the BLA than do their more timid conspecifics, suggesting that a developmental pattern of the number of amygdala neurons can influence behavioural traits of an adult animal. Furthermore, post hoc correlations revealed that individuals performing with higher arousal, as reflected by their frequency of startle behaviour, have more CeA neurons. Our results support a direct link between the number of neurons in amygdala regions and aspects of animal personality.

*Brain Struct Funct.* 2018 May; 223(4):1989-1998. Doi: 10.1007/s00429-018-1606-4. Epub 2018 Jan 9.

## HEALTH AND DISEASE

### Prevalence of fecal viruses and bacteriophage in Canadian farmed mink (*Neovison vison*)

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<sup>2</sup>*Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), Elora, ON, Canada.*

Recent viral metagenomic studies have demonstrated the diversity of eukaryotic viruses and bacteriophage shed in the feces of domestic species. Although enteric disease is a major concern in the commercial mink farming industry, few etiologic agents have been well characterized. This study aimed to identify viruses shed in the fecal matter of clinically healthy commercial mink from 40 southern Ontario farms. Viral RNA was extracted from 67 pooled fecal samples (30 adult female mink and 37 kit) and amplified for Illumina sequencing on the NextSeq platform, and the resulting contigs were trimmed and assembled using Trimmomatic 0.36.0 and Spades 3.8.0 in iVirus (CyVerse, AZ, USA) and SeqMan NGen 12 (DNASTar, WI, USA). Identification of assembled sequences >100 bp (Geneious 10.1.3) showed an abundance of bacteriophage sequences, mainly from families Siphoviridae (53%), Podoviridae (22%), Myoviridae (20%), Inoviridae (1%), Leviviridae (0.04%), Tectiviridae (0.01%), and Microviridae (0.01%). A diverse range of vertebrate viruses were detected, of which posavirus 3, mink bocavirus, gyroviruses, and avian-associated viruses were most abundant. Additionally, sequences from nonvertebrate viruses with water and soil-associated amoebal and algal hosts were also highly prevalent. The results of this study show that viruses shed in the fecal matter of healthy commercial mink are highly diverse and could be closely associated with diet, and that more research is necessary to determine how the detected viruses may impact mink health.

*Microbiologyopen.* 2018 Apr 10: e00622.

Doi: 10.1002/mbo3.622. Epub ahead of print.

### Genetic diversity and phylogenetic analysis of Aleutian mink disease virus isolates in North-east China

Leng X.<sup>1</sup>, Liu D.<sup>2</sup>, Li J.<sup>1</sup>, Shi K.<sup>1</sup>, Zeng F.<sup>1</sup>, Zong Y.<sup>1</sup>, Liu Y.<sup>1</sup>, Sun Z.<sup>1</sup>, Zhang S.<sup>1</sup>, Liu Y.<sup>1</sup>, Du R.<sup>3</sup>

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Aleutian mink disease is the most important disease in the mink-farming industry worldwide. So far, few large-scale molecular epidemiological studies of AMDV, based on the NS1 and VP2 genes, have been conducted in China. Here, eight new Chinese isolates of AMDV from three provinces in north-east China were analyzed to clarify the molecular epidemiology of AMDV. The seroprevalence of AMDV in north-east China was 41.8% according to counterimmunoelectrophoresis. Genetic variation analysis of the eight isolates showed significant non-synonymous substitutions in the NS1 and VP2 genes, especially in the NS1 gene. All eight isolates included the caspase-recognition sequence NS1:285 (DQTD↓S), but not the caspase recognition sequence NS1:227 (INTD↓S). The LN1 and LN2 strains had a new 10-amino-acid deletion in-between amino acids 28-37, while the JL3 strain had a one-amino-acid deletion at position 28 in the VP2 protein, compared with the AMDV-G strain. Phylogenetic analysis based on most of NS1 (1755 bp) and complete VP2 showed that the AMDV genotypes did not cluster according to their pathogenicity or geographic origin. Local and imported ADMV species are all prevalent in mink-farming populations in the north-east of China. This is the first study to report the molecular epidemiology of AMDV in north-east China based on most of NS1 and the complete VP2, and further provides information about polyG deletions and new variations in the amino acid sequences of NS1 and VP2 proteins. This report is a good foundation for further study of AMDV in China.

*Arch Virol.* 2018 Feb 17.

Doi: 10.1007/s00705-018-3754-5.

Epub ahead of print.

### Development and application of an indirect enzyme-linked immunosorbent assay based on recombinant capsid protein for the detection of mink circovirus infection

Ge J.<sup>1,2</sup>, Cui X.<sup>1</sup>, Shi Y.<sup>1</sup>, Zhao L.<sup>3</sup>, Wei C.<sup>1</sup>, Wen S.<sup>1</sup>, Xia S.<sup>1</sup>, Chen H.<sup>4</sup>

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#### Background

Mink circovirus (MiCV) is a newly discovered pathogen associated with mink diarrhea. The prevalence and economic importance of this virus remain poorly understood, and no specific serological assay has been developed for the diagnosis of MiCV infection.

#### Results

In this study, a recombinant capsid protein antigen expressed in *Escherichia coli* was utilized to establish an indirect enzyme-linked immunosorbent assay (iELISA). Results revealed that the assay had no cross-reactivity with other related pathogens, and the respective sensitivity and specificity of the proposed iELISA were 92.31% and 91.67% compared with those obtained of Western blot on 138 serum samples from minks. The correlation coefficient between iELISA and Western blot was 0.838 ( $p > 0.05$ ). iELISA was applied to detect MiCV antibodies in 683 clinical serum samples from different farms from the major mink industry province in China, and 21 of 24 farms with 163 of 683 (23.87%) individuals were tested positive for MiCV antibodies. The positive rates of each of the 21 flocks ranged from 2.33% to 73.68%.

#### Conclusions

These results indicated that iELISA was a sensitive and specific method suitable for the large-scale detection of MiCV infections in mink. This study provided an effective method for the serological diagnosis and positive rate investigation of MiCV infection.

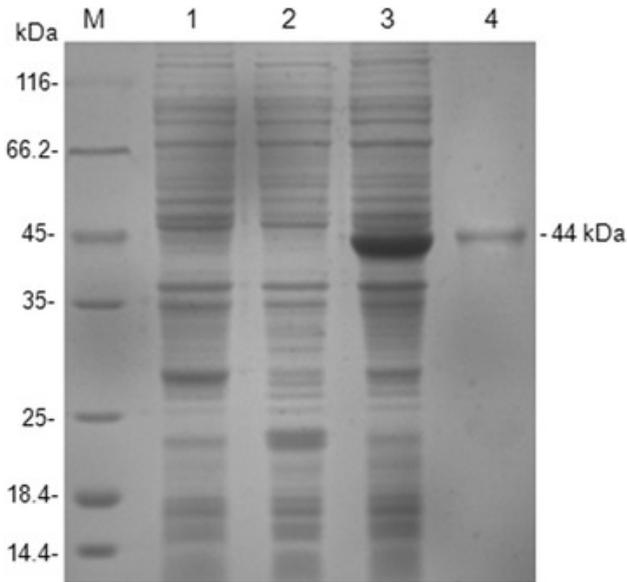


Fig. 1. From: Development and application of an indirect enzyme-linked immunosorbent assay based on recombinant capsid protein for the detection of mink circovirus infection.

SDS-PAGE analysis of the rCap fusion protein. Lane M: protein molecular weight marker; Lane 1: Rosetta control; Lane 2: pET32a vector control; Lane 3: pET32a-cap bacterial lysate; Lane 4: Purified fusion protein

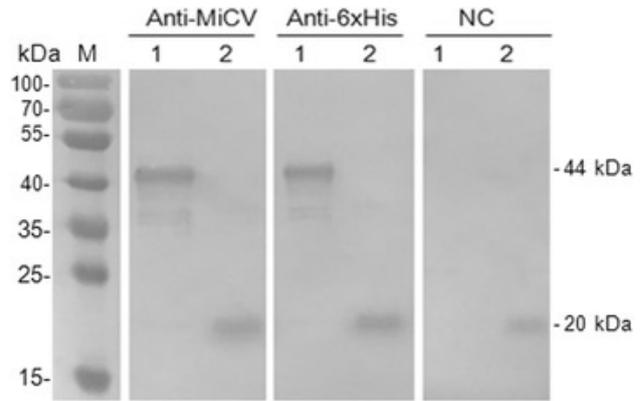


Fig. 2. From: Development and application of an indirect enzyme-linked immunosorbent assay based on recombinant capsid protein for the detection of mink circovirus infection.

