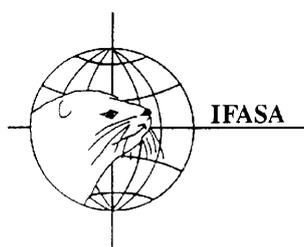
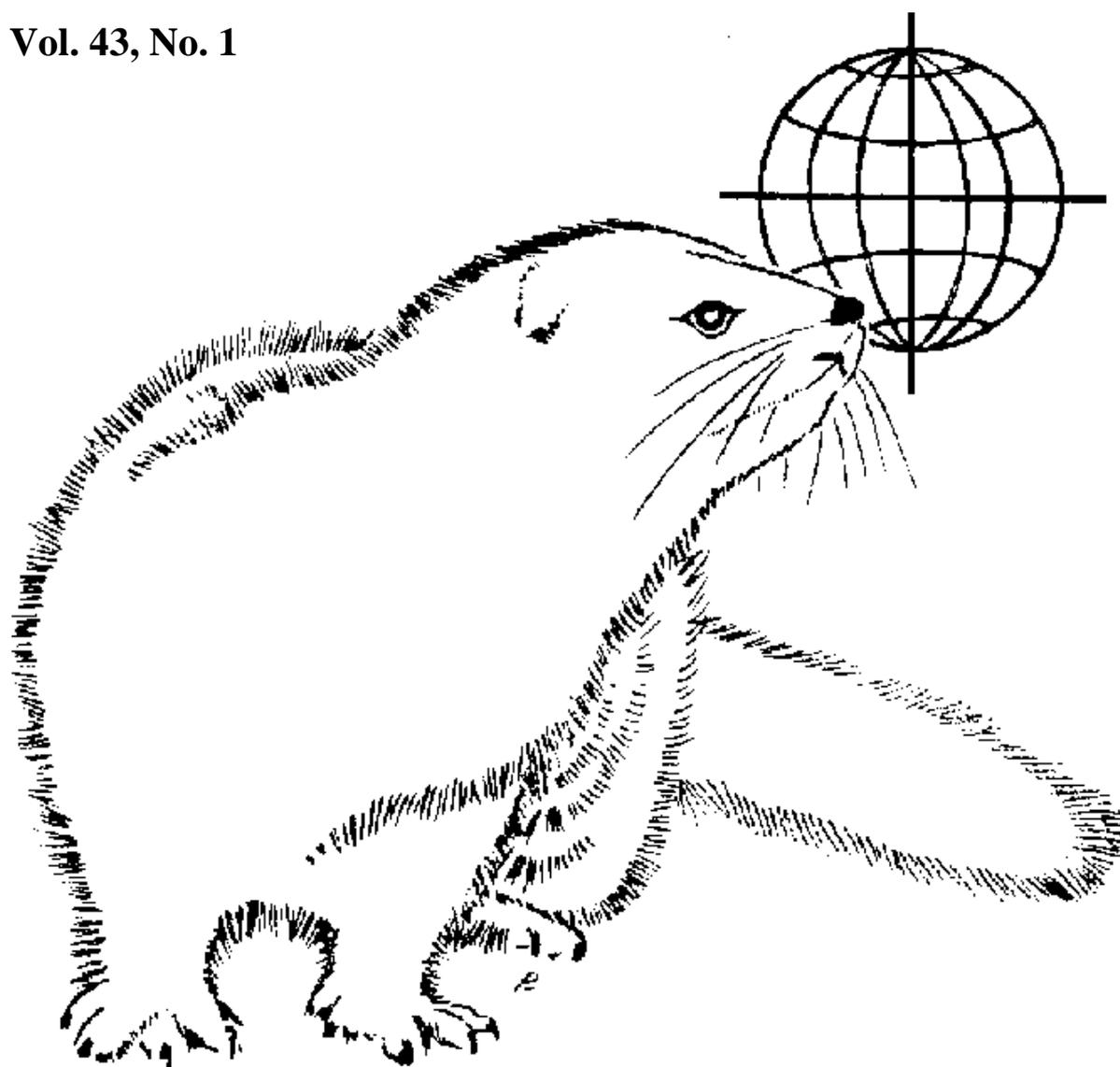


