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Detection of Aleutian mink disease virus DNA and antiviral antibodies in American mink (Neovison vison) 10 days postinoculation
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4. PhD Dissertation

Indirect Genetic Effects for Group-Housed Animals
Setegn Worku Alemu
Notes from the Editor

Focus on resource use is becoming increasingly important in farm animal production including fur animal production. New research studying longitudinal body weight, feed consumption and residual feed intake (RFI) in mink is presented in Scientifur 39,2. The results show the potential of improving resource efficiency in mink production by selection for RFI.

This issue of Scientifur also refers to publications dealing with plasmacytose which causes serious losses to the fur industry worldwide. Early detection of the virus causing the disease is important in eradication programs. A recent study shows that Aleutian mink disease virus (AMDV) DNA can be detected efficiently using PCR while counterimmunoelectrophoresis (CIEP) is not a reliable test for early detection of AMDV infection in mink. Another study has demonstrated several different AMDV strains. Pododermatitis is also a disease of concern for mink breeders worldwide. New results suggest that Arcanobacterium phocae is involved in the pathogenesis of pododermatitis.

It is a pleasure to present a summary of a PhD thesis dealing with fur animal research completed jointly under the auspices of Aarhus University and Wageningen University. In the thesis social interactions in group-housed mink were studied.

The Nordic NJF meeting will be held in Åbo in Finland from 29 September to 1 October 2015. Further information can be obtained at: tml@kopenhagenfur.com

Vivi Hunnicke Nielsen
Editor Scientifur
BREEDING, GENETICS AND REPRODUCTION

Longitudinal analysis of residual feed intake and BW in mink using random regression with heterogeneous residual variance

M. Shirali, V.H. Nielsen, S.H. Møller, J. Jensen

The aim of this study was to determine the genetic background of longitudinal residual feed intake (RFI) and BW gain in farmed mink using random regression methods considering heterogeneous residual variances. The individual BW was measured every 3 weeks from 63 to 210 days of age for 2139 male+female pairs of juvenile mink during the growing-furring period. Cumulative feed intake was calculated six times with 3-week intervals based on daily feed consumption between weighing's from 105 to 210 days of age. Genetic parameters for RFI and BW gain in males and females were obtained using univariate random regression with Legendre polynomials containing an animal genetic effect and permanent environmental effect of litter along with heterogeneous residual variances. Heritability estimates for RFI increased with age from 0.18 (0.03, posterior standard deviation (PSD)) at 105 days of age to 0.49 (0.03, PSD) and 0.46 (0.03, PSD) at 210 days of age in male and female mink, respectively. The heritability estimates for BW gain increased with age and had moderate to high range for males (0.33 (0.02, PSD) to 0.84 (0.02, PSD)) and females (0.35 (0.03, PSD) to 0.85 (0.02, PSD)). RFI estimates during the growing period (105 to 126 days of age) showed high positive genetic correlations with the pelting RFI (210 days of age) in male (0.86 to 0.97) and female (0.92 to 0.98). However, phenotypic correlations were lower from 0.47 to 0.76 in males and 0.61 to 0.75 in females. Furthermore, BW records in the growing period (63 to 126 days of age) had moderate (male: 0.39, female: 0.53) to high (male: 0.87, female: 0.94) genetic correlations with pelting BW (210 days of age). The result of current study showed that RFI and BW in mink are highly heritable, especially at the late furring period, suggesting potential for large genetic gains for these traits. The genetic correlations suggested that substantial genetic gain can be obtained by only considering the RFI estimate and BW at pelting, however, lower genetic correlations than unity indicate that extra genetic gain can be obtained by including estimates of these traits during the growing period. This study suggests random regression methods are suitable for analysing feed efficiency and BW gain; and genetic selection for RFI in mink is promising.


Estimation of indirect genetic effects in group-housed mink (Neovison vison) should account for systematic interactions either due to kin or sex

S.W. Alemu, P. Berg, L. Janss, P. Bijma

Social interactions among individuals are abundant, both in wild and in domestic populations. With social interactions, the genes of an individual may affect the trait values of other individuals, a phenomenon known as indirect genetic effects (IGEs). IGEs can be estimated using linear mixed models. Most IGE models assume that individuals interact equally to all group mates irrespective of relatedness. Kin selection theory, however, predicts that an individual will interact differently with family members versus non-family members. Here, we investigate kin- and sex-specific non-genetic social interactions in group-housed mink. Furthermore, we investigated whether systematic non-genetic interactions between kin or individuals of the same sex influence the estimates of genetic parameters. As a second objective, we clarify the relationship between estimates of the traditional IGE model and a family-based IGE model proposed in a previous study. Our results indicate that male siblings in mink show different non-genetic interactions than female siblings in mink and that this may impact the estimation of genetic parameters. Moreover, we have shown how estimates from a family-based IGE model can be translated to the ordinary direct-indirect model and vice versa. We find no evidence for genetic differences in interactions among related versus unrelated mink.