Western blot was performed with MiCV positive sera (Anti-MiCV), Anti-6xHis HRP conjugated (Anti-6xHis) or MiCV negative sera control (NC). Lane M: protein molecular weight marker; Lane 1: Purified protein rCap; Lane 2: pET32a vector control

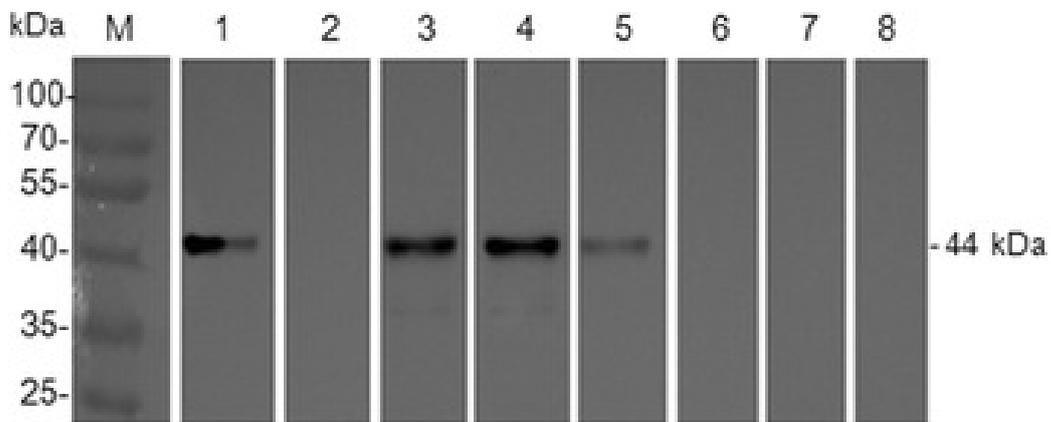


Fig. 3. From: Development and application of an indirect enzyme-linked immunosorbent assay based on recombinant capsid protein for the detection of mink circovirus infection.

Western blot analysis of the rCap fusion protein. Lane M: protein molecular weight marker; Lane 1: Positive serum control; Lane 2: Negative serum control; Lane 3: Serum 1; Lane 4: Serum 2; Lane 5: Serum 3; Lane 6: Serum 4; Lane 7: Serum 5; Lane 8: Serum 6

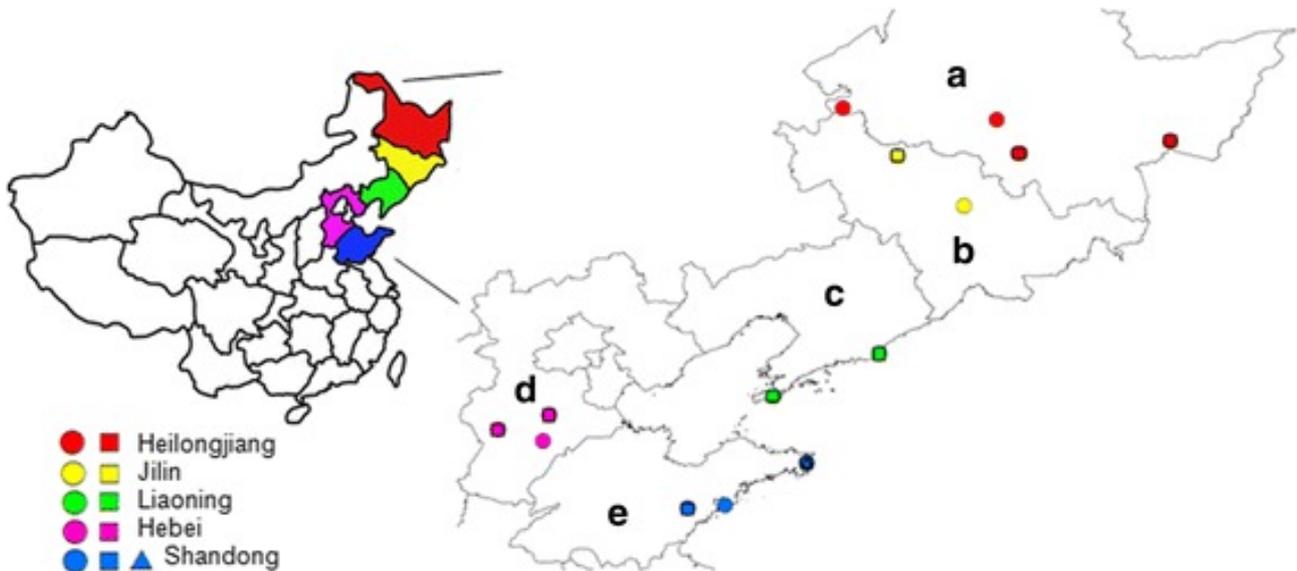


Fig. 4. From: Development and application of an indirect enzyme-linked immunosorbent assay based on recombinant capsid protein for the detection of mink circovirus infection. Geographical distribution of the sampled mink farms. The area covers 5 provinces in China: (a) Heilongjiang, (b) Jilin, (c) Liaoning, (d) Hebei, and (e) Shandong. The farms are marked with circles, squares, and triangles

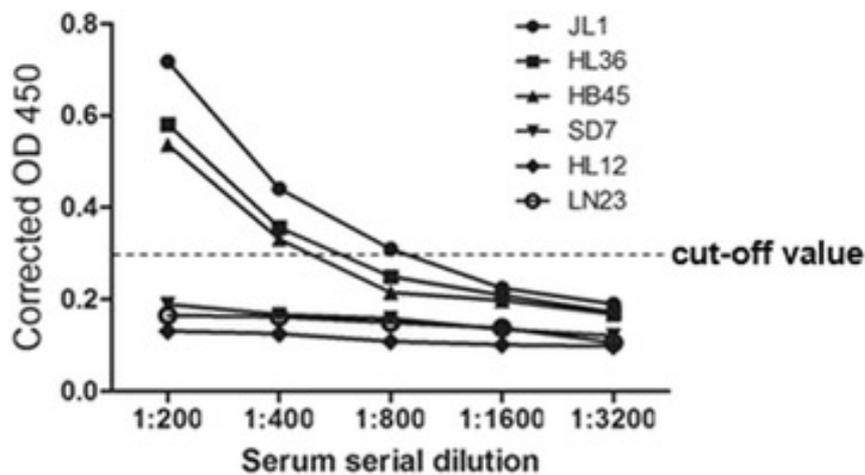


Fig. 5. From: Development and application of an indirect enzyme-linked immunosorbent assay based on recombinant capsid protein for the detection of mink circovirus infection. Anti-MiCV antibody titres in positive and negative mink serum samples. Positive and negative mink serum samples were prepared in dilutions of 1:200, 1:400, 1:800, 1:1600 and 1:3200 used in an ELISA assay. The cut-off value cutoff was determined by counting the mean OD value of the ELISA negative samples plus 3 standard deviation (SD)

*BMC Vet Res.* 2018 Jan 26; 14(1):29.  
Doi: 10.1186/s12917-018-1337-z.

### Rapid and sensitive detection of mink circovirus by recombinase polymerase amplification

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To date, the pathogenic role of mink circovirus (MiCV) remains unclear, and its prevalence and economic importance are unknown. Therefore, a rapid and sensitive molecular diagnosis is necessary for disease management and epidemiological surveillance. However, only PCR methods can identify MiCV infection at present. In this study, we developed a nested PCR and established a novel recombinase polymerase amplification (RPA) assay for MiCV detection. Sensitivity analysis showed that the detection limit of nested PCR and RPA assay was 10<sup>1</sup> copies/reaction, and these methods were more sensitive than conventional PCR, which has a detection limit of 10<sup>5</sup> copies/reaction. The RPA assay had no cross-reactivity with other related viral pathogens, and amplification was completed in less than 20 min with a simple device. Further assessment of clinical samples showed that the two assays were accurate in identifying positive and negative conventional PCR samples. The detection rate of MiCV by the RPA assay in clinical samples was 38.09%, which was 97% consistent with that by the nested PCR. The developed nested PCR is a highly sensitive tool for practical use, and the RPA assay is a simple, sensitive, and potential alternative method for rapid and accurate MiCV diagnosis.