SCIENTIFUR

SCIENTIFIC INFORMATION IN FUR ANIMAL PRODUCTION

Vol. 43, No. 1



INTERNATIONAL FUR ANIMAL SCIENTIFIC ASSOCIATION

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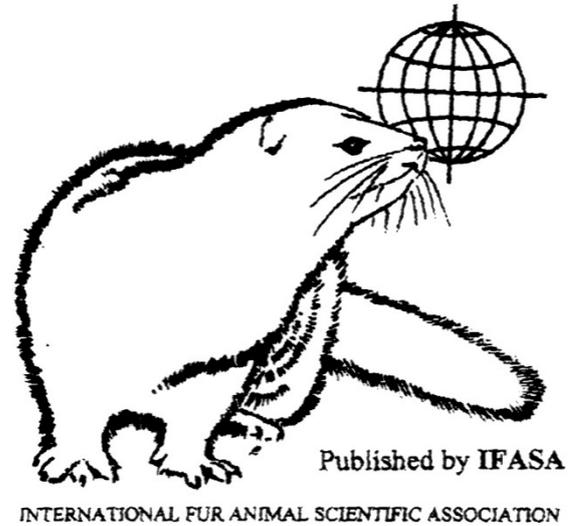
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Notes from the Editor

In recent years, concern has been raised about the loss of biodiversity in nature. Agricultural activities are attributed part of the loss. This challenge must also be considered in fur animal production. This is especially the case where the production of fur animals takes place in areas outside the natural habitat of the animals. Escape of animals from fur farms either by accident or by deliberate release of animals by animal-rights organizations poses threats to small mammals, reptiles, amphibians and ground nesting birds as well as competition to similar sized carnivores. A sustainable production with public accept is based on effective fencing with regular monitoring and awareness of access to the farm. In case of escapees, actions need to be taken e.g. capture of escaped animals in traps.

WelFur is a science-based animal welfare assessment programme voluntarily initiated by the European fur sector. I am glad to publish an abstract from a recent Danish PhD-thesis: “On-farm animal welfare assessment – handling challenges of implementing the wel-

fur-mink protocol on a large scale” dealing with recording of welfare in mink. One outcome of the study is a new semi-random sampling method suggested to obtain a feasible, unbiased and representative sampling in WelFur-Mink. Besides, it is suggested to derive conversion factors for adjustment to ensure the most correct welfare scores independent of date of recording in the winter and growth period. Finally, it is suggested to perform recordings of stereotypic behavior before feeding to get the most reliable results. The thesis was defended at Department of Animal Science, Faculty of Science and Technology, Aarhus University, Denmark.

Summaries of reports from two student projects conducted at Aarhus University in cooperation with the fur industry are also presented in this issue of *Scientifur*. One of the projects deals with the environmental conditions in the nestbox and its relation to early kit mortality. The second project shows the possible application of weights in the surveillance of mink on the farm.

Vivi Hunnicke Nielsen

Editor *Scientifur*

BREEDING, GENETICS AND REPRODUCTION

SNP markers associated with body size and pelt length in American mink (*Neovison vison*)

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Background

Identification of genes underlying production traits is a key aim of the mink research community. Recent availability of genomic tools have opened the possibility for faster genetic progress in mink breeding.

Availability of mink genome assembly allows genome-wide association studies in mink.

Results

Identification of genes underlying production traits is a key aim of the mink research community. Recent availability of genomic tools have opened the possibility for faster genetic progress in mink breeding. Availability of mink genome assembly allows genome-wide association studies in mink.

Conclusions

Combining association results with existing functional information of genes and mammalian phenotype databases, we proposed WWC3, MAP2K4, SLC7A1 and USP22 as candidate genes for body weight and pelt length in mink.