Short Communication Development of novel microsatellite markers for conservation genetic studies of *Vulpes vulpes* (*Canidae*) by using next-generation sequencing method

J.N. Yu, C.U. Chung, K.H. Oh, B.K. Lee, C.E. Lim

The red fox, *Vulpes vulpes* (*Canidae*), is the most widely distributed terrestrial carnivore worldwide, but this species is classified as endangered in Korea. In this study, we developed 25 polymorphic microsatellite markers that included 3-13 (mean = 6.32) alleles per locus using 22 red fox individuals. The most polymorphic locus was FR(59)TG (13 alleles) and the least polymorphic loci were FR(70)TG and FR(182)AG (3 alleles each). No significant deviation from Hardy-Weinberg equilibrium (P < 0.05) was observed for the 25 markers. Observed (HO) and expected (HE) heterozygosity varied from 0.182 to 1.000 and from 0.175 to 0.929, respectively. These newly developed microsatellite markers will be useful for investigating the genetic diversity and population genetic structure of *V. vulpes* and will aid in developing conservation strategies for this species.


Complementary expression and phosphorylation of Cx46 and Cx50 during development and following gene deletion in mouse and in normal and orchitic mink testes

R.M. Pelletier, C.D. Akpovi, L. Chen, N.M. Kumar, M.L. Vitale


Effect of pre-fixation delay and freezing on mink testicular endpoints for environmental research

E. Spörndly-Nees, E. Ekstedt, U. Magnusson, A. Fāhrzadeh, C.L. Luengo Hendriks, L. Holm

There is growing interest in using wild animals to monitor the real-life cocktail effect of environmental chemicals on male reproduction. However, practical difficulties, such as long distances to the laboratory, generally prolong the time between euthanisation and specimen handling. For instance, tissue fixation is often performed on frozen material or on material where deterioration has started, which may affect tissue morphology. This study examined the effect of pre-fixation delay and freezing on mink testicular endpoints in order to determine robust endpoints in suboptimally handled specimens. Sexually mature farmed mink (n=30) selected at culling were divided into six groups and subjected to different time intervals between euthanisation and fixation or freezing: 0 hours (fixed immediately post mortem), 6 hours, 18 hours, 30 hours, 42 hours, or frozen 6 hours post mortem and thawed overnight. Unaffected endpoints when pre-fixation storage was extended to 30 hours included: area and diameter of the seminiferous tubules, length and weight of the testes, and acrosomes marked with Gata-4. Epithelial height, Sertoli cells marked with Gata-4 and cell morphology were affected endpoints after 6 hours of storage. Freezing the tissue prior to fixation severely altered cell morphology and reduced testicular weight, tubular diameter and area. Morphological changes seen after 6 hours included shredded germ cells and excess cytoplasm in seminiferous tubular lumen, chromatin rearrangements and increased germ cell death. Extended delay before fixation and freezing affected many endpoints in the mink testicular tissue. Some of these endpoints may mimic chemically induced effects, which is important to consider when evaluating specimens from wild animals for environmental toxicity.


**NUTRITION, FEEDING AND MANAGEMENT**

Species-specific features of tocopherol content in carnivorous mammals during autumn period

T.N. il'ina, I.V. Baishnikova

A comparative study of the major liposoluble antioxidant, vitamin E (α-tocopherol), content was conducted in carnivorous mammals (polar fox, silver fox, raccoon dog, mink, sable) during late autumn under natural lowering of temperature. The highest vitamin E content in liver and kidney was found in polar fox and raccoon dog, obviously due...
to seasonal metabolic changes and ecological specialization determining the accumulation of considerable amounts of tocopherol both in polar and hibernating species. To investigate the features of vitamin E accumulation, the tocopherol loading experiment was implemented on three species (polar fox, silver fox, mink). It was shown that polar fox, as compared to silver fox and mink, exhibits a higher ability to reserve vitamin E. The established differences in tocopherol content and distribution in different species should be considered as a genetically fixed reaction to the effect of environment underlain by metabolic differences in animals with different ecological specialization. The ability of tocopherol to accumulate in tissues and organs allows considering its essential role in stabilization of the antioxidant system and ensuring thereby a seasonal cold resistance.