*J Virol Methods*. 2018 Mar 6; 256:1-5.

Doi: 10.1016/j.jviromet.2018.02.022.

Epub ahead of print.

### Detection and characterization of Distemper virus in a mink (*Neovison vison*) in Turkey

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In this study, a mink showing hard pad disease like symptoms was euthanised. Heart blood and various

tissue samples collected during necropsy and tested by specific RT-PCR were found positive for CDV. H and F gene segments of the CDV strain was also partially sequenced using the appropriate primers, and subsequently the sequences were analysed and compared with same gene fragment sequence of other CDV isolates from different countries. The results of the phylogenetic analysis showed that the Turkish-Mink distemper strain is closely related to European CDV strains of lineage 1. Additionally, the distemper antigen was also detected when the tissue samples were examined by histology or immunohistochemistry.

*Vet Ital*. 2018 Mar 31; 54(1):79-85.

Doi: 0.12834/VetIt.936.4787.4.

### Quantitative Analysis of Cellular Proteome Alterations in CDV-Infected Mink Lung Epithelial Cells

Tong M.<sup>1</sup>, Yi L.<sup>1</sup>, Sun N.<sup>1</sup>, Cheng Y.<sup>1</sup>, Cao Z.<sup>1</sup>, Wang J.<sup>1</sup>, Li S.<sup>1</sup>, Lin P.<sup>1</sup>, Sun Y.<sup>1</sup>, Cheng S.<sup>1</sup>

<sup>1</sup>State Key Laboratory for Molecular Biology of Special Economic Animals, Institute of Special Economic Animal and Plant Science, Chinese Academy of Agricultural Sciences, Changchun, China.

Canine distemper virus (CDV), a paramyxovirus, causes a severe highly contagious lethal disease in carnivores, such as mink. Mink lung epithelial cells (Mv.1.Lu cells) are sensitive to CDV infection and are homologous to the natural host system of mink. The current study analyzed the response of Mv.1.Lu cells to CDV infection by iTRAQ combined with LC-MS/MS. In total, 151 and 369 differentially expressed proteins (DEPs) were markedly up-regulated or down-regulated, respectively. Thirteen DEPs were validated via real-time RT-PCR or western blot analysis. Network and KEGG pathway analyses revealed several regulated proteins associated with the NF-κB signaling pathway. Further validation was performed by western blot analysis and immunofluorescence assay, which demonstrated that different CDV strains induced NF-κB P65 phosphorylation and nuclear translocation. Moreover, the results provided interesting information that some identified DEPs possibly associated with the pathogenesis and the immune response upon CDV infection. This study is the first overview of the responses to CDV infection in Mv.1.Lu cells, and the findings will help to analyze

further aspects of the molecular mechanisms involved in viral pathogenesis and the immune responses upon CDV infection.

*Front Microbiol.* 2017 Dec 22; 8:2564.

Doi: 10.3389/fmicb.2017.02564. eCollection 2017.

### **H9N2 influenza virus isolated from minks has enhanced virulence in mice**

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<sup>3</sup>Key Laboratory of Animal Epidemiology and Zoonoses, Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, Beijing, China.

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H9N2 is one of the major subtypes of influenza virus circulating in poultry in China, which has a wide host range from bird to mammals. Two H9N2 viruses were isolated from one mink farm in 2014. Phylogenetic analysis showed that internal genes of the H9N2 viruses have close relationship with those of H7N9 viruses. Interestingly, two H9N2 were separated in phylogenetic trees, indicating that they are introduced to this mink farm in two independent events. And further mice studies showed that one H9N2 caused obvious weight loss and 20% mortality in infected mice, while another virus did not cause any clinical sign in mice infected at the same dose. Genetic analysis indicated that the virulent H9N2 contain a natural mutation at 701N in PB2 protein, which was reported to contribute to mammalian adaptation. However, such substitution is absent in the H9N2 avirulent to mice. Circulation of H9N2 in mink may drive the virus to adapt mammals; continual surveillance of influenza virus in mink was warranted.

*Transbound Emerg Dis.* 2018 Jan 14.

Doi: 10.1111/tbed.12805. Epub ahead of print.

### **Amdoparvovirus Infection in Red Pandas (*Ailurus fulgens*)**

Alex C.E.<sup>1,2</sup>, Kubiski S.V.<sup>1,2</sup>, Li L.<sup>3,4</sup>, Sadeghi M.<sup>3</sup>, Wack R.F.<sup>1</sup>, McCarthy M.A.<sup>1</sup>, Pesavento J.B.<sup>5</sup>, Delwart E.<sup>3</sup>, Pesavento P.A.<sup>1</sup>

<sup>1</sup>University of California, Davis, Davis, CA, USA.

<sup>2</sup>Institute for Conservation Research, San Diego Zoo Global, San Diego, CA, USA.

<sup>3</sup>Blood Systems Research Institute, San Francisco, CA, USA.

<sup>4</sup>California Department of Public Health, Microbial Diseases Laboratory, Richmond, CA, USA.

<sup>5</sup>California Animal Health and Food Safety Laboratories, Davis, CA, USA.

Aleutian mink disease virus is the type species in the genus Amdoparvovirus, and in mink and other Mustelidae can cause either subclinical disease or fatal chronic immune stimulation and immune complex disease. The authors describe a novel amdoparvovirus in the endangered red panda (*Ailurus fulgens*), discovered using viral metagenomics. The authors analyzed the prevalence, tissue distribution, and disease association by PCR, in situ hybridization, electron microscopy, and histology in a group of 6 red pandas from a single zoological collection. The study incorporates a fecal shedding survey and analysis of tissues from 4 necropsied animals over a 12-year span. The tentatively named red panda amdoparvovirus (RpAPV) was detected in the feces and/or tissues of all animals tested. At necropsy of 1 geriatric animal, infection was associated with pyogranulomatous peritonitis, pancreatitis, and myocarditis. Other animals had detectable low-level viral nucleic acid in lymph nodes and both oral and intestinal epithelium at the time of necropsy. Full-length genome sequences of RpAPV strains from 2 animals had 12% sequence divergence, demonstrating genetic diversity even among in-contact animals. RpAPV is a persistent infection in this cohort of red pandas, and has variable clinical expression.

*Vet Pathol.* 2018 Jan 1: 300985818758470.

Doi: 10.1177/0300985818758470.

Epub ahead of print.

## Vaccine resistant pseudorabies virus causes mink infection in China

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<sup>5</sup>School of Life Sciences, Shandong University, 27 Shanda Road South, Jinan, 250100, China.

### Background

Pseudorabies, a highly contagious infectious disease of swine is caused by pseudorabies virus (PRV). PRV can cause fatal infection in other animal species.

### Results

We report a deadly outbreak of pseudorabies that killed 87.2% (3522/4028) minks in a farm in 2014 in Shandong Province, China. PRV was isolated by using Vero cell culture and detected in mink samples by PCR from minks died during the outbreak. Epidemiological analysis indicated that 5.8% of minks (33/566) were PCR positive to PRV in Shandong Province. Phylogenetic analysis indicated that the PRV strains isolated from minks in this study were in the same clade with the Chinese porcine PRV isolates, which are resistant to the PRV vaccine.