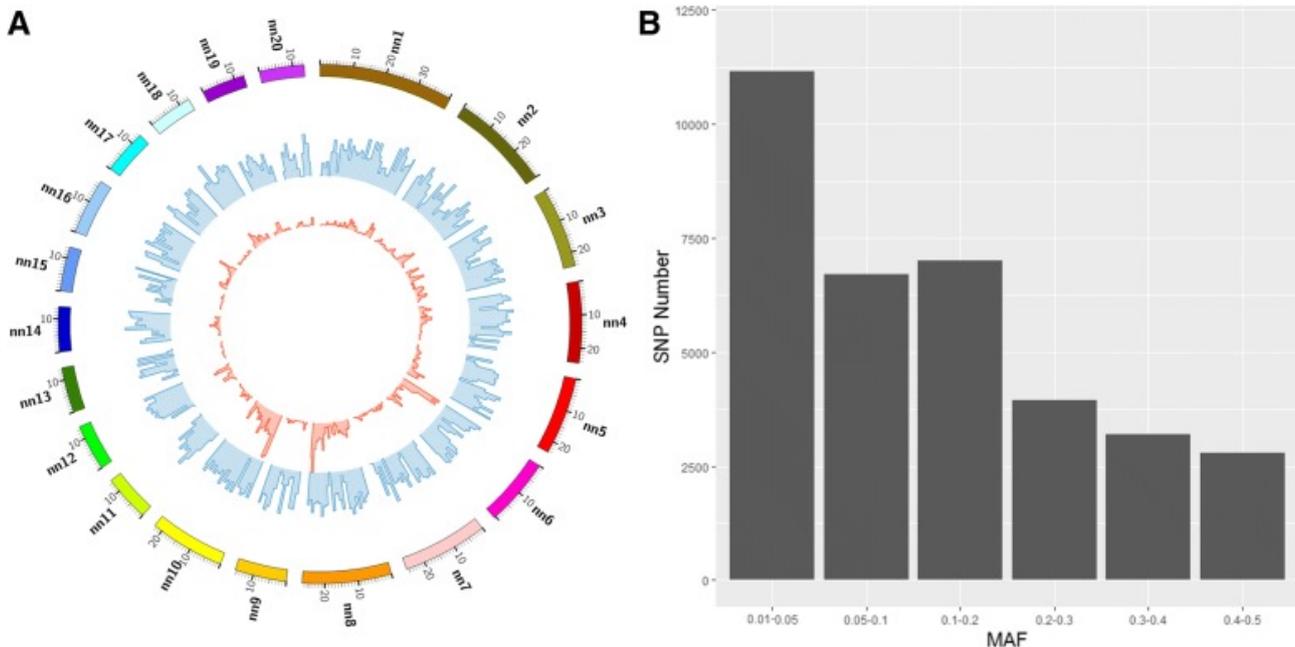


Fig. 1. The basic statistic of SNPs called from GBS data. **a** The Circos plot shows SNP and gene densities for the first 20 scaffolds of the mink genome assembly. The ‘nn’ in the figure represents ‘*Neovison vison* scaffold’ for easy display on figure. The blue track shows the gene density in each 1 Mb block. The red track shows the SNP density of each 1 Mb block. **b** The histogram shows the number of SNP in different MAF classes

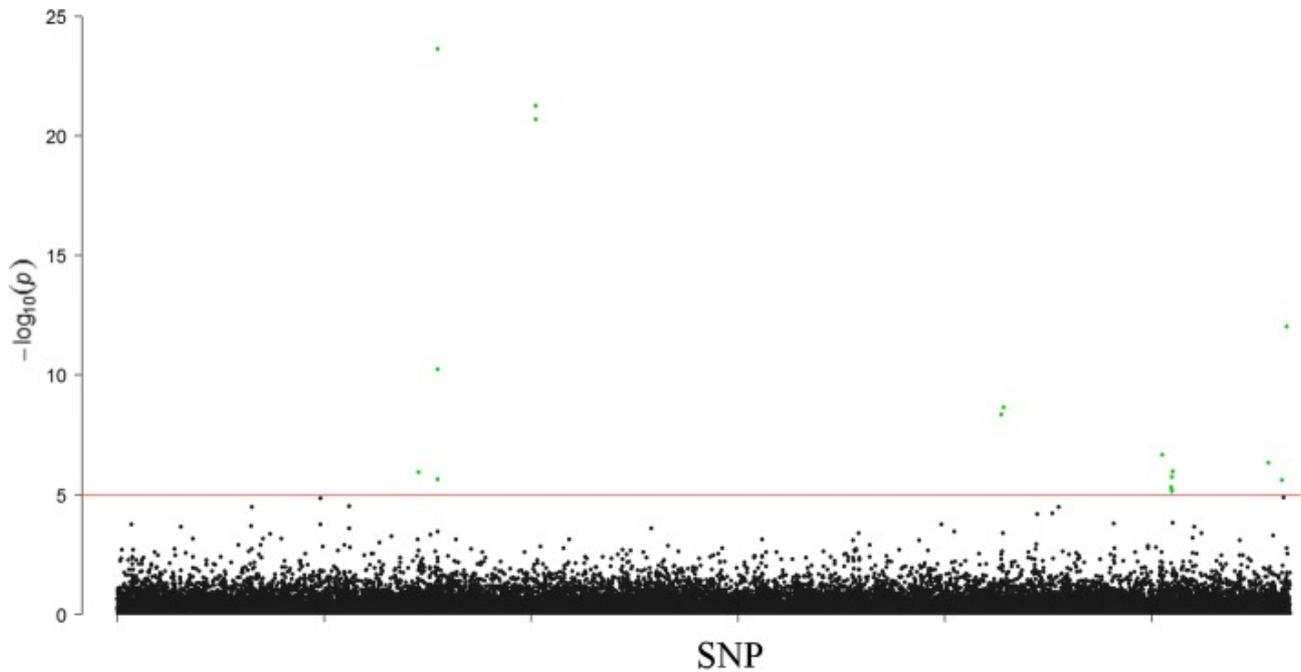


Fig. 2. Manhattan plot for association of SNPs with body weight in mink. The red horizontal line indicates genome-wide significance level ($P < 1.0e-5$). Green dots are the genome-wide significant SNPs.

