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

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

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SUBSCRIPTION: Free of charge: http://www.ifasanet.org

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Notes from the Editor

Environmental sustainability as a whole needs to be considered as still more important in all livestock productions. The focus should be on primary production but also on product processing, use and waste and/or disposal. In fur farming possible improvements of all stages of the production need to be considered. Thus, attention should be paid to the reproduction and production period with optimization of feed production, feed and feed efficiency, health and welfare and fur quality. At pelting, attention should also be paid to the circular bioeconomy with use of the whole body e.g. as fertilizer and biodiesel. The final product, the fur, should be for long-term use with options for changes with new fashions. At the final stage, a recent investigation has shown fur being able of biodegradation:

Research presented in this issue of Scientifur supports improvements of several of the above mentioned areas. Thus, it is proposed to improve litter weight traits using genetic or genomic selection to increase mink maternal abilities for better kit survival rate. Furthermore, specific factors are shown to regulate the development of hair follicles in mink, which contributes to the understanding of growth of hair and thus fur quality.

Healthy animals are important for sustainability as well as for good welfare. Mink maternal serum immunoglobulin G concentration is shown to correlate positively with the concentration in the kits, which is assumed to improve resistance against a number of infections. In cases with Aleutian disease, the difference between farms observed with clinical and subclinical Aleutian disease, respectively, can be ascribed to virus nucleotid polymorphism but also to the stage of infection of the virus.

The Fur Animal Research Autumn Meeting of the NJF Fur Animal Subsection – the Nordic NJF Seminar 505 – is held in Malmö in Sweden 3-5 October 2018. Further information can be found at: https://www.slu.se/hmh/NJFseminarie

Vivi Hunnicke Nielsen

Editor Scientifur
BREEDING, GENETICS AND REPRODUCTION

Genetic and Phenotypic Parameters for Litter Size, Survival Rate, Gestation Length and Litter Weight Traits in American Mink

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The economic efficiency of mink production is greatly influenced by reproductive performance. Therefore, the objective of this study was to estimate phenotypic and genetic parameters for reproduction traits including total number of kits born (TB), number of live kits at birth (LB), number of live kits at weaning (LW), survival rate at birth (SB), survival rate at weaning (SW), gestation length (GL), average kit weight per litter at birth (AWB), average kit weight per litter at week three (AW3) and average kit weight per litter at weaning (AWW) in American mink. Data included records of 3,046 litters collected by the Canadian Centre for Fur Animal Research at Dalhousie Faculty of Agriculture between 2002 and 2016. Significance (P<0.05) of fixed effects (year, number of matings, color type, age of dam, origin of dam, sex ratio and number of live kits) and random effect of permanent environment were determined using univariate repeatability models. A significant effect of permanent environment was only found for survival rate traits (P<0.05). Subsequently, genetic and phenotypic parameters for all traits were estimated by fitting a set of bivariate models using ASREML 4.0. Heritabilities (±SE) were estimated to be 0.07±0.03 for TB, 0.07±0.02 for LB, 0.09±0.04 for LW, 0.13±0.03 for SB, 0.10±0.02 for SW, 0.29±0.03 for GL, 0.28±0.05 for AWB, 0.19±0.04 for AW3 and 0.10±0.04 for AWW. Moderate positive genetic correlation was observed between AWB with SB (0.66±0.10) and SW (0.61±0.13). Furthermore, genetic correlations of LB with SW and AWB were 0.55±0.16 and 0.53±0.18, respectively. On the other hand, negative and moderate genetic correlations were observed between GL and survival rates at birth (-0.43±0.14) and at weaning (-0.37±0.15). These results indicated that selection for higher litter weights at birth can effectively improve survival rate and number of live kits in mink farms. It was suggested to incorporate litter weight traits as a selection criterion to increase maternal ability in mink breeding programs. Unfavorable genetic trends were observed for the studied traits indicating that phenotypic selection with low selection intensity had not been an efficient method to improve them over the last ten years. It was recommended to use genetic or genomic evaluation methods for mink selection.