### Conclusions

We demonstrated that pseudorabies virus caused an outbreak of minks in a farm in Shandong Province of China and the virus has a very high infection rate in minks in Shandong Province, which is a challenge for the fur industry in China

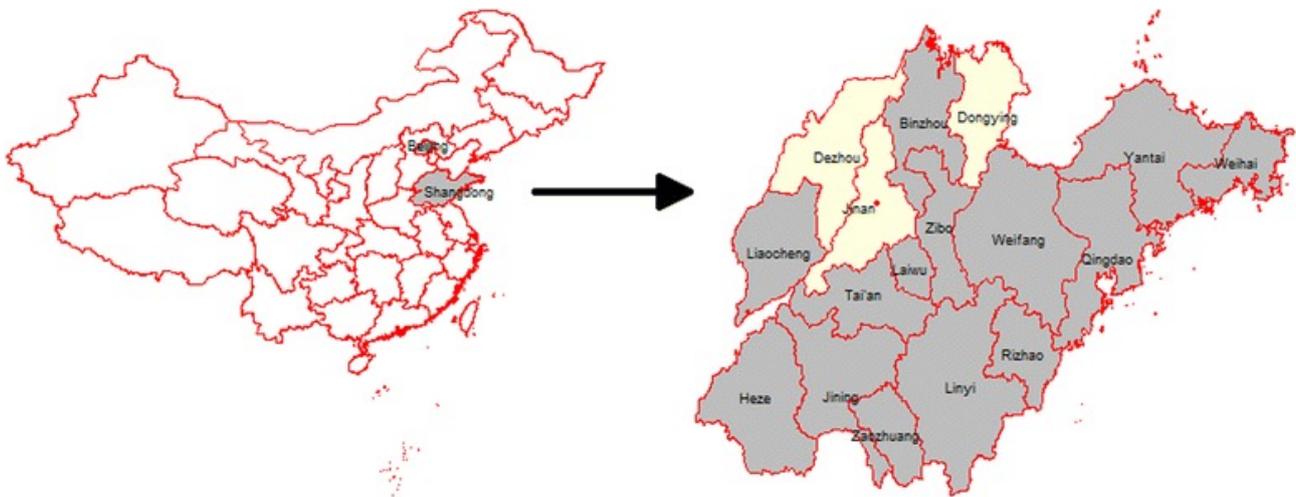


Fig. 1. From: Vaccine resistant pseudorabies virus causes mink infection in China.

Geographic location of Shandong Province of China (left) and the mink sample collection sites (right). Mink samples were collected from 14 (grey areas) of 17 cities in Shandong Province. The maps were drawn using the R Project for Statistical Computing (<https://www.r-project.org/>)

Abstracts

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W-MPRV1  ACCGGGAGGTGGTGAACACTACTGGTACCGCAAGAACGGCCGGACGCTCCCGCGGGCCTACG  780
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
W-MPRV2  ACCGGGAGGTGGTGAACACTACTGGTACCGCAAGAACGGCCGGACGCTCCCGCGGGCCTACG  780

W-MPRV1  CCGCCGC-----  787
          |||||
W-MPRV2  CCGCCGCCACGCCGTACGCCATCGACCCCGCGCGGCCCTCGGCGGGCTCGCCGAGGCCCA  840

W-MPRV1  -----

W-MPRV2  GGCCCCGGCCCCGGCCCCAGGCCCGGCCGAAGCCCCGAGCCCCCGCCGACGCCCGCGC  900

W-MPRV1  -----

W-MPRV2  CCCCCGGCCGCTGCCCGAGCCGGGACGCGGGACCACGCCCGGGGGGGCGCCCCACGC  960

W-MPRV1  -----

W-MPRV2  CGGACCCCCGAGGCCCGAGACGCCGACCGCCCTTCGCCCGCCGGCCGTCGTGCCCA  1020

W-MPRV1  -----GCCGGGCGTCT  798
          |||||||||
W-MPRV2  GCGGGTGGCCGAGCCCGGGAGCCGTTCCCGCCCCGGACCACCGCCGCGCCGGGCGTCT  1080

W-MPRV1  CGGCCACCGCTCGGTGATCGTCGGCACGGGCACCGCGATGGGCGCGCTCCTGGTGGGCG  858
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
W-MPRV2  CGGCCACCGCTCGGTGATCGTCGGCACGGGCACCGCGATGGGCGCGCTCCTGGTGGGCG  1140

W-MPRV1  TGTGCGTCTACATCTTCTCCGCCTGAGGGGGGCGAAGGGGTATCGCCTCCTGGGCGGTC  918
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
W-MPRV2  TGTGCGTCTACATCTTCTCCGCCTGAGGGGGGCGAAGGGGTATCGCCTCCTGGGCGGTC  1200

W-MPRV1  CCGCGGACCGCGACGAGCTAAAAGCGCAGCCCGGTCCGTAGCCTCCGCAGTA  978
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
W-MPRV2  CCGCGGACCGCGACGAGCTAAAAGCGCAGCCCGGTCCGTAGCCTCCGCAGTA  1252
    
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Fig. 2. From: Vaccine resistant pseudorabies virus causes mink infection in China. DNA sequence alignment of gD gene of mink isolates of PRV W-MPRV1 and W-MPRV2. W-MPRV1 had a deletion of 281 nucleotides from 787 nucleotide to 1069 nucleotide

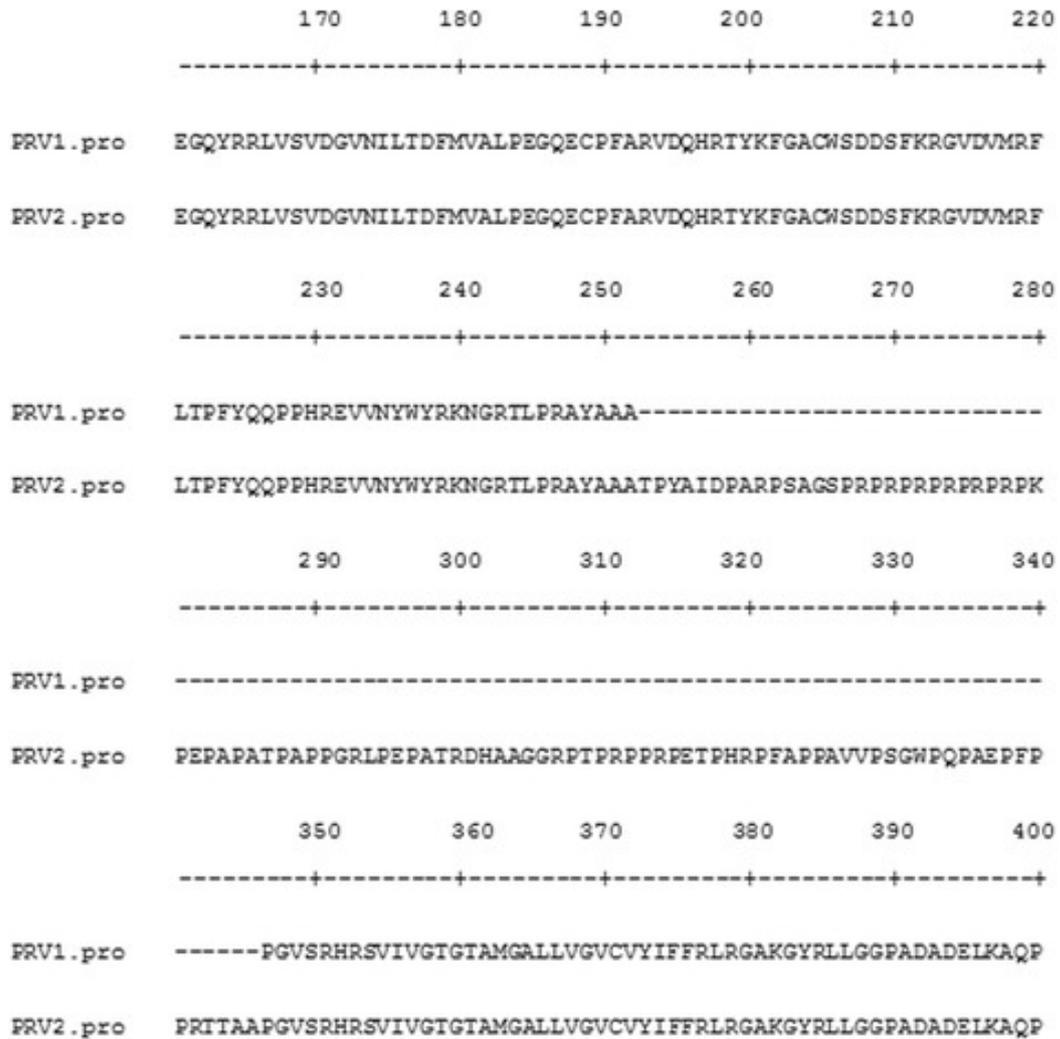


Fig. 3. From: Vaccine resistant pseudorabies virus causes mink infection in China. Amino acid sequence alignment of gD of mink isolates of PRV W-MPRV1 and W-MPRV2. W-MPRV1 had a deletion of 93 amino acids near the C-terminal

