SUMMER 2019

Introduction: Dr. Hugh Hildebrandt

Fur Commission USA and Joint Mink Research Committee Adviser

This edition of the research newsletter contains three general categories of topics. The first is Aleutian Disease which our North American funded research has shown for the first time that the virus has been identified in a fox. It has also shown that there are AD-like viruses that are present in the wildlife with unknown significance to our industry. These studies reinforce how important biosecurity is for protection of the animals.

The second category is health with research articles about kit diarrhea, tail tip lesions, and a toxicology study.

The last category is behavior. It is a diverse group of studies from around the world along with studies done by researchers supported by the Joint Mink Research Committee. These studies are done to help us better understand the animal and its interaction with its environment. It is noted in the stereotypic behavior article that the studies may help zoos, breeding centers and mink farms improve the design of their enclosures and efficacy of their enrichments.

All studies are available in their entirety at www.FurResearch.org, a free repository for academic research addressing health, welfare, and disease issues affecting the husbandry of fur bearing animals. Originally launched in 2005 by Fur Commission USA as an archive of the work done by the first generation of leading fur animal scientists in North America, we now include more recent material from sources across the globe, to be used as reference tools for current and future researchers, veterinarians, and fur farmers throughout the world. Fur Commission USA does not receive any compensation for this service.
Application of Molecular Methods to the Study of Infectious Diseases in the Mink Industry

Dr. Marta Canuti and Dr. Andrew S. Lang: Department of Biology, Memorial University of Newfoundland

Our research project studied infectious diseases affecting mink that are relevant for the mink industry and was focused on three main areas: Aleutian mink disease virus (AMDV) and related viruses, mink enteritis virus (MEV) and related viruses, and footpad necrosis (FPN).

AMDV is a very contagious virus that causes a chronic disease in mink and other terrestrial carnivores. Since the virus can cause the death of the animal it infects and since there is no vaccine available, this constitutes a serious problem for the mink industry and a cause of economic loss for farms. AMDV can be introduced into a farm by another infected farm or from wild animals. In our investigation we have identified several different lineages of viruses circulating in Newfoundland, which were similar to viruses circulating in other parts of Canada (Ontario and British Columbia), and we compiled a catalogue of strains for each infected farm. This allowed us to monitor the movement of viruses on the island. We observed that different farms (even from different Canadian provinces) share the same viruses, probably because they exchanged animals or contaminated materials, and that viruses very similar to those in farms circulate in wild animals. These viruses probably originated from (past or recent) animal escapes from farms. We observed the presence of two different viral strains that were probably introduced into Newfoundland at two different time points. Finally, we have identified a group of viruses that only affect wild animals, and this can be considered a marker virus that will help us determine in the future if viruses move from the wild to the farm.

In recent years, several AMDV-like viruses were identified in wild animals and it is likely that these can infect mink as well. The distribution of these viruses in the wild is unknown so we developed a diagnostic test that can detect all of these viruses and potentially also viruses never identified before. We used this method to screen several wild animals from various Canadian provinces and territories and identified four novel species of viruses re-
lated to AMDV. These viruses were found in skunks and mink in British Columbia and in foxes and pine martens in Labrador. These results allowed us to increase our knowledge about the different species of AMDV-like viruses and to update the diagnostic tests to detect these viruses and hopefully thereby reduce the risk of their introduction into farms.

MEV causes a severe disease in mink and other terrestrial carnivores, characterized by hemorrhagic enteritis and immune depression. Although there is a vaccine, this is not 100% effective and vaccinated animals that come in contact with this virus can still get infected. Our research identified for the first time the presence of MEV-like viruses in Canadian wildlife. Two different viral types were found in raccoons in British Columbia and other variants were found in Newfoundland in domestic dogs and wild coyotes. These studies demonstrate that MEV-related viruses are widespread in Canadian wildlife and their distribution should be monitored to avoid their introduction into farms.

FPN is a disease that causes serious dermatitis localized to the foot pads of mink and the cause is currently unknown. This condition causes pain and discomfort to the animals and is associated with reduced reproduction rates and consequential economic loss for farms. During this research we developed diagnostic methods to identify possible candidates responsible for this disease (both bacteria and viruses) but, unfortunately, we were not successful at identifying an etiological agent. At this point we cannot exclude that the initial cause for the disease is due to environmental factors, rather than an infectious agent. However, the methods devolved will be very useful in the event of future pathogen emergence as they will be readily available to allow a prompt identification of the involved pathogen.