Ecological risk assessment for mink and short-tailed shrew exposed to PCBs, dioxins and furans in the Housatonic River area


HEALTH AND DISEASE

Detection of Aleutian mink disease virus DNA and antiviral antibodies in American mink (Neovison vison) 10 days postinoculation

A.H. Farid, I. Hussain, I. Arju


Research into the antibody detection technology of mink plasmacytosis and its current applications

H. Wan, E. Feng, H. Wu, Y. Yang, J. Ni, L. Chen

Aleutian mink disease virus in free-ranging mink from Sweden


Aleutian mink disease (AMD) is a chronic viral disease in farmed mink and the virus (AMDV) has been found in many free-ranging mink (Neovison vison) populations in Europe and North America. In this study, AMDV DNA and AMDV antibodies were analysed in 144 free-ranging mink hunted in Sweden. Associations between being AMDV infected (defined as positive for both viral DNA and antibodies) and the weight of the spleen, liver, kidneys, adrenal glands and body condition were calculated and the sequences of ten AMDV isolates were analysed in order to characterize the genetic relationships. In total, 46.1% of the mink were positive for AMDV antibodies and 57.6% were positive for AMDV DNA. Twenty-two percent of the mink tested on both tests (n = 133) had dissimilar results. The risk of having AMDV antibodies or being positive for AMDV DNA clearly increased with age and the majority of the mink that were two years or older were infected. Few macroscopic changes were found upon necropsy. However, the relative weight of the spleen was sexually dimorphic and was found to be slightly, but significantly (p = 0.006), heavier in AMDV infected male mink than uninfected. No association between AMDV infection and body condition, weight of the kidneys, liver or adrenal glands were found. Several different strains of
AMDV were found across the country. Two of the AMDV sequences from the very north of Sweden did not group with any of the previously described groups of strains. In summary, AMDV seems to be prevalent in wild mink in Sweden and may subtly influence the weight of the spleen.


**Corrigendum to "Identification of biosecurity measures and spatial variables as potential risk factors for Aleutian disease in Danish mink farms"**


**Recombinant Newcastle disease viral vector expressing hemagglutinin or fusion of canine distemper virus is safe and immunogenic in minks**


**Pneumonia associated with Acinetobacter baumannii in a group of minks (Neovison vison)**

R.J. Molenaa, E. van Engelen

**Case report. doi:10.1080/01652176.2015.1030714**

**Staphylococcus spp., Streptococcus canis, and Arcanobacterium phocae of healthy Canadian farmed mink and mink with pododermatitis**


**The transmissible spongiform encephalopathies of livestock**

J.J. Greenlee, M.H. Greenlee

Prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal proteinmisfolding neurodegenerative diseases. TSEs have been described in several species, including bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats, chronic wasting disease (CWD) in cervids, transmissible mink encephalopathy (TME) in mink, and Kuru and Creutzfeldt-Jakob disease (CJD) in humans. These diseases are associated with the accumulation of a protease-resistant, disease-associated isoform of the prion protein (called PrP(Sc)) in the central nervous system.
Abstracts

Cloning, expression and antiviral activity of mink alpha-interferons


As a key link between innate and adaptive immune responses, the interferon (IFN) system is the first line of defense against viral infection. IFN, and in particular, IFN-α, has been used clinically as an effective therapeutic agent for viral infections. However, different subtypes of IFN-α demonstrate distinct antiviral activity. Therefore, it is important to identify IFN-α subtypes with high antiviral activity for the development of genetically engineered antiviral drugs.

In this study, we cloned the genes for 13 IFN-α subtypes from peripheral blood lymphocytes of the mink. The homologies of the 13 mink IFN-α genes were 93.6-99.3% and 88.8-98.4% at the nucleotide and amino acid sequence levels, respectively. In contrast to human and canine IFN-α subtypes, most mink IFN-α subtypes contained two N-glycosylation sites. We expressed and purified 13 mink IFN-α subtypes in Escherichia coli. The cytopathic effect inhibition assay showed that all the 13 recombinant mink IFN-α subtypes inhibited the propagation of vesicular stomatitis virus in WISH cells, with IFN-α2 and IFN-α12 demonstrating the highest activities. Furthermore, recombinant mink IFN-α2 and IFN-α12 significantly suppressed the propagation of canine distemper virus in Vero cells, with IFN-α2 demonstrating the highest activity. We identified the mink IFN-α2 subtype as a promising candidate for the development of effective antiviral drugs.