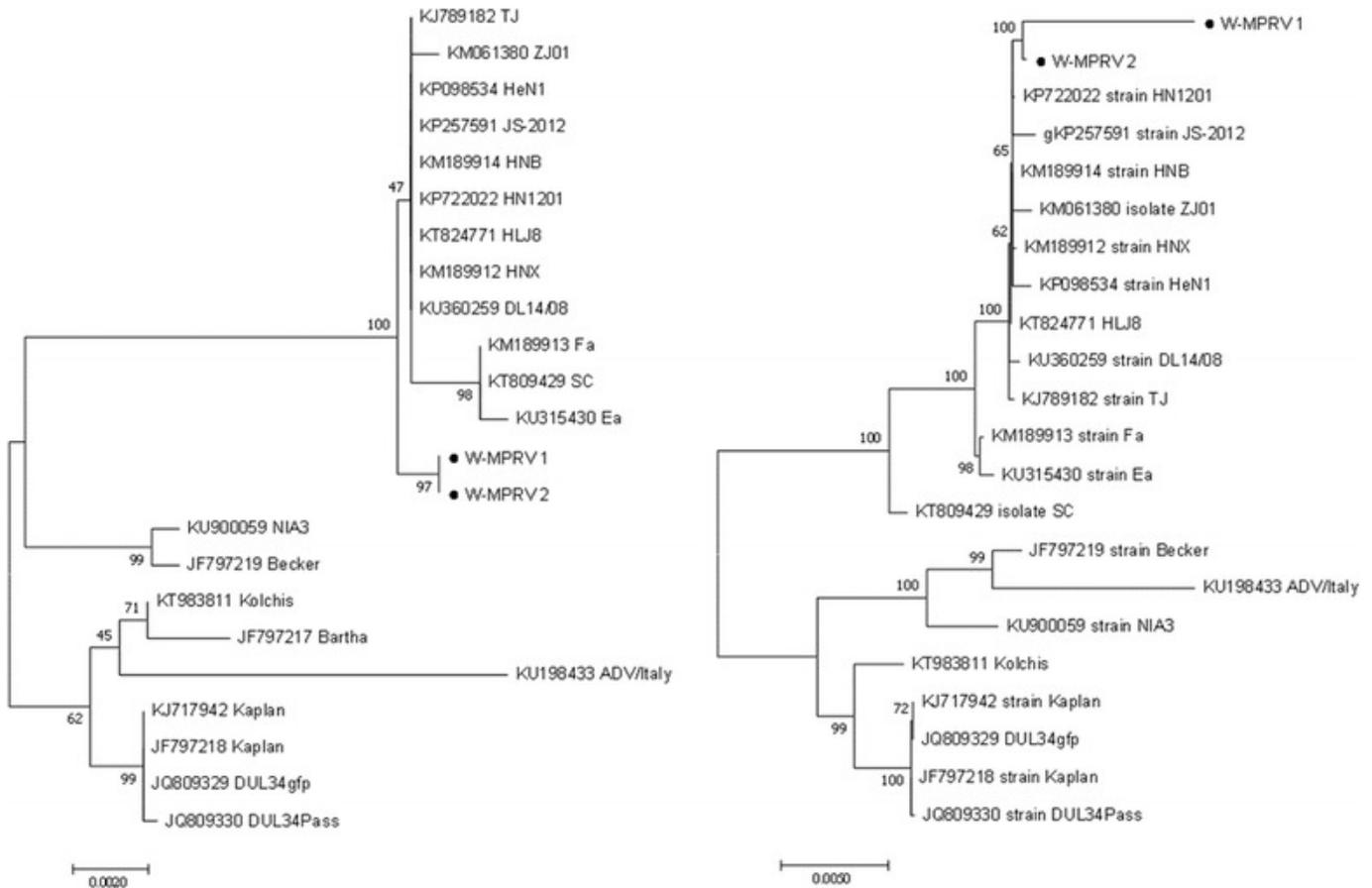


Fig. 4. From: Vaccine resistant pseudorabies virus causes mink infection in China. Phylogenetic tree of Pseudorabies virus. The phylogenetic tree was constructed with gB gene sequence (left) and the concatenated sequence of gB, gC, gD, gE, gH and TK genes (right) using MEGA5 software with 1000 replicates for bootstrap testing. Numbers (> 50) above or below branches are posterior node probabilities. The GenBank number was labeled on each line. Dots indicated sequences obtained in this study. Scale bar indicates nucleotide substitutions per site

*BMC Vet Res.* 2018 Jan 19; 14(1):20.

Doi: 10.1186/s12917-018-1334-2.

### Occurrence of *Giardia duodenalis* assemblages in farmed long-tailed chinchillas *Chinchilla lanigera* (*Rodentia*) from Romania

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<sup>2</sup>Parasitology and Parasitic Diseases Department Cluj-Napoca, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, Cluj-Napoca, Romania.

### Background

*Giardia duodenalis* is a parasitic protist that infects a large number of species, being localized in the small intestine. Two of the eight recognized assemblages have zoonotic potential, but studies regarding their distribution in less important pet or farm species are scarce. Of these species, the long-tailed chinchilla is a host for *Giardia* spp., although data on the spread of infection and assemblages involved are confined. The present work aimed to determine the prevalence of *Giardia* infection and assemblage identification in farmed chinchillas in Romania. A total of 341 fecal samples were collected from 5 farms and microscopically examined using flotation test based on satu-

rated sodium chloride solution. DNA from all positive samples was extracted and identified by PCR targeting the *gdh* gene.

### **Results**

The overall prevalence of *Giardia* infection was 55.7% (190/341); there was no statistically significant difference ( $P=0.25$ ) in prevalence between young animals (58.8%) and adults (52.6%). Assemblages B (151/190), D (33/190) and E (6/190) were identified. Among assemblage B, sub-assemblages BIII (6/151) and BIV (145/151) were determined.

### **Conclusions**

This study demonstrates that *Giardia* spp. infection is highly prevalent in farmed chinchillas from Romania, and the sub-assemblages identified are potentially zoonotic.

*Parasit Vectors*. 2018 Feb 7; 11(1):86.

Doi: 10.1186/s13071-018-2652-8.

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# FAGLIG ÅRSBERETNING 2017



KOPENHAGEN FUR RESEARCH

**ANNUAL REPORT 2017**



### **How to ensure a correct evaluation of the minks' temperament by the 'stick test'**

*B.I.F. Henriksen, J. Malmkvist & S.H. Møller*

A shelf formed as a bunk in the front part of the cage may function as a place of refuge, with the mink reacting less timid and with more confidence if they do not have to leave this refuge during the test situation. The size of the wooden stick influences the minks' reaction in the test, indicating that the mink pay less attention to a thin stick compared with a tongue spatula. When performing a stick test, it is therefore important to keep a distance between a refuge and the stick during the test, and to use the standardized wooden tongue spatula to be able to compare results between farms or tests.

*Annual Report 2017, 5-7. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Relocation shortly after mating does not have a major impact on stress responses and reproduction in farm mink**

*T.M. Schou & J. Malmkvist*

We investigated the influence of relocation of breeding mink females to the maternity units. We compared behaviour, stress response and reproductive outcome in breeding females in three groups of c 40 double-mated first-year dams: NON: no relocation, i.e. they stayed in their home cage, DIRECT: relocated directly after their own last mating, and EARLY: relocated early after the end of the mating season, March 23. Treatment EARLY is the best-known practise, based on the current knowledge, and the Danish legislative demands on having an empty cage between mink dams from mid April. All mink got straw in the cage, and thereby nest building possibility, March 23. Group NON dams tended to have a lower concentration of stress hormone (FCM) in mid-April ( $P = 0,088$ ), however, NS different before and after the deliveries. We report no or only minor difference in nest building, in nest climate, behaviour and reproductive outcome, which are attributed to the different group treatments. Thus, the results do not lead to novel recommendations for the relocation of mated mink females.