**A Comparison Between Intraperitoneal Injection and Intranasal and Oral Inoculation of Mink with Aleutian Mink Disease Virus**

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**ABSTRACT:** Intranasal, with (INS) and without (IN) sedation, and oral inoculation were compared with intraperitoneal (IP) injection for establishing infection with a local isolate of
Aleutian mink disease virus (AMDV) in 35 American mink. Blood samples were collected on 0, 21, 36 and 56 day post-inoculation (dpi). Antiviral-antibodies and viral DNA in plasma and tissues were measured by counter-immunoelectrophoresis (CIEP) and PCR, respectively. The presence of AMDV DNA was tested by PCR in saliva, rectal and fecal samples collected on 0, 6, 10, 15, 21, 28, 36 and 56 dpi. Animals were killed at 56 dpi, samples of six organs were tested for antibody and AMDV DNA, and samples of the lungs, liver, kidneys and heart were subjected to histology. Viral DNA was detected in the spleen, lungs and lymph nodes of all inoculated mink on 56 dpi, indicating that all inoculation routes caused infection in mink. Viral DNA and antibodies were detected in plasma of all IP and INS inoculated mink by 36 dpi, but some animals which were inoculated orally or via IN remained seronegative by 56 dpi. It was concluded that INS route was the most effective method for establishing infection in mink without breaking the integrity of the animals' anatomical barriers. Viremia was short-lived in some mink, whereas antibody production persisted in seroconverted animals during the duration of the experiment. Saliva, rectal and fecal samples did not accurately detect infection. Histologic lesions of AD were observed on the four organs of most mink.

**Dam Characteristics Associated with Pre-weaning Diarrhea in Mink (Neovison vison)**

*Julie Melsted Birch1*, Jens Frederik Agger1, Bent Aalbæk1, Tina Struve2, Anne Sofie Hammer1 and Henrik Elvang Jensen1

**Abstract:** Background: Pre-weaning diarrhea (PWD) in mink, also known as “sticky kits”, is a frequently occurring syndrome in suckling mink kits on commercial mink farms. Outbreaks of PWD result in weakened kits, increased mortality and reduced growth and welfare as well as considerable economic losses for the farmers. The syndrome is regarded as multifactorial with a complex etiology, and studies have focused on associations with environment, management and dam characteristics. The present study was conducted from May to
June 2015 and included 70 dams with mink litters with and without PWD. The aims were to examine associations between PWD and mastitis (bacterial infection and histological signs of inflammation or other lesions in the mammary gland), and to examine associations between PWD and other dam-related characteristics (age, litter size, body mass index, and weight and number of active mammary glands of the dam).

**Results:** Using multivariable mixed logistic regression analyses with farm id as a random intercept, we found that the odds for PWD in the litter were significantly higher in 1 year old dams versus > 1 year old (OR = 13.3, CI 2.0–90.2, P = 0.01), higher if litter size observed after birth was > 5 kits versus ≤ 5 kits (OR = 16.5, CI 2.2–123.7, P = 0.01), higher if the number of active mammary glands per kit was ≤ 1.5 versus > 1.5 glands per kit (OR = 6.5, CI 1.2–36.0, P = 0.03), and higher in farms with high prevalence of PWD versus low prevalence (OR = 16.8, CI 2.9–97.6, P = 0.002). There were no significant associations between PWD and bacterial infection, histological signs of inflammation or other lesions of the mammary gland, body mass index or weight of mammary gland per kit.

**Conclusion:** Pre-weaning diarrhea had a statistically significant association with age of the dam, litter size and the number of active mammary glands per kit. However, PWD was not associated with mastitis, body mass index and weight of mammary gland tissue per kit.