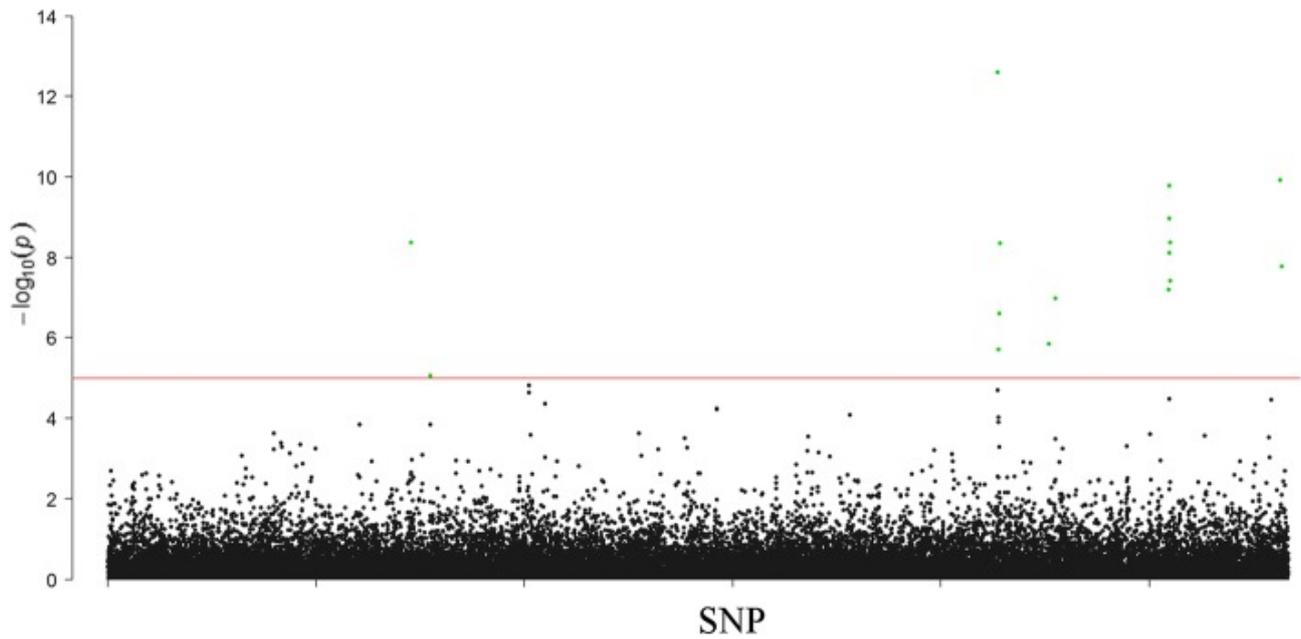


Fig. 3. Manhattan plot for association of SNPs with pelt length in mink. The red horizontal line indicated the genome-wide significance level ($P < 1.0e-5$). The green dots indicate significantly associated SNPs

BMC Genet. 2018 Nov 12; 19(1):103.

Doi: 10.1186/s12863-018-0688-6.

Detection of self-biting behavior of mink by loop-mediated isothermal amplification (LAMP) and sequence-characterized amplified regions (SCAR)

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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 sergent 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. χ^2 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.

Pol J Vet Sci. 2018 Jun; 21(2):371-376.
Doi: 10.24425/122601.

Establishment of mink heart identification method based on mitochondrial cytochrome b gene and development of its detection kit

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In this study, the mink heart mitochondrial DNA was used as the target gene to design the specific primers of mink heart mtDNA Cytb, the extraction and detection reagents of mink heart DNA were developed using DNA fingerprinting technique, and the specificity, reproducibility, and stability of the reagents were investigated. Molecular cloning and sequencing technique were used to clone the standard substance of mink heart DNA detection, then a DNA fingerprint detection method of mink heart and the quality standard for mink heart were established, and a DNA detection kit of mink heart was developed. The results showed that the structure of DNA extracted from mink heart by self-developed reagents was complete, and both the concentration and purity of DNA were high. A specific amplification band of the original mink samples was found at 337 bp. The sequence of mink heart DNA was consistent with that of mink heart mtDNA specific fingerprint region. The mink heart DNA fingerprint identification method established, in this study, is accurate and reliable, and the procedure of the developed DNA kit is easy, the results obtained using this kit is stable and the method is suitable for popularization and application.

Mitochondrial DNA A DNA Mapp Seq Anal. 2018 Oct 13:1-7.

Doi:10.1080/24701394.2018.1504932.

Epub ahead of print.