*Annual Report 2017, 8-11. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Effect of reducing litter size just after birth on the survival of kits raised by 1st and 2nd year females during the nursing period**

*T.N. Clausen & P.F. Larsen*

The purpose of the experiment was to investigate the survival of mink kits relocated to another female during the first days after birth. Kits from litter size 1 were relocated and litters with more than 10 kits were reduced to a maximum of 10. It was the largest kits that was relocated in litters with more than 10 kits. The kits were relocated within the first few days after birth. Kits were relocated to females with litter size 2 - 8. 1 - 3 kits were relocated, but only up to 9 kits in the new litter were allowed. In 2017 the kits were chip-marked and weighed before relocation and observed the rest of the period.

Results showed that the large relocated kits had as good a survival as the foster mother's own kits, but it was best for the whole litter that the female only received one kit extra. The survival of relocated kits did not depend on whether they came from a first or second year female and when they were relocated (investigated within the first five days after birth). On the other hand, the weight of the kits was important as the heaviest of the relocated kits had the best chance of survival. A large proportion of the kits that came from litter size 1 died, whereas kits from litters greater than 10, had very low mortality. The best foster mothers were the second-year females.

*Annual Report 2017, 12-15. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Selection for more confident mink does not result in setbacks for quality, size and fecundity**

*T. Villumsen, J.T. Thirstrup, J. Malmkvist & M.S. Lund*

We investigated the consequences of selection toward more confident mink, which is required in the Danish legislation. We estimated heritability for behavior to determine the selection potential for more confident behavior in mink. Furthermore, we estimated the genetic correlation between behavior and pelt/skin traits and fecundity - all traits of major economic importance. Data consisted of 26,327 brown mink (*Neovison vison*), all born in 2013-2016 at AU Foulum research farm. We found a moderate heritability (standard error in brackets) on 0.19 (0.03) for behavior in both sexes. The genetic correlation between males and females was 0.95 (0.06), indicating identical genetic background for the

two sexes, and same potential for selection. We found no significant genetic correlations between behavior and production/fertility traits. Based on these results, we conclude that breeding for more confident mink is possible without any detrimental effect on the traits of major economic importance, such as quality, size and fecundity. However, the genetic progress for these traits will be reduced when a part of the selection potential is used to select confident mink.

*Annual Report 2017, 16-21. Copenhagen Forskning, Agro Park 15, DK-8200 Aarhus N, Denmark.*

### **Effect of sex and color type on the digestibility of nutrients in mink**

*K. Byskov & F.L. Larsen*

The purpose of this study is to compare the apparent total tract digestibility coefficients (ATTDC) of protein, fat, and carbohydrate in complete diets fed to Brown type males and females and to males of Brown and White color types, to investigate the effect of sex and color type on the ability of the mink (*Neovision vision*) to digest nutrients. The trial comprised 5 males and 5 females of Brown color type and 5 males of White color type. The 5 Brown color type males were used as control group against both Brown females and White males. The trial showed that females contaminate feces with urea to a greater degree than males, affecting ATTDC of protein and carbohydrate with 7.4 and +9.7 percent units, respectively. ATTDC of carbohydrate is affected, as carbohydrate content is calculated as a function of analyzed content of protein. When nitrogen content in feces was corrected for the contribution of urea, there was no significant difference in ATTDC between males and females for protein, fat or carbohydrate. Furthermore, there was no significant difference between ATTDC of protein or carbohydrate between males of Brown and White color type, while ATTDC of fat was 2.5 percentage units higher for Brown males compared to White males. The results demonstrate that sex and color type of the mink have no or only little effect on the ability to digest nutrients.

*Annual Report 2017, 23-25. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **The effect of animal fat and sunflower oil on fat digestibility and mink kit growth from 6-11 weeks of age**

*C.F. Matthiesen, C. Marcussen & A.H. Tauson*

The metabolisable energy (ME) depends on diet composition and nutrient digestibility. The fat digestibility is usually high in carnivores, including the mink. The fat digestibility depends on fatty acid composition, chain length and may also be affected by dietary fat amount among kits. The aim was to determine the fat digestibility of lard and sunflower oil with either a medium (45% of ME) or high (55% of ME) fat content. Further to investigate the effect of fat source and amount on kit growth from 6 -11 weeks of age. Based on preliminary results, it can be concluded that fat digestibility and kit growth not was affected significantly by fat source or the amount of dietary fat. However, numerical differences in fat digestibility between groups were found. The analyzed fat content of 57% and 47% of ME from lard and sunflower oil, respectively, did not reduce the fat digestibility compared to a fat content of 45% and 44% of ME. The fat digestibility, ME intake, daily gain and kit growth was influenced by kit age where the fat digestibility increased with age and was numerically different between feeding groups. These finding however needs to be compared with results from ongoing analyzes.

*Annual Report 2017, 26-31. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Methionine in the last part of the growing furring period**

*T.N. Clausen & P.F. Larsen*

The purpose of the experiment was to investigate the consequences of reducing the content of digestible methionin (met) in the feed from 0.16 to 0.14 g / 100 kcal from late September to pelting. We used three groups of 135 brown male mink each. Two of the groups were control groups (K1, K2) where the content of digestible met in the feed was 0.16 g / 100 kcal throughout the whole period. The experimental group (Umet) was fed as the control groups until September 25, after which the content of digestible met in the feed was reduced from 0.16 to 0.14 g / 100 kcal until pelting. The results showed no adverse effect on skin size and quality when the content of digestible met in the feed was reduced from 0.16 to 0.14 g/ 100 kcal from late September to pelting.

*Annual Report 2017, 33-37. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

**Mink fed without addition of vitamins and minerals during the growing period – consequences in urine and blood with focus on vitamin B**

*M.S. Hedemann, P.F. Larsen, T.N. Clausen & S.K. Jensen*

The purpose of the investigation was to study the excretion of B vitamins and metabolites in urine and the concentration of B vitamins in blood in mink fed either control feed containing the recommended amount of vitamins and minerals or a similar diet without vitamins and minerals during the growing period. The results showed that vitamin B2 (riboflavin), metabolites of vitamin B3 (niacin), vitamin B5 (pantothenic acid) and vitamin B6 (pyridoxal) were excreted in the urine. The excretion was highest in male mink fed the control diet in September. The excretion was lower in November, which indicates a higher need for B-vitamins at this time. Male mink fed without addition of vitamins and minerals had a lower excretion of vitamin B in both September and November. There were no signs of vitamin B deficiency during the experimental period. In blood samples, riboflavin and a metabolite of niacin were detected. The differences observed in blood were comparable to those observed in urine. Apart from vitamin B, amino acid metabolites were excreted as well. Male mink fed a diet without addition of vitamins and minerals had a lower excretion that may be related to the better growth observed in this group. In conclusion, the addition of B2, B3, B5 and B6 vitamins can be reduced.

*Annual Report 2017, 38-43. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

**Effect of increasing content of Furpro 200 in mink feed during the growing furring period**

*T.N. Clausen & P.F. Larsen*

The purpose of the study was to investigate the effect of up to 12% Furpro 200 in the feed during the growing and furring period on body weight and skin length and quality. 5 groups of each 135 brown male mink kits were used. Furpro 200 was used with 4, 8 or 12 % in the feed and tested against two identical control groups. There was no negative impact on body weight and skin length and quality when up to 12 % Furpro 200 was included in the feed from mid-July to pelting.

*Annual Report 2017, 44-53. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

**Effect of increasing content of Scanbio cat 2 in mink feed during the growing furring period**

*T.N. Clausen & P.F. Larsen*

The purpose of the experiment was to investigate the effect of increasing inclusion of Scanbio cat 2 silage in mink feed during the growing and furring period. Five groups of 135 brown males were used, of which 2 control groups were without Scanbio. All experimental groups received from mid-July 3% Scanbio cat 2, two of the groups increased to 6% in August and one of these increased to 9% in September and the rest of the period.

The results showed that the quality of Scanbio cat 2 silage was good and no negative effect on body weight, skin length and quality was observed with up to 6% in the feed from mid-August and 9% from mid-September until pelting.