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**Tail Tip Lesions in Mink (Neovison vison): Effects of an Additional Hammock in Multilevel Cages**

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**Simple Summary:** There are several animal welfare concerns in farmed mink, including the occurrence of wounds, such as tail tip lesions. However, little is known about how these lesions develop. An increasing tendency to develop tail tip wounds was reported by Norwegian mink farmers after the introduction of multilevel cages. It appears that the mink jump directly at a presumably high speed from the upper level towards the nest box on ground

(continued on Page 6)
INTRODUCTION

Orotic acid (OA) has been extensively utilized to induce fatty liver in rats. However, the capacity of OA to cause liver steatosis is species-specific, which may be due to the regulation of transcription factors mediating hepatic lipogenic events. Despite this, previous research evaluating OA-mediated hepatocyte lipidosis in cell types other than primary rat hepatocytes remains sparse, and there are no previous data about the detailed fatty acid (FA) composition of the OA-treated cells or about how the available FA are processed.

AIM

In this study, we have re-elucidated the usefulness of OA to investigate the cellular mechanisms in non-alcoholic fatty liver disease (NAFLD) pathogenesis and in the protection against lipid accumulation. In addition, alterations in detailed FA profiles of hepatocytes and culture media were studied.

METHOD

We used HepG2 cells exposed to OA. Bacterial endotoxin, lipopolysaccharide (LPS), was used to mimic hepatic inflammation. The suitability of the model was assessed by labeling cellular lipids with the Nile red stain, investigating cells with fluorescence confocal microscopy and performing image quantifications. The expression levels of key enzymes involved in de novo lipogenesis (DNL) (acyl-coenzyme A carboxylase [ACC], fatty acid synthase [FASN]) and inflammatory markers related to the disease development were studied by qRT-PCR. Furthermore, the effects on FASN activity were assessed by activity-based protein profiling, and FA profiles of cells as well as culture media were determined from total lipids with gas chromatography-mass spectrometry.
The carcinoma cells resist steatosis via fatty acid metabolism

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University of Eastern Finland, Kuopio, Finland
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CONCLUSIONS

- Our results indicate that OA triggers inflammatory responses and promotes the first stage of DNL, but is not capable of causing actual lipidosis in HepG2 cells.
- The elevated proportions of 20:4n-6, 22:6n-3, total PUFA and 18:0 DMA suggest that OA-treated cells could alleviate lipidosis by increasing the secretion of lipoproteins from the endoplasmic reticulum and by releasing extracellular vesicles.
- The decreased 18:0 levels may result from highly active delta9-desaturation, characteristic of HepG2 cells, which may be protective against lipotoxicity.
- These results suggest that apart from the transcription-level events reported by previous studies, FA metabolism may also be involved in the prevention of OA-mediated steatosis. Thus, the secretion of lipoproteins and extracellular vesicles emerges as a new promising hypothesis to be studied for preventing lipid accumulation and NAFLD.

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(+) The results were obtained from six independent experiments. OA = oleic acid, LPS = lipopolysaccharide, 18:0 = stearic acid, DMA = monounsaturated fatty acid, significantly different from each other (Tukey–Wolf test ANOVA).

ACKNOWLEDGEMENTS

Financial support for this study was provided by the University of Eastern Finland, the Joint Mink Research Committee (Canada) and the Academy of Finland. The authors want to thank Kirsti Rouvinen-Watt (Dalhousie University), Jarno Laitinen and Juha Savinainen (University of Eastern Finland) for collaboration.

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level, causing the tail to hit the wire mesh several times. This study investigates whether cage design may be involved in the development of tail tip lesions. Specifically, effects of installing an additional hammock in standard multilevel cages, intended to reduce speed during transitions between cage levels and thereby assumed to lower the incidence and severity of tails hitting the wire mesh, were investigated in 600 mink at three farms (300 with hammocks and 300 without hammocks). More tail tip lesions were found in mink housed in cages without a hammock. Further studies are needed to understand the causal relationship between cage design and tail tip lesions in mink, in order to develop recommendations for improved cage designs and thereby improving animal welfare.

Abstract: The occurrence of wounds in different anatomical regions, such as tail tip lesions, is an important welfare concern in farmed mink. This study investigated whether mechanical factors attributed to cage design in multilevel cages may be involved in the etiology of tail tip lesions.