Balance between fibroblast growth factor 10 and secreted frizzled-relate protein-1 controls the development of hair follicle by competitively regulating β-catenin signaling

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Growth of hairs depends on the regular development of hair follicles which are hypothesized to be regulated by fibroblast growth factor 10 (FGF10) and secreted frizzled-relate protein-1 (sFRP1). In the current study, the effect of FGF10 or sFRP1 on hair follicle cells was assessed and the possible mechanism mediating the interaction between FGF10 and sFRP1 in hair follicle cells was explored. Out root sheath (ORS) and dermal papilla (DP) cells were isolated...
from mink skin tissues and subjected to administrations of FGF10 (50 ng/ml) or sFRP1 (10 ng/ml). Then proliferation, cell cycle distribution, and migration potentials of both cell types were detected. Moreover, the nuclear translocation of β-catenin was determined. The results showed that the administration of FGF10 increased cell proliferation and migration potential in both cell types, which was associated with the up-regulated nuclear level of β-catenin. To the contrary, the administration of sFRP1 decreased cell proliferation and migration potentials while induced the G1 cell cycle arrest in both cell types by inhibiting nuclear translocation of β-catenin. Compared with the sole administrations, the co-treatment of FGF10 and sFRP1 had a medium effect on cell proliferation, cell cycle distribution, cell migration, and nuclear β-catenin level, representing an antagonistic interaction between the two factors, which was exerted by competitively regulating β-catenin pathway. Conclusively, the cycle of hair follicles was promoted by FGF10 while blocked by sFRP1 and the interplay between the two factors controlled the development of hair follicles by competitively regulating β-catenin signaling.


NUTRITION, FEEDING AND MANAGEMENT

Large-scale variation in density of an aquatic ecosystem indicator species

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Monitoring indicator species is a pragmatic approach to natural resource assessments, especially when the link between the indicator species and ecosystem state is well justified. However, conducting ecosystem assessments over representative spatial scales that are insensitive to local heterogeneity is challenging. We examine the link between polychlorinated biphenyl (PCB) contamination and population density of an aquatic habitat specialist over a large spatial scale using non-invasive genetic spatial capture-recapture. Using American mink (Neovison vison), a predatory mammal and an indicator of aquatic ecosystems, we compared estimates of density in two major river systems, one with extremely high levels of PCB contamination (Hudson River), and a hydrologically independent river with lower PCB levels (Mohawk River). Our work supports the hypothesis that mink densities are substantially (1.64-1.67 times) lower in the contaminated river system. We demonstrate the value of coupling the indicator species concept with well-conceived and spatially representative monitoring protocols. PCBs have demonstrable detrimental effects on aquatic ecosystems, including mink, and these effects are likely to be profound and long-lasting, manifesting as population-level impacts. Through integrating non-invasive data collection, genetic analysis, and spatial capture-recapture methods, we present a monitoring framework for generating robust density estimates across large spatial scales.
Figure 1. From: Large-scale variation in density of an aquatic ecosystem indicator species. Variation in detectability. Baseline detection probability varied by session (unique river-year combinations: Hudson 2013, Hudson 2014, Mohawk 2013, Mohawk 2014), by visit (first, second and third visits), and by sex, although the latter received marginal support. Red and blue points are model averaged predictions of visit-specific baseline detection probability for each session and sex (red: males, blue: females). Bold black lines are unconditional standard errors of the predictions and the thin grey lines are the unconditional 95% confidence intervals.

Figure 2. Variation in density. Density varied primarily by river (Hudson and Mohawk), although there was some support for year (2013, 2014) and session (unique river-year combinations: Hudson 2013, Hudson 2014, Mohawk 2013, Mohawk 2014) differences. Here we provide, on the left of each session, session-specific density for each of the 116 models as blue circles, where the size of the circles is proportional to the AIC model weight (AIC + ω in Table 2). On the right of each session, red points are model averaged predictions of session-specific density holding all continuous covariates, that had very little support and coefficient estimates not significantly different from 0, at their mean value (i.e., 0 because covariates were standardized). Bold black lines are unconditional standard errors of the predictions and the thin grey lines are the unconditional 95% confidence intervals.