Purging of Strongly Deleterious Mutations Explains Long-Term Persistence and Absence of Inbreeding Depression in Island Foxes

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The recovery and persistence of rare and endangered species are often threatened by genetic factors, such as the accumulation of deleterious mutations, loss of adaptive potential, and inbreeding depression [1]. Island foxes (*Urocyon littoralis*), the dwarfed descendants of mainland gray foxes (*Urocyon cinereoargenteus*), have inhabited California's Channel Islands for >9,000 years [2-4]. Previous genomic analyses revealed that island foxes have exceptionally low levels of diversity and elevated levels of putatively deleterious variation [5]. Nonetheless, all six populations have persisted for thousands of generations, and several populations rebounded rapidly after recent severe bottlenecks [6, 7]. Here, we combine morphological and genomic data with population-genetic simulations to determine the mechanism underlying the enigmatic persistence of these foxes. First, through analysis of genomes from 1929 to 2009, we show that island foxes have remained at small population sizes with low diversity for many generations. Second, we present morphological data indicating an absence of inbreeding depression in island foxes, confirming that they are not afflicted with congenital defects common to other small and inbred populations. Lastly, our population-genetic simulations suggest that long-term small population size results in a reduced burden of strongly deleterious recessive alleles, providing a mechanism for the absence of inbreeding depression in island foxes. Importantly, the island fox illustrates a scenario in which genetic restoration through human-assisted gene flow could be a counterproductive or even harmful conservation strategy. Our study sheds light on the puzzle of island fox persistence, a unique success story that provides a model for the preservation of small populations.

*Curr Biol. 2018 Nov 5; 28(21):3487-3494.e4.
Doi: 10.1016/j.cub.2018.08.066. Epub 2018 Oct 25.*

Comparison of the characteristics of chinchilla epididymal semen after collection, storage at 5°C and cryopreservation

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The aim of the study was to compare the characteristics of chinchilla epididymal sperm: fresh, stored at liquid state and cryopreserved. Epididymal spermatozoa obtained from 11 males were assessed for subjective motility, concentration, motility parameters measured by CASA, viability, morphology, membrane integrity, acrosome integrity, mitochondrial potential, lipid peroxidation, chromatin structure, apoptotic changes and capacitation. Then half of the spermatozoa were stored at 5°C for 30 hr, and the second half was cryopreserved. After storage and thawing the same parameters as in fresh semen were assessed. Fresh semen showed good quality, with low levels of lipid peroxidation, chromatin fragmentation and capacitation. CASA evaluation showed significantly lower values for MOT, PMOT, RAPID, VCL, VAP and VSL after both storage at liquid state and cryopreservation ($p < 0.05$). Cold storage did not induce membrane and acrosome damage ($p > 0.05$), conversely to cryopreservation ($p < 0.05$). After storage, there was a drop in high mitochondrial potential in live cells ($p < 0.05$) and an increase in the percentage of non-apoptotic, capacitated cells ($p < 0.05$). These changes were not seen after cryopreservation ($p > 0.05$). Lipid peroxidation in live cells and chromatin structure remained unchanged both after storage and cryopreservation ($p > 0.05$). The study showed that examined methods of semen preservation exerted different patterns of changes in spermatozoa and that sperm quality after both of them allowed for further use of preserved spermatozoa in artificial reproductive techniques.

*Reprod Domest Anim. 2018 Nov; 53 Suppl 3:29-36.
Doi: 10.1111/rda.13329.*

BEHAVIOUR AND WELFARE

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

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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.

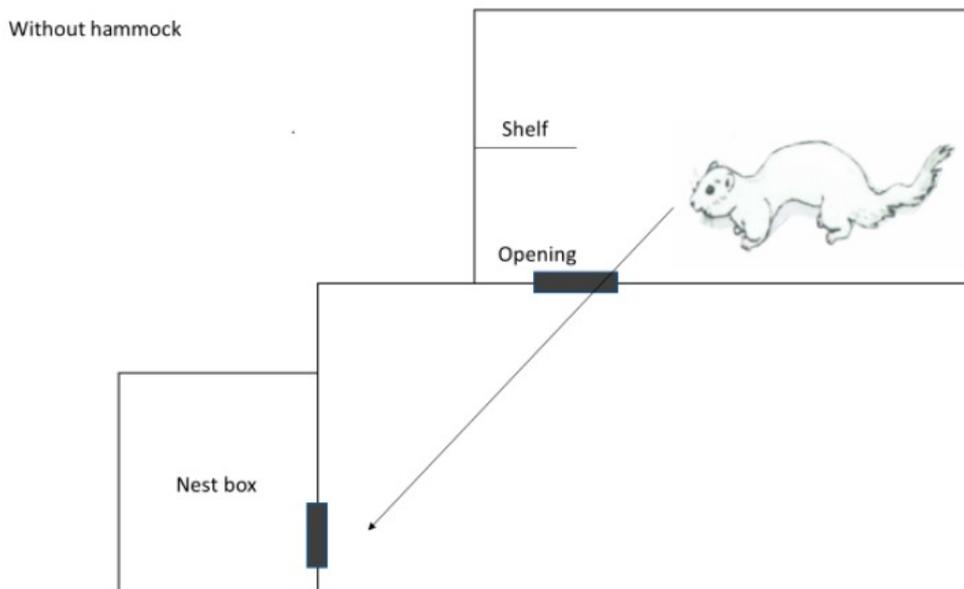


Fig. 1. Standard multilevel mink cage (control), view from the left side. The arrow indicates the chosen route of the mink from the upper floor to the nest box.