Specifically, effects of an additional hammock intended to reduce speed during transitions between cage levels and thereby assumed to lower the incidence and severity of tails hitting the wire mesh were investigated. Three mink farms and a total of 600 mink participated in the study. On each farm, brown female mink (n = 100) were either housed in multilevel cages equipped with plastic hammocks (placed either perpendicular or parallel to the sidewalls) or in standard multilevel cages without hammocks (n = 100). The study was conducted from December to March using singly housed females. Significant differences in the number of tail tip wounds were found between groups with a hammock installed in the cage vs. control groups in two of the farms (p = 0.029 and p = 0.031), with more wounds developing in cages without a hammock. Furthermore, there was a trend towards difference in the number of tail tip wounds in groups with hammocks installed perpendicular vs. groups with hammocks installed parallel to the cage sidewalls, but a potential farm effect cannot be ruled out. This study is the first to suggest that mechanical factors associated with cage design may play a role in the etiology of tail tip lesions in farmed mink. Further studies are needed to understand the causal relationship between cage design and tail tip lesions in mink.
**Induction of Maxillary and Mandibular Squamous Epithelial Cell Proliferation in Mink (Neovison vison) by b-Naphthoflavone**

Danielle K. Matz, Joanna Chuck, Robin J. Hosmer, Hannah C. Piper, Jane E. Link, Scott D. Fitzgerald, Juan P. Steibel, and Steven J. Bursian

Abstract: A jaw lesion reported in mink exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and TCDD-like chemicals is considered a potential indicator of exposure to these chemicals. Many of the effects of TCDD-like chemicals are induced through interaction with the aryl hydrocarbon receptor. The present study indicates that mink dosed with b-naphthoflavone, which is an aryl hydrocarbon receptor ligand but not a TCDD-like chemical, also develop the lesion.

Environ Toxicol Chem

**Prolonged Nest Building Increase the Reproductive Outcome in American Female Mink**

Toke Munk Schou-a, Rupert Palme-b, Jens Malmkvist-r

Abstract: The timing of the onset of maternal-related nest building in American mink is unknown, and it is expected that farmed mink are highly motivated to perform nest building during times with restricted access to nesting material on mink farms. Not fulfilled motivations due to thwarting can lead to stress response with increased HPA- axis output and performance of abnormal behaviour. We aimed to
investigate when maternal nest building have onset and to test how prolonged access to nesting material influences dams stress response and reproductive outcome. We set up two groups that differed in timing of allocation of nesting material in the cage: group ALWAYS from 15 January and group REGULAR from 23 March. On 16 January after allocation of nesting material to ALWAYS, the mink built advanced nests, and their nest scores (P < 0.001) and nest temperature (P < 0.001) stayed significantly higher through to 23 March. When both groups had access to nesting material, from 23 March through birth, no difference was found in nest score (P = 0.28) and temperature (P = 0.27). ALWAYS mink females had better reproduction outcome: offspring survival (P = 0.007) and litter size (in average +1.2 young) Day 7 after birth (P = 0.021). Additionally, access to nesting material during winter/early spring (group ALWAYS) tended to reduce females’ faecal cortisol metabolite concentrations (FCM; P = 0.075) and the performance of active behaviour including stereotypic behaviour (P = 0.008). After mating, maternal nest building was documented as the nest scores increased through the period 24 March to Day 2 after birth. During this period, when both groups had equal access to nesting material, no differences was found in FCM, stereotypic and active behaviour, nest score and nest climate. In conclusion, the current study demonstrated for the first time that adult mink females are motivated for nest building prior to mating, during winter/early spring, thus it is not only a maternal behaviour. Further, prolonged access to nesting material in the cage had a stress-reducing effect and a positive long-term effect on the reproductive outcome in farmed mink.

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**Early Recognition of Offspring Vocalisation by Mink Mothers**