*Annual Report 2017, 54-62. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

**No negative effect of omitting mineral and vitamin addition during the growing and furring period on growth, skin quality and health**

*T.N. Clausen, R.B. Kjørup & P.F. Larsen*

The purpose of the experiment was to investigate the effect of omitting the addition of minerals or minerals and vitamins in the feed fed during the growing and furring period on growth, skin length, skin quality and health. The experiment comprised 4 groups of 135 male mink kits. All groups were fed the same basic feed. Two identical control groups (K1 and K2) were fed a feed added the recommended level of vitamins and minerals. One experimental group received a feed added the recommended level of vitamins but no minerals (UM). The second experimental group received a feed that was neither added vitamins nor minerals (UVM). With the composition of the basic feed used in this experiment, no adverse effects on growth, skin length, skin quality and health were observed, neither when only addition of minerals was omitted or when addition of both minerals and vitamins was omitted. The experiment showed that some minerals and especially vitamins were greatly overdosed when the recommended level of minerals and vitamins was added to the feed (K1 and K2). It should therefore be considered

whether this overdose causes the slightly higher mortality in the control groups compared to the experimental groups without additions. Similarly, in the future, it should be considered to reduce the recommended level of zinc and E vitamin during the growing and furring period.

*Annual Report 2017, 63-75. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Histological changes after experimental infection with Aleutian mink disease virus**

*T.H. Jensen, M. Chriél & M.S. Hansen*

The aim of this study was to give a histopathological description of the progression of a chronic experimental infection with a currently circulating Danish strain of AMDV, Saeby/DEN/799.1/05.

This experimental AMDV infection resulted in only minor unspecific clinical signs (decreased appetite and soft or discolored feces) mostly within the first eight weeks after AMDV inoculation. Gross pathology revealed few and inconsistent findings mainly associated with liver, spleen and kidney. Primarily, infiltrations of mononuclear cells in liver, kidney and brain, reduced density of lymphocytes and increased numbers of plasma cells in lymph node and spleen was observed. Natural infection, as occurred in the sentinel sapphire mink progressed similar to the experimentally inoculated mink.

The observed histopathology substantiates AMDV infection and no correlation to time of inoculation was found. The study confirms that diagnosing AMDV infection requires serology or PCR or ideally a combination of the two. The histopathological findings of the Saeby/DEN/799.1/05 AMDV strain resembles findings with other AMDV strains. This work has been published in details in *Acta Vet Scand* 2016, 58:35.

*Annual report 2017, 77-82. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark*

### **Antimicrobial drugs: consumption and resistance patterns**

*N.K. Nikolaisen, M. Chriél, T. Struve, V.F. Jensen, G. Larsen & K. Pedersen*

There are only few antimicrobial compounds registered for use in mink and no general treatment guidelines. Improper treatment of the animals may lead to issues

regarding animal welfare, skin quality and antimicrobial resistance. Here is presented the existing resistance patterns in pathogenic bacteria in Danish mink in relation to the antimicrobial consumption.

The consumption of antimicrobials increased from 2007 to 2012 and has since fluctuated at high levels.

The main prescribed antimicrobials were aminopenicillins followed by tetracyclines and macrolides, to which the bacterial pathogens generally had the highest resistant levels.

In general, antimicrobial resistance was recorded in many pathogens. *E. coli* showed high level of resistance to ampicillin. Half of the *Staphylococcus* spp. was resistant to tetracycline. The *Streptococcus* spp. showed high levels of resistance to tetracycline and erythromycin.

Antimicrobial resistance was commonly found in bacterial pathogens from mink. This expresses the need for treatment guidelines to ensure future prudent use of antimicrobials.

*Annual Report 2017, 83-88, Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **The early development of the gut microbiota in mink kits (*Neovison vison*) and the effect of early antibiotic intervention**

*A. Jakobsen, S. Skønager, M.I. Bahl, L. Andresen, O.L. Honoré & A.S. Hammer*

Many publications have highlighted the importance of the gut microbiota in health and disease, along with the potential repercussions of disturbances in its early establishment. On mink farms, antibiotics are primarily used in the kit season where the mink kits are still establishing their gut microbiota. Since antibiotics are known to have pervasive effects here on, it was the aim of this study to investigate the establishment and development of the gut microbiota in newborn mink kits as well as the effect of early antibiotic intervention (EAI). Both on kits who received antibiotics directly and on the offspring of antibiotic treated adult females. The dominating gut bacteria of mink kits were determined and a great similarity between the microbiota of the female's skin surrounding the teats and the offspring's gut microbiota was found. Alterations in bacterial community composition and diversity were revealed secondary to EAI. Furthermore, it was concluded that maternal antibiotic treatment affects the gut microbiota of the offspring, but in a different way than directly treated kits.

*Annual Report 2017, 89-94. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Feed quality and other factors relevant to the consumption of antibiotics on mink farms**

*V.F. Jensen, H.M. Sommer, T. Struve, J. Clausen & M. Chriél*

The consumption of antimicrobials in the mink breeding in Denmark has increased gradually over the past decade, and consumption on the individual farm has been shown to be affected by a number of factors (Jensen et al, 2016). In previous studies, the feed producer has been found to affect the occurrence of gastrointestinal disease and antimicrobial use in mink (Jensen et al., 2016, Rattenborg et al., 1999). The objective of this study (Jensen et al., 2017) was to investigate potential effects of specific feed parameters on prescription of antimicrobials. The study was cross-sectional, including 12 mink feed producers and 1472 mink farms during the study period, 2012–2014. The examined feed parameters included both chemical parameters and microbiological parameters. A multi-variable variance analysis was carried out analysing the effect of the feed parameters. Two binomial models were developed, adjusting for significant effects ( $p < 0.0001$ ) of *Ps. aeruginosa* infection, herd size, month (season) and year. The response variable was the prescription of antimicrobials on a given farm (Model A) or a proportion of farms within feed producer (Model B) for a period of 3.5 or 7 days from the day following the delivery of the given batch. In all models, prescription of antimicrobials was significantly ( $p < 0.0001$ ) associated with the count of faecal cocci.

*Annual Report 2017, 95-97. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Morphological and molecular characterization of an Eimeria-species isolates from Danish mink**

*H.H. Petersen, R. Yang, M. Chriél, M.S. Hansen & U.M. Ryan*

A survey was conducted on 30 Danish mink farms to determine the prevalence and species of *Eimeria* in Danish farmed mink (*Neovison vison*) from April to October 2016. Morphological analysis of sporulated oocysts revealed that they resemble the species *Eimeria vison*. However, the newly identified specie is a bit smaller than *E. vison*. Afterwards, the new oocyst

where molecularly analysed, and compared with sequences available in Genbank. Unfortunately, mink-derived sequences were not available from GenBank and the novel identified species is named *E. vison-like*. A total of 2.6% mink samples (108/4,141) were positive for *E. vison-like* oocysts by microscopy, equalling 78 (23.9%) mink excreting oocysts at least once during the study period.

*Annual report 2017, 99-101. Copenhagen Research, Agro Food Park 15, DK-8200, Aarhus N, Denmark.*

### **Mink may also have the flu**

*C.K. Hjulsgager, J.S. Krog, G. Larsen, M. Chriél & L.E. Larsen*

Influenza virus has been detected in mink with complicated and lethal pneumonia on several occasions in recent years. Test for influenza virus is only rarely requested in diagnostic submissions from diseased mink in Denmark. Thus, test for influenza virus is performed only if suspicion is raised based on autopsy findings, or if a known outbreak of influenza is ongoing. To initiate measures to control influenza in farmed mink, knowledge about the prevalence of influenza virus in farmed mink in Denmark is a prerequisite.

The aim of this study was to perform a systematic survey of influenza virus in the lungs from mink submitted for diagnostic examinations. Farmed mink submitted in 2016 to the National Veterinary Institute at DTU for diagnostic examinations, regardless of the reason, and samples collected at a mink autopsy course as well as from wild mink, were included in the study. Influenza A virus was detected in mink from eight different farms. Genetic analyses suggested these to be of both swine and human origin. To prevent transmission of influenza virus to mink, it is recommended to avoid contact between mink and humans with influenza-like symptoms and to ensure that mink feed containing swine byproducts are properly heat treated or otherwise inactivated.