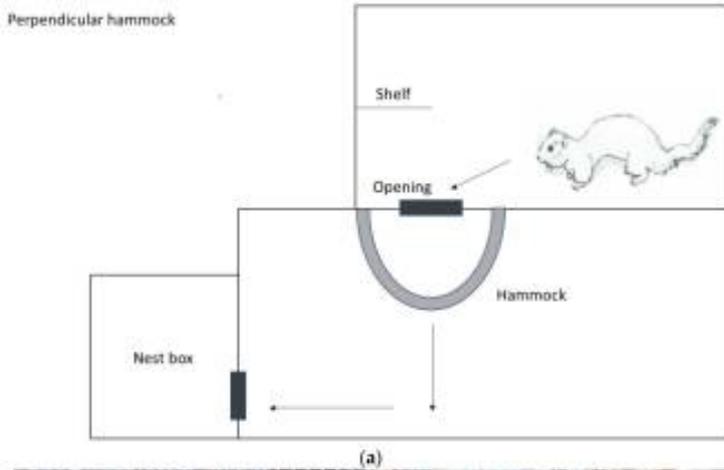


Fig. 2. (a) Multilevel cage with a hammock installed perpendicular to the sidewalls, view from the left side. The arrows indicate the chosen route of the mink from the upper floor to the nest box. (b) Front view of a hammock placed perpendicular to the sidewalls.

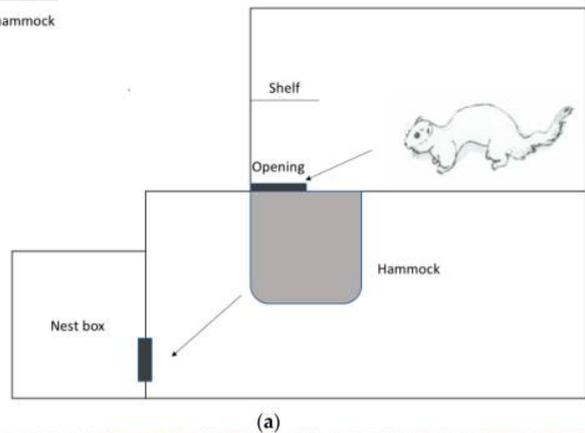
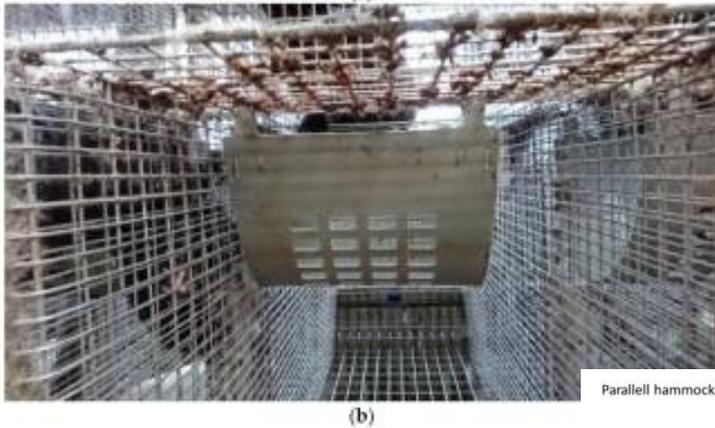


Fig. 3. (a) Multilevel cage with a hammock installed parallel to the sidewalls, view from the left side. The arrows indicate the chosen route of the mink from the upper floor to the nest box. (b) Front view of a hammock placed parallel to the sidewalls.

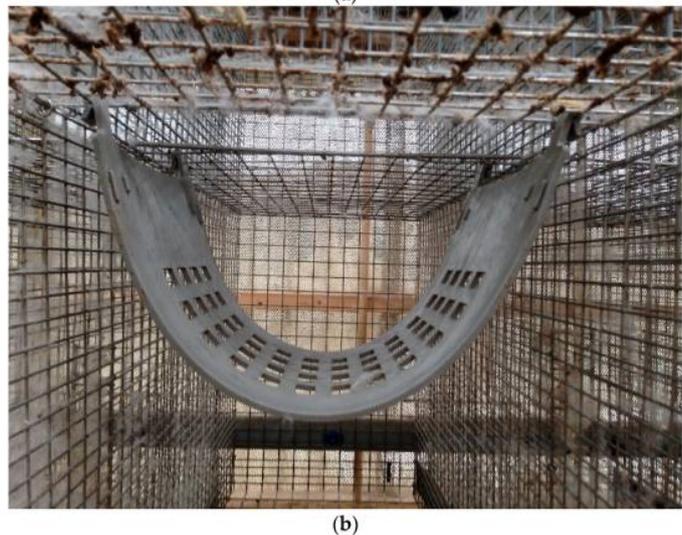




Fig. 4. Typical morphology of a wound probably caused by tail hitting the wire mesh, located on the ventral side of the mink tail.

Animals (Basel). 2018 Nov 19; 8(11). pii: E214.
Doi: 10.3390/ani8110214.

NUTRITION, FEEDING AND MANAGEMENT

Determination of endogenous fat loss and true total tract digestibility of fat in mink (*Neovison vison*)

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The objective of this study was to determine the endogenous fat loss (EFL) and to calculate true total tract digestibility (TTTD) of fat in mink (*Neovison vison*) using soybean oil-based diets with different fat levels. In the digestibility assay, four diets with 6.30%, 13.9%, 22.0% and 34.0% fat in dry matter were used. Sixteen adult male mink were distributed in a complete randomised design. The apparent total tract digestibility (ATTD) of dietary fat was 90.8%, 95.9%, 96.9% and 97.8%, respectively. The apparent total digestible fat was linearly related to dietary fat intake ($r^2 = 0.99$). The EFL was estimated from the slope of the regression equation and was determined to be 5.09 g/kg DM intake. The TTTD of soybean oil was determined to be 99.3%. Therefore, TTTD values will have negligible impact in feed formulation as they are close to ATTD values with the dietary fat levels normally used for mink.

Arch Anim Nutr. 2019 Feb; 73(1):67-73.
Doi: 10.1080/1745039X.2018.1545501.
Epub 2018 Nov 21.

Distribution of α -tocopherol stereoisomers in mink (*Mustela vison*) organs varies with the amount of all-rac- α -tocopheryl acetate in the diet

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Synthetic α -tocopherol has eight isomeric configurations including four 2R (RSS, RRS, RSR, RRR) and four 2S (SRR, SSR, SRS, SSS). Only the RRR stereoisomer is naturally synthesised by plants. A ratio of 1:36:1 in biopotency of RRR- α -tocopheryl acetate to all-rac- α -tocopheryl acetate is generally accepted; however, studies indicate that neither biopotency of α -tocopherol stereoisomers nor bioavailability between them is constant, but depend on dose, time, animal species and organs. A total of forty growing young male mink were, after weaning, assigned one of the following treatments for 90 d: no α -tocopherol in diet (ALFA_0), 40 mg/kg RRR- α -tocopheryl acetate (NAT_40), 40 mg/kg all-rac- α -tocopheryl acetate (SYN_40) and 80 mg/kg feed all-rac- α -tocopheryl acetate (SYN_80). Mink were euthanised in CO₂ and blood was collected by heart puncture. Mink were pelted and liver, heart, lungs, brain and abdominal fat were collected for α -tocopherol stereoisomer analysis. The proportion of RRR- α -tocopherol decreased in all organs and plasma with increasing amount of synthetic α -tocopherol stereoisomers in the diet ($P \leq 0.05$), whereas the proportion of all synthetic α -tocopherol stereoisomers increased with increasing amount of synthetic α -tocopherol stereoi-

somers in the diet ($P \leq 0.05$). The proportion of α -tocopherol stereoisomers in plasma, brain, heart, lungs and abdominal fat showed the following order: RRR>RRS, RSR, RSS> Σ 2S, regardless of α -tocopherol supplement. The liver had the highest proportion of Σ 2S stereoisomers, and lowest proportion of RRR- α -tocopherol. In conclusion, distribution of α -tocopherol stereoisomers differs with dose and form of α -tocopherol supplementation. The results did also reveal the liver's role as the major organ for accumulation of Σ 2S α -tocopherol stereoisomers.

Br J Nutr. 2018 Dec; 120(12):1332-1337.

Doi: 10.1017/S0007114518002878.

Epub 2018 Oct 17.