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**ABSTRACT:** Mink deliver around the same time of year. Consequently, mink dams are exposed to other delivering dams and litters nearby in the same housing facility on farms. In addition, farmers often transfer kits between litters within the first week
of life, as levelling large litters is believed to increase kit survival. It is, however, unknown whether farm mink mothers react to the exposure of unfamiliar offspring, or even are able to differentiate between own versus unfamiliar kits, for instance based on vocalisations being particularly distinctive early in life. Therefore, I studied mink mothers’ (N = 18 second-parity brown, litter size 6 to 9; complete data set: N = 16) behaviour towards playbacks of own versus unfamiliar kit vocalisations day 2 post-partum. Each dam was assigned to a block of playbacks of offspring vocalisations recorded from individual male kits the previous day (post-partum day 1). The playback loudspeaker – with a high linearity output in the range 1–125 kHz – was placed just outside the end of the home cage opposite to the nest box opening. Each dam was exposed to fixed orders of own and one unfamiliar male kit in call bouts of 1 min, controlled for order in the presentation of own and unfamiliar calls. Dam behaviour was recorded blind from video and data analysed taking repeated measures per dam into account. Dams reacted to the playback with approach, moving from the nest box with their Litter into the wire cage closer to the source of the sound (time spent in wire cage: 69.1% with vocalisation, 14.2% when silent, P < 0.001), also spending more time in contact with the loudspeaker (39.8%) during kit vocalisation than when silent (0.3%; P < 0.001). The time spent in the cage was negatively associated with the litter size (P = 0.035). The dams explored (touched, sniffed) the back wall area with the loudspeaker for significantly longer time during the playback of her own kit (19.0 ± 2.11%) versus an unfamiliar kit (12.5 ± 2.20%; P = 0.040). The dams contacted the loudspeaker area significantly quicker if the playback was of their own kit (P = 0.016). The estimated hazard ratio for dam latency time to contact was 2.117 for own relative to alien kit playback. This preference – based on kit vocalisation postnatal days 1–2 – is the first evidence of mink mothers’ recognition of own compared to unfamiliar kits.
Stereotypic Behaviours are Heterogeneous in their Triggers and Treatments in the American Mink, *Neo vison*, a Model Carnivore

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**ABSTRACT:** Stereotypic behaviours (SBs) are common in confined animals including captive Carnivora, which display diverse forms of SB: often whole-body movements (e.g. pacing), but also head-only movements (e.g. head twirling) and ‘scrabbling’ (scratching at enclosure boundaries). Although often pooled together, emerging evidence indicates that SBs are heterogeneous, suggesting that subtypes differ in their causes, triggers, and consequently treatments. In mink, a model carnivore, scrabbling seems to be elicited by neighbouring conspecifics. We tested this hypothesis via three studies of 32 males (individually caged in rows and separated by solid partitions). Study 1 investigated whether neighbour proximity affects the location of any SBs, and Study 2, whether removing neighbours reduces any SBs. Results revealed that although mink typically avoided proximity to their neighbours, scrabbling was uniquely directed towards neighbours who were close to the shared cage partition. It was also the only SB significantly elevated by having all-male neighbours, and reduced by removing neighbours. Study 3 then investigated whether environmental enrichment, a standard SB treatment, would reduce or abolish different SBs equally. To assess whether scrabbling is simply easier to alleviate than other SBs. Enrichment reduced all SB subtypes, but logistic regressions revealed that the odds of complete abolition were higher for whole-body and head-only SBs than for scrabbling. Overall, these naturally solitary carnivores thus seem to avoid conspecific proximity, but they specifically direct their stereotypic scrabbling at neighbours; and their scrabbling is reduced by neighbour removal, while their whole-body and head-only SBs are instead better alleviated with enrichment. Understanding that carnivore SBs are heterogeneous in their triggers and most effective treatments may help zoos, breeding centres and mink farms improve the design of their enclosures and the efficacy of their enrichments.
Detection of self-biting behavior of mink by loop-mediated isothermal amplification (LAMP) and sequence-characterized amplified regions (SCAR)

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Abstract

Self-biting disease occurs in most farmed fur animals in the world. The mechanism and rapid detection method of this disease has not been reported. We applied bulked sergeant analysis (BSA) in combination with RAPD method to analyze a molecular genetic marker linked with self-biting trait in mink group. The molecular marker was converted into SCAR and loop-mediated isothermal amplification (LAMP) marker for rapid detection of this disease. A single RAPD marker A10 amplified a specific band of 1000bp in self-biting minks. The sequences of the bands exhibited 73% similarity to the Canis Brucella. SCAR and LAMP marker were designed for the specific fragment of RAPD marker A10 and validated in 30 self-biting minks and 30 healthy minks. c2 test showed difference (p<0.05) with SCAR and significant difference (p<0.01) with LAMP in the detection rate between the two groups, but LAMP method was more accurate than SCAR method. This indicated that LAMP can be used as a positive marker to detect self-biting disease in minks.