HEALTH AND DISEASE

Quantitative immunoassay for mink immunoglobulin in serum and milk

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Background

The significance of maternal immunoglobulin G (IgG) for the resistance against a number of infections affecting the health of young mink offspring is not known. Here, we present a validated immunoassay for quantification of mink IgG in serum and milk, using a commercially available polyclonal goat anti-ferret IgG antibody cross-reactive with mink IgG as both the catching and the detection antibody, in a sandwich format enzyme linked immunosorbent assay (ELISA). Using this ELISA, serum IgG concentrations was analyzed over time in both mothers and kits in order to establish a correlation between maternal IgG serum concentrations and those of the offspring.

Results

Intra-assay coefficient of variation (CV) for a serum sample ranged from 2.15 to 5.97% depending on the dilution, while the inter-assay CV ranged from 5.17 to 17.78%. In addition, the range of milk intra-assay CV was 2.71-5.92%, while the range of the inter-assay CV was 4.20-16.03%. Calibrating the ELISA with purified mink IgG (an in-house preparation purified from mink serum) the lower limit of detection was found to be 5 ng/mL for serum and 1 ng/mL for milk. Both serum and milk showed high precision and good linearity over a two-log dilution range. When comparing the serum IgG concentrations of the mink kits a clear within litter effect was seen, while the mean serum IgG concentrations of litters differed significantly between some of the litters (P = 0.0013). Mean maternal serum IgG concentrations correlated positively with the IgG serum concentration of the corresponding offspring sampled over a 3 week period (R² = 0.63).

Conclusions

A calibrated and reproducible sandwich ELISA for quantifying mink IgG concentrations in both milk and serum with high analytical sensitivity was developed and validated. The results in this study corroborate previous investigations supporting the usability of the ELISA, paving the way for investigations into the importance of maternal IgG in milk and in serum for the welfare and health of the offspring.
magnitude between the number of copies of the viral DNA on the farm with the clinical course of the disease (10^5) and the farm with the subclinical course (10^3). The sequencing results confirm a high level of homogeneity within each farm and variation between them. The phylogenetic analysis indicates that the variants belonging to different farms are closely related and occupy different branches of the same clade. The in silico analysis of the effect of differences in the sequence encoding the VP2 protein between the farms revealed no effect of the polymorphism on its functionality. The close phylogenetic relationship between the isolates from the two farms, the synonymous nature of most of the polymorphisms and the potentially minor effect on the functionality of the protein indicate that the differences in the clinical picture may be due not only to polymorphisms in the nucleotide and amino acid sequences, but also to the stage of infection on the farm and the degree of stabilization of the pathogen-host relationship.


Whole genome analysis of a novel neurotropic bovine astrovirus detected in a Japanese black steer with non-suppurative encephalomyelitis in Japan

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While neurotropic bovine astroviruses (BoAstVs) have been identified in North America and Europe, their presence has never been reported in Asia. In this study, we detected BoAstV in the brain of a steer showing neurological signs. Phylogenetic analysis revealed that the identified virus belongs to the Virginia/Human-Mink-Ovine clade, which contains most of the neurotropic astroviruses including the neurotropic BoAstVs. Similarity plot analysis showed that the virus was closely related to the American BoAstV NeuroS1 strain with respect to the ORF regions and to the European BoAstV CH13 strain in the 3' untranslated region, suggesting the occurrence of intra-genotypic recombination events.