BMC Vet. Res. 2015; 11(1): 42

Emetic responses to T-2 toxin, HT-2 toxin and emetine correspond to plasma elevations of peptide YY3-36 and 5-hydroxytryptamine


Trichothecene mycotoxins are a family of potent translational inhibitors that are associated with foodborne outbreaks of human and animal gastroenteritis in which vomiting is a clinical hallmark. Deoxynivalenol (DON, vomitoxin) and other Type B trichothecenes have been previously demonstrated to cause emesis in the mink (Neovison vison), and this response has been directly linked to secretion of both the satiety hormone peptide YY3-36 (PYY3-36) and neurotransmitter 5-hydroxytryptamine (5-HT). Here, we characterized the emetic responses in the mink to T-2 toxin (T-2) and HT-2 toxin (HT-2), two highly toxic Type A trichothecenes that contaminate cereals, and further compared these effects to those of emetine, a natural alkaloid that is used medicinally and also well known to block translation and cause vomiting. Following intraperitoneal (IP) and oral exposure, all three agents caused vomiting with evident dose-dependent increases in both duration and number of emetic events as well as decreases in latency to emesis. T-2 and HT-2 doses causing emesis in 50% of treated animals (ED50s) were 0.05 and 0.02 mg/kg BW following IP and oral administration, respectively, whereas the ED50 for emetine were 2.0 and 1.0 mg/kg BW for IP and oral exposure, respectively. Importantly, oral administration of all three toxins elicited marked elevations in plasma concentrations of PYY3-36 and 5-HT that corresponded to emesis. Taken together, the results suggest that T-2 and HT-2 were much more potent than emetine and that emesis induction by all three translational inhibitors co-occurred with increases in circulating levels of PYY3-36 and 5-HT.

Arch Toxicol. 2015: [Epub ahead of print]
Susceptibility of domestic animals to a pseudotype virus bearing RD-114 virus envelope protein


Trichinella britovi in a red fox (Vulpes vulpes) from Portugal

A.P. Lopes, M.F. Vila-Viçosa, T. Coutinho, L. Cardoso, B. Gottstein, N. Müller, H.C. Cortes

doi: 10.1016/j.vetpar.2015.03.025. [Epub 2015 Apr 8]

A molecular survey of vector-borne pathogens in red foxes (Vulpes vulpes) from Bosnia and Herzegovina

A. Hodžić, A. Alić, H.P. Fuehrer, J. Harl, W. Wille-Piazzai, G.G. Duscher

Red foxes (Vulpes vulpes) have recently been recognized as potential reservoirs of several vector-borne pathogens and a source of infection for domestic dogs and humans, mostly due to their close vicinity to urban areas and frequent exposure to different arthropod vectors. The aim of this study was to investigate the presence and distribution of Babesia spp., Hepatozoon canis, Anaplasma spp., Bartonella spp., “Candidatus Neoehrlichia mikurensis”, Ehrlichia canis, Rickettsia spp. and blood filaroid nematodes in free-ranging red foxes from Bosnia and Herzegovina.

Spleen samples from a total of 119 red foxes, shot during the hunting season between October 2013 and April 2014 throughout Bosnia and Herzegovina, were examined for the presence of blood vector-borne pathogens by conventional PCRs and sequencing.

In the present study, three species of apicomplexan parasites were molecularly identified in 73 red foxes from the entire sample area, with an overall prevalence of 60.8%. The DNA of B. canis, B. cf. microti and H. canis was found in 1 (0.8%), 38 (31.9%) and 46 (38.6%) spleen samples, respectively. In 11 samples (9.2%) co-infections with B. cf. microti and H. canis were detected and one fox harboured all three parasites (0.8%). There were no statistically significant differences between geographical region, sex or age of the host in the infection prevalence of B. cf. microti and H. canis, although females (52.9%; 18/34) were significantly more infected than males (32.9%; 28/85). The presence of vector-borne bacteria and filaroid nematodes was not detected in our study.

This is the first report of B. canis, B. cf. microti and H. canis parasites in foxes from Bosnia and Herzegovina and the data presented here provide a first insight into the distribution of these pathogens among the red fox population. Moreover, the relatively high prevalence of B. cf. microti and H. canis reinforces the assumption that this wild canid species might be a possible reservoir and source of infection for domestic dogs.