*Annual Report 2017, 102-105. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **The influence of the female on diarrhea in the pre-weaning period in the mink**

*J.M. Birch, J.F. Agger, B. Aalbæk, T. Struve, A.S. Hammer & H.E. Jensen*

The aim of the study was to examine the association between patho-anatomical conditions in mink females and the presence of pre-weaning diarrhea in the mink litter. In total, 40 females with litters with diarrhea and 48 females with healthy litters from 30 mink farms were enclosed in this case control study. The females and 2-3 kits from each litter were euthanized and cultivation for bacteria from mammary tissue and the gut of the mink kits was carried out. Sections from formalin fixated mammary tissue were histologically evaluated for the presence of pathological lesions, and associations with disease status of the litter were tested with univariate and multivariable statistical analyses. Diarrhea in the mink litter was significantly associated with the age of the female and litter size. Positive bacterial culture from the mammary tissue and mastitis, defined as infiltration with neutrophil leucocytes, was common but not significantly associated with disease status in the litter in the multivariable statistical model.

*Annual Report 2017, 106-109. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Microbiological quality of mink feed**

*U. Lyhs, H. Frandsen, B. Andersen, B. Nonnemann, C.K. Hjulsager, K. Pedersen & M. Chriél*

Both the nutritional composition of mink feed as well as the microbiological quality are essential for the health and the breeding of mink. Mink feed is a fresh but perishable commodity. This study has focused on the microbiological quality of raw materials and mink feed from 3 Danish mink food producers. The samples were collected in November 2016 and May 2017. The results showed that there is a large variation in the bacterial load in the raw materials. The risk of degradation of the mink feed should be kept in mind during storage and use on the mink farms. MRSA and Salmonella were isolated in swine products, but not in the finished products. Products like barley and maize may have contained fungal toxins which are known to cause disease in other animals. The methods have a limitation, as only a microscopic part of the large amounts of ingredients used on a daily basis can be examined prior to use in the production. The raw materials are con-

stantly changing, and cocktail effects should be assessed when introducing new ingredients in the feed production.

*Annual Report 2017, 111-117. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Uptake of IgG in three-day-old kits**

*R. Mathiesen, M. Chriél, T. Struve, Å. Uttenthal & P.M.H. Heegaard.*

Mink kits are born with an immature immune system and with low serum concentrations of circulating immunoglobulins (IgG) (antibodies). It is crucial that the kits reach high concentrations of IgG in the circulation after birth by passive immunization via the mother's IgG found in colostrum and milk. This is vital for the mink kits' resistance against infection (bacteria and virus) from their near environment. In this study, we investigated the transfer of IgG by giving IgG orally to three-day-old kits. The transfer of IgG to the blood circulation was evaluated after three hours. To analyze the specific uptake of mink IgG to the blood circulation some kits received porcine IgG and others mink IgG. Preliminary results indicate that IgG uptake is not specific for mink IgG, as porcine IgG is also taken up. However, the percentage of mink IgG uptake is higher than for porcine IgG, which indicates the presence of a specific Fc receptor for mink IgG located in the intestinal wall of the mink kits. Future studies will elucidate if there is a correlation between high IgG serum concentration and increased protection against "pre-weaning diarrhea" syndrome.

*Annual Report 2017, 119-122. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

### **Preliminary results of pathological and microbiological investigations of mink kits with cystitis and urolithiasis**

*K. Mundbjerg, P.E. Pedersen, O.L. Honoré, F. Foged, C. Berner, A. Jakobsen, I. Sebbelov, A. Tolver & A.S. Hammer*

Urinary tract disease, including cystitis and urolithiasis are some of the most common causes of death in mink kits during the growth season on Danish farms. Only few systematic pathological and microbiological studies have been conducted in relation to urinary tract disease in mink. This report presents preliminary results

from an investigation of all mink kits found dead or euthanized during July 2017 on 3 mink farms (n = 452). Lesions in the urinary tract detected at necropsy were compared with results from microbial examination of bladder swaps. A total of 59 healthy animals were examined as a control, using the same procedure at the time of pelting in 2 of the 3 mink farms.

In conclusion, we found a significant ( $p < 0.05$ ) association between detection of bacterial species belonging to the *Staphylococcus intermedius* group (SIG) in the bladder and the presence urinary lesions at necropsy. This finding indicates that these bacteria may play a significant role in the development of urinary tract disease in mink in July. Further studies are necessary in order to investigate the role of these bacteria in development of urinary tract disease in mink. The majority (82.5%) of the mink kits dying of urinary tract lesions in July were males.

*Annual Report 2017, 123-127. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

#### **Factors affecting the occurrence of dermatitis in the abdominal region (wet belly) in female mink**

*A.S. Hammer, O.L. Honoré, F. Foged, C. Berner, A. Jakobsen & I. Sebbelov*

A severe type of dermatitis in the abdominal region has been reported in female mink. The dermatitis, referred to as “wet belly”, is associated with exudative skin lesions in the caudal abdominal region, inguinal area as well as inner thighs and may be associated with multiple ulcerations.

This report presents results of gross pathological and microbiological investigations of female mink on a farm with a high prevalence of dermatitis in the abdominal region in 2017. The prevalence of wet belly was estimated in two groups of 6 months old female mink of brown color type. Group 1 contained offspring of mothers found free of dermatitis before the breeding season (n=127) and group 2 contained offspring of mothers diagnosed with mild dermatitis in the abdominal region registered before the breeding season (n=122). In this study, a higher prevalence of dermatitis in the abdominal region was found among the offspring of mothers with dermatitis compared to offspring of animals without dermatitis. This finding was however not significant when applying a 5 % significance level ( $P=0.06$ ). There was a significantly higher prevalence of dermatitis in the abdominal region in females with high BMI and body weight (BMI exceeding 25 and body weight exceeding 2500 g). Long animals were

more predisposed for dermatitis, though length seemed to have less effect than BMI and weight.

*Annual Report 2017, 129-134. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

#### **Causes of mortality in Danish farm chinchilla (*Chinchilla lanigera*)**

*A.S. Hammer, A. Jakobsen & W. Weiss*

Until date, the main causes of death on Danish chinchilla farms were unknown since chinchilla are rarely sent in to full necropsy. By establishing the main causes of death, measures could be taken in order to prevent disease. To gain an overview of the primary causes of death among Danish farm chinchilla, 28 individuals from 4 different farms were necropsied. Histology and microbiology was performed in order to determine the cause of death and elaborate a necropsy manual for chinchilla. It was found that gastrointestinal illness along with dental disease seem to be the primary causes of death.

*Annual Report 2017, 135-138. Copenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

#### **Effect of feed and housing strategy during the growing period of the second-year females**

*T.N. Clausen & P.F. Larsen*

The purpose of the study was to investigate whether young females housed alone and feed restrictively (to body score 3) during the growing season performed better in the following reproduction period than young females housed with a male kit and fattened during the growing period. We used 473 brown first year females to the investigation.

The body weight during the winter period differed between the two groups of females until February. There was no significant difference in litter sizes and kit weights between the two groups, but females housed with a male kit had significantly more stillborn kits and a significantly greater weight loss from day 28 to day 42. Furthermore, there were a tendency to more kits day 28 in the group where the females had been housed alone. Based on the results of the investigation, it appears that moderately restrictive feeding during the prior growth period may have a positive effect on the following nursing period.

*Annual Report 2017, 139-142. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

**Investigation of two different water systems for mink kits in the nursing period**

*T.N. Clausen & P.F. Larsen*

The purpose of the study was to investigate whether installation of an extra water nipple in front of the nest box has a positive effect on growth and survival of mink kits and females. The study comprised 500 white first year females divided into 3 groups. In the control group females and kits were housed in traditional cages with the water nipple at the end of the cage, in the two experimental groups one of two different water systems were installed where the nipple was placed in the cage in front of the nest box.

The use of additional water close to the nest box had in this investigation a positive effect on weight development of kits and females during the nursing period.

*Annual Report 2017, 143-147. Kopenhagen Research, Agro Food Park 15, DK-8200 Aarhus N, Denmark.*

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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.