Fecal bacterial microbiota of Canadian commercial mink (*Neovison vison*): Yearly, life stage, and seasonal comparisons

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The gastrointestinal microbiome is known to play a critical role in animal health but has been relatively poorly characterized in commercial mink, an obligate carnivore. Whether the microbiota can be manipulated in mink to improve pelt quality, health, and well-being is unknown. The objectives of this study

were to characterize the fecal microbiota of commercial mink, and to evaluate potential changes due to year (2014 vs 2015), life stage (adult female vs weaned kit), season (summer vs winter), and between Canadian farms. Pooled fecal samples were collected from adult females and weaned kits in the summers of 2014 ($n = 173$) and 2015 ($n = 168$), and from females in the winter of 2016 ($n = 39$), a time when females undergo marked calorie restriction, from 49 mink farms in Ontario. Bacterial DNA was extracted and the V4 region of the 16S rRNA gene was amplified. Approximately 22 million sequences were identified following quality control filtering. A total of 31 bacterial phyla were identified; however, only 3 comprised >1% of the total sequences identified, with Firmicutes and Proteobacteria together comprising 95% of the total sequences. Comparisons were made by life stage, season and year; no differences were found in the relative abundance of any taxa between samples collected from adult females and weaned kits from the same year and the greatest number of differences at each taxonomic level were noted between 2014 and 2015. Significantly more operational taxonomic units (OTUs) were found in 2014 than 2015 or 2016 ($p < 0.05$) and samples from 2014 were more even, but less diverse than in 2015 ($p = 0.002$ and 0.001 , respectively). There were significant differences in community population and structure by year and season (all p -values < 0.001). The predominant phyla and genera at the farm level were similar from year to year. Together, these indicate that mink environment, season, and time are important factors in the stability of gastrointestinal microbiota, once mink reach maturity.

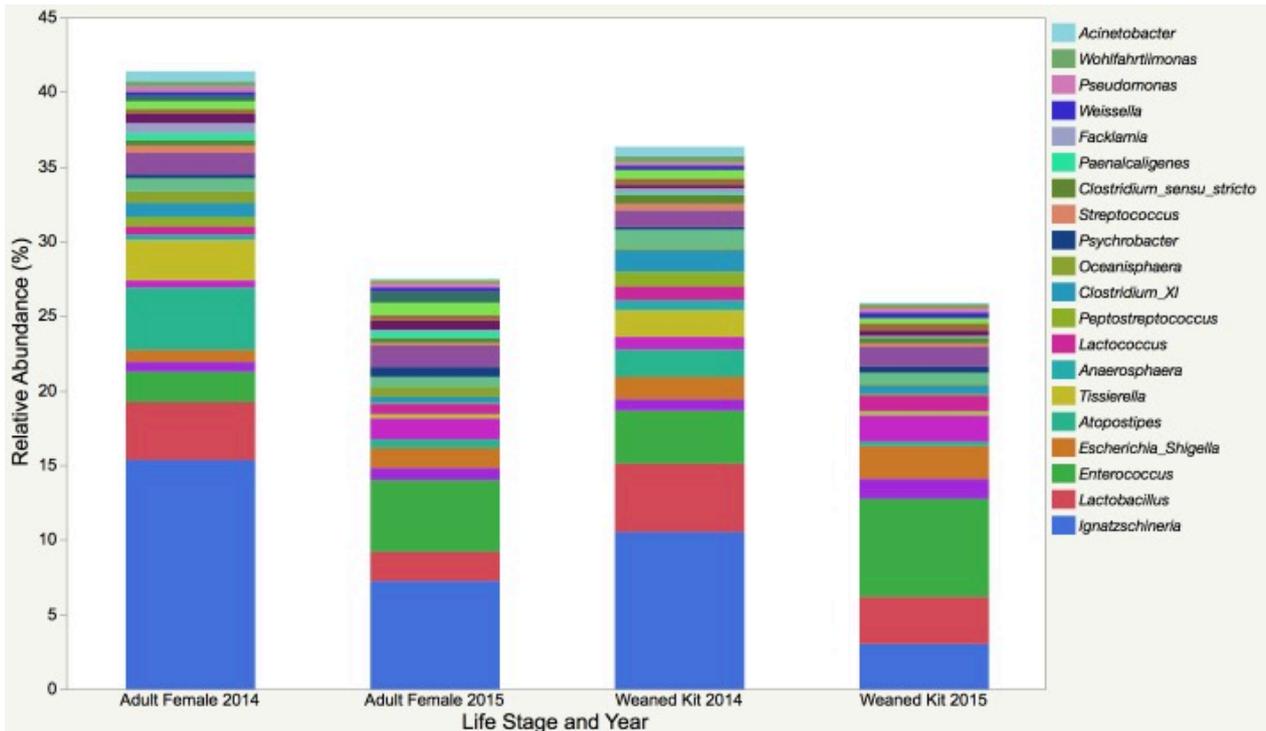


Fig. 1. Relative abundance of the 20 most predominant bacterial genera present in the feces of commercial mink (n = 366). Samples were obtained from 43, 46, and 39 farms in 2014, 2015, and 2016, respectively.