Indirect Genetic Effects for Group-Housed Animals

PhD thesis by Setegn Worku Alemu

Joint PhD Project

between

Department of Molecular Biology and Genetics
Faculty of Science and Technology, Aarhus University, Denmark

and

Wageningen University, Wageningen, Holland

2015
Naturally, mink is a solitary and territorial species. Traditionally mink are housed in a pair of full sibs, one male and one female. This type of housing limits the aggressive interactions among cage mates. However, this housing has some limitations, such as limited space for the individuals, which makes the mink stressful. Group housing of mink was recommended by the Council of Europe (European Commission, 1999). This is because it may improve welfare from ‘social enrichment’ as outlined in (European Commission, 2001), and it increases the stocking density in the cages and thereby decreases housing investments. Though group housing offers some advantages, it has still some limitations such as increased competition for food and aggressive behaviour (Pedersen and Jeppesen, 2001; Moller et al., 2003).

The aggressive behaviour in group-housed mink is higher than in pair-wise housing and it is reflected by increased bite marks and bite wounds. Thus, for continuity of group housing, the welfare of mink should be improved. For example, mink in group housing should have a lower level of biting. One solution to improve the welfare in group-housed mink is to improve the management by the use of environmental enrichment, such as plastic tubes. However, this still does not improve the welfare sufficiently.

Another promising solution is genetic selection. Genetic selection can reduce bite marks in group-housed mink. Thus, this thesis addresses genetic selection with indirect genetic effect models, and investigates whether this can be a solution to reduce bite mark in group-housed mink. Bite marks on one individual reflect the aggression of its group members, which means that the number of bite marks carried by one individual depends on the behaviour of other individuals and that it may have a genetic basis so called indirect genetic effect.

In 2009, a selection experiment was initiated to select for a reduced number of bite marks at pelting, at the mink farm at the Research Centre Foulum in Denmark. The experiment lasted three generations from 2009 till 2011.

We analysed data from the first three generations of that selection experiment using the model that includes both direct genetic effects (DGE) and indirect genetic effects (IGE). The genetic parameters for the bite mark traits in the neck, body and tail region as well as total bite mark score were estimated.

We found that both DGE and IGE contribute to genetic variation of Bite Mark Score (BMS) on all regions of the body. IGE contributed a significant proportion of the heritable variation available for variance to total heritable variation, measured by the ratio \( (n-1)^2 \sigma^2_D / \sigma^2_T \), ranged from 30% for tail bite marks to 52% for neck bite marks, while that of DGE variance was about 16% for all regions of the body. Moreover, there was a strong positive correlation between DGE and IGE, which further increased total heritable variance. Thus, most of the heritable variation in BMS relates to IGE. For instance, for total BMS, the variance in IGE and the direct-indirect genetic covariance together contributed 85% of the heritable variation. Estimated genetic correlations between direct and indirect genetic effects were strong and positive and ranged from 0.55 to 0.99, i.e. significantly different from zero, except for bite marks in the neck region. Thus, these results suggest that if a genotype causes an individual to bite more, it also leads the individual to be more bitten, which, in turn, suggests that an individual benefits from not harming others. Thus, there are good prospects for genetic improvement of the trait. We predicted the response to selection. Using mass selection, the accuracy of selection based on estimates equals ~0.4 and if we used 10% of the population used for breeding the predicted response to selection will be equal to ~3.07 and the total BMS is predicted to reduce from ~6.47 to ~3.4, which is a very substantial reduction in a single generation of selection. When using group selection for groups of four sibs, two males and two females that all belong to the same family, it is possible to reach an even higher accuracy of ~0.65, and thus the predicted response to selection will be ~5 and the total BMS is predicted to reduce from ~6.47 to ~1.47 in a single generation of selection. However, mass selection and group selection are difficult to use, since currently bite mark scores are recorded on the pelts of the dead animals. Hence, recording the trait would require sacrificing the selection candidates. Thus sib selection is more appropriate. The predicted accuracy of sib selection for groups of four sibs, two males and two females that all belong to the same family will be equal to ~ 0.54 and the predicted response to selection to ~4.14. Thus, total BMS will be reduced from ~6.47 to ~ 3.33, again a very
substantial reduction in a single generation of selection. Thus, although in practice response to selection is usually lower than the theoretical predicted value, our results indicate that it is possible to select mink that have a considerably lower level of biting in a few generations.
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Manuscripts must be in English, typed double spaced with page and line numbering and consisting of:

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Authors name(s) as well as name(s) and address(es) of the institutions to which the work is attributed. E-mail address of the corresponding author should preferably be included.

Summary/Abstract, which should not exceed 150 words.

Keywords in alphabetic order if not included in the title.

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