Application of Real-Time Quantitative PCR to Detect Mink Circovirus in Naturally and Experimentally Infected Minks

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The mink circovirus (MiCV), a newly discovered pathogen, is associated with diarrhea in farmed minks. The prevalence and economic importance of this virus remain poorly understood, and a quantitative method for diagnosis of MiCV infection has not been established. This research aims to develop a highly specific, sensitive, and quantitative assay for MiCV. A Real-Time quantitative polymerase chain reaction (qPCR) assay was developed to detect different isolates of the MiCV in mink samples. The qPCR system is highly sensitive with a detection limit of as low as 10 viral DNA copies. The specificity of this qPCR assay was supported by the absence of cross-reaction with other pathogens. The coefficients of variation were low for both inter-assay and intra-assay variabilities. In addition, the results also expressed the distribution of MiCV in infectious mink tissues with high levels of virus in the skeletal
muscle and heart. The heart occupied a higher proportion than other tissues, which can be considered the primary source of test material. This qPCR method could be a useful tool for epidemiological studies and disease management. This method for MiCV is highly specific, sensitive, repeatable, quantitative, and can rapidly determine viral load levels in different tissues samples.

Figure 1. Amplification curve and standard curve of MiCV. (A): Standard curve (Slope: −3.155, Y-Intercept: 39.202, and Efficiency: 107.447) was analyzed with the ABI7500 software. (B): Specificity of the qPCR. Ten other viral pathogens were used for the specificity test. 1: MiCV HEB15 strain DNA; 2–11: DNA and RNA samples of AMDV, MEV, CDV, PRV, MCV, CAV2, RV, PCV1, PCV2, DogCV, and H2O. (C): Amplification curves. Ten-fold dilutions of standard DNA ranging from 10⁸ copies/μL to 10⁶ copies/μL were used as standard controls.

Genomic characterization of circoviruses associated with acute gastroenteritis in minks in northeastern China

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Mink circovirus (MiCV), a virus that was newly discovered in 2013, has been associated with enteric disease. However, its etiological role in acute gastroenteritis is unclear, and its genetic characteristics are poorly described. In this study, the role of circoviruses (CVs) in mink acute gastroenteritis was investigated, and the MiCV genome was molecularly characterized through sequence analysis. Detection results demonstrated that MiCV was the only pathogen found in this infection. MiCVs and previously characterized CVs shared genome organizational features, including the presence of (i) a potential stem-loop/nonanucleotide motif that is considered to be the
origin of virus DNA replication; (ii) two major inversely arranged open reading frames encoding putative replication-associated proteins (Rep) and a capsid protein; (iii) direct and inverse repeated sequences within the putative 5' region; and (iv) motifs in Rep. Pairwise comparisons showed that the capsid proteins of MiCV shared the highest amino acid sequence identity with those of porcine CV (PCV) 2 (45.4%) and bat CV (BatCV) 1 (45.4%). The amino acid sequence identity levels of Rep shared by MiCV with BatCV 1 (79.7%) and dog CV (dogCV) (54.5%) were broadly similar to those with starling CV (51.1%) and PCVs (46.5%). Phylogenetic analysis indicated that MiCVs were more closely related to mammalian CVs, such as BatCV, PCV, and dogCV, than to other animal CVs. Among mammalian CVs, MiCV and BatCV 1 were the most closely related. This study could contribute to understanding the potential pathogenicity of MiCV and the evolutionary and pathogenic characteristics of mammalian CVs.


Loop-mediated Isothermal Amplification-Single Nucleotide Polymorphism Analysis for Detection and Differentiation of Wild-type and Vaccine Strains of Mink Enteritis Virus

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Broad coverage of mink enteritis virus (MEV) vaccination program in northeast of China has provided effective protection from mink viral enteritis. Nevertheless, MEV vaccine failures were reported due to continually evolving and changing virulence of field variants or wild-type MEV. In this study, a combined loop-mediated isothermal amplification (LAMP) and single nucleotide polymorphism (SNP) method, named LAMP-SNP assay, was developed for detection and differentiation of wild-type and vaccine strains of MEV. Four primers in MEV-VP2-LAMP were used to detect both wild-type and vaccine strains of MEV in our previous publication, and other four primers in LAMP-SNP were designed to amplify the NS1 gene in wild-type MEV and only used to detect wild-type viruses. The LAMP-SNP assay was performed in a water bath held at a constant temperature of 65 °C for 60 min. LAMP-SNP amplification can be judged by both electrophoresis and visual assessment with the unaided eyes. In comparison with virus isolation as the gold standard in testing 171 mink samples, the percentage of agreement and relative sensitivity and specificity of the LAMP-SNP assay were 97.1, 100%, and 94.0%, respectively. There were no cross-reactions with other mink viruses. The LAMP-SNP assay was found to be a rapid, reliable and low-cost method to differentiate MEV vaccine and field variant strains.
Figure 1. Detection MEV and non-MEV viruses by the LAMP-SNP assay. Lane M: DNA Marker DL2000 (TaKaRa, Dalian, China). Lane 1: LAMP amplification products with MEVB DNA as the template; Lanes 2–4: LAMP amplification products with MEV SD, MEV-Z6, and MEV-LN10 DNAs as templates, respectively; Lanes 5–7: LAMP amplification products with DNA/cDNAs of MPRV-J, AMDV-G, and CDV3 as templates, respectively. Full-length gel is presented in Supplementary Fig.

Figure 2. Visual inspection LAMP products by using SYBR Green I. (A) P: positive control (MEVB), N: negative control (double distilled water), V: vaccine MEVB strain, W: wild-type MEV SD isolate. (B) P: positive control (MEV SD), N: negative control (double distilled water), V: vaccine MEVB strain, W: wild-type MEV SD isolate. For visual inspection of the LAMP products, 4 μL diluted SYBR Green I dye 10,000× concentration in double distilled water was added to the reaction tube after the reaction termination. Yellowish green and reddish orange represent positive and negative reactions, respectively. Full-length tubes are presented in Supplementary Fig.
Abstracts

Figure 3
Detection limit of LAMP-SNP assay. (A) Electrophoresis. Lane M: DNA Marker DL2000 (TaKaRa, Dalian, China). Lanes 1–8 represent $2 \times 10^6$–$2 \times 10^1$ copies/mL of pT-MEV-NS1 plasmid, respectively. (B) Visual inspection. Tubes 1–8 represent $2 \times 10^6$–$2 \times 10^1$ copies/mL of pT-MEV-NS1 plasmid, respectively. Both methods exhibited the same results, the detection limit was $10^1$ copies/mL, with no differences between electrophoresis and visual inspection. Full-length gels and tubes are presented in Supplementary Fig.

Figure 4
Evaluation of clinical specimens by (A) LAMP-SNP and (B) virus isolation. (A) P: positive control (MEV SD), N: negative control (double distilled water), tubes 1–10 represent different clinical fecal samples. (B) Virus isolation (MEV-Z6) was identified by an indirect immunofluorescence assay using a monoclonal antibody against the VP2 protein of MEV and goat anti-mouse IgG-FITC conjugated secondary antibody. Specific immunofluorescent signals were detected in cells exposed to the respective tissue filtrates but not in mock-infected control cells. Full-length tubes and immunofluorescence pictures are presented in Supplementary Fig.

Figure 5
Partial sequence alignment of NS1. NS1 sequences from wild-type strains and vaccine strain MEVB were analyzed. In wild-type strains, there is a G at position 1846 of NS1, whereas there is an A at that position in the vaccine strain. This nucleotide difference was used to design the differentiation primers used in the LAMP-SNP assay.
Figure 6
Schematic of single nucleotide polymorphism typing using the LAMP method. The BIP and FIP primers were designed to contain the wild-type SNP allele. With wild-type DNA template and these primers, a dumbbell-like structure is formed and amplification occurs. If the template DNA is from the vaccine strain MEVB or from a non-MEV virus, a dumbbell-like structure will not form and amplification will not occur.


Molecular identification of Sarcocystis lutrae (Apicomplexa: Sarcocystidae) in muscles of five species of the family Mustelidae

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Carnivores usually act as definitive hosts of Sarcocystis species. However, the number of reports on sarcocyst formation in musculature of predators is on the increase. In the present study, muscle samples of 68 mustelids collected in Lithuania were examined for sarcocysts of Sarcocystis species. Sarcocysts were detected in diaphragm, tongue and limb muscles of ten animals (14.7%) but were not discovered in the heart. Based on 18S rDNA, 28S rDNA, cox1 and ITS1 sequence analysis, Sarcocystis lutrae was identified in three American minks (Neovison vison), two beech martens (Martes foina), three Eurasian badgers (Meles meles), one Eurasian otter (Lutra lutra) and one European polecat (Mustela putorius). The intraspecific variability of this Sarcocystis species was determined only in ITS1 region. Based on the phylogenetic analysis, no clear separation of S. lutrae by intermediate hosts or geographical locations was established. This paper represents the first identification of S. lutrae in the American mink, the beech marten and the European polecat. Current results indicate that S. lutrae is a common species in the muscles of various European mustelids.


Tracking Toxoplasma gondii in freshwater ecosystems: interaction with the invasive American mink (Neovison vison) in Spain


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Water-borne transmission may play an important role in the epidemiology of Toxoplasma gondii. Mammals closely related to freshwater ecosystems, such as the American mink (Neovison vison), are potentially valuable sentinels for T. gondii. To assess the importance of freshwater ecosystems in T. gondii epidemiology, sera of 678 American minks collected during the 2010 to 2015 Spanish national eradication campaigns were tested for the presence of T. gondii antibodies using the modified agglutination test (MAT, cut-off 1:25). A high prevalence of samples, 78.8% (CI95%: 75.5-81.8), were seropositive. In addition, a specific real-time PCR was performed in 120 brain samples and the parasite DNA was detected in 9.2% (CI95%: 5.2-15.7). Significant differences in seroprevalence were detected among bioregions, with the highest levels detected in coastal areas, and by age. The higher seroprevalence observed in older animals (80.0% adults versus 68.7% juveniles) confirms the importance of the horizontal transmission. These results indicate a widespread presence of T. gondii oocysts in freshwater ecosystems from Spain and further support the importance of water-borne transmission in the epidemiology of T. gondii.
two of two kodkods (Leopardus guigna). Toxoplasma gondii DNA was detected in tissues from one American mink and one Southern river otter. The present study confirms the widespread distribution of T. gondii in Southern Chile, and shows a high exposure of semiaquatic mustelids and domestic cats to the parasite. Cats and anthropogenic disturbance have a role in the maintenance of T. gondii infection in ecosystems of southern Chile.


Multiple-strain Trichophyton mentagrophytes infection in a silver fox (Vulpes vulpes) from a breeding farm

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Dermatophyte infections are extremely frequent worldwide, and their epidemiological features and distribution make them one of the most frequent infections all over the world. We identified and analysed multiform T. mentagrophytes strains isolated from a silver fox (Vulpes vulpes) kept on a breeding farm. Identification of dermatophyte strains was carried out traditionally by correlating both the clinical manifestations of the infection with a micro- and macroscopic examination. To confirm the species affiliation fully, molecular differentiation methods were used. DNA was isolated from the dermatophytes with the phenol-chloroform method. The reaction of chitin synthase 1 (chs1) amplification was carried out to confirm the dermatophytes. The phylogenetic analysis was based on the ITS sequences. The polymerase chain reaction melting profile (PCR-MP) procedure was used for differentiation of dermatophyte genomes. Direct analysis of the material sampled from the clinical lesions revealed the presence of arthrospores in the samples collected from all animals with skin lesions. The macromorphology of the colonies obtained from material sampled from the same individual was not homogeneous. The PCR-MP electrophoregram indicated high variability of their genomes. Although the dermatophytes were isolated from one infected fox, no two identical genomic profiles were obtained. The PCR-MP result corresponds with the phenotypic diversity of the isolates. The findings about the multiple dermatophyte infection in one individual complicate any future epidemiology work and other clinical investigation. Previously, using only morphological characteristics, it had been assumed that one fungal isolate per patient could be diagnosed. The novel findings encourage application of the newly developed molecular typing methods in the diagnosis of dermatophytosis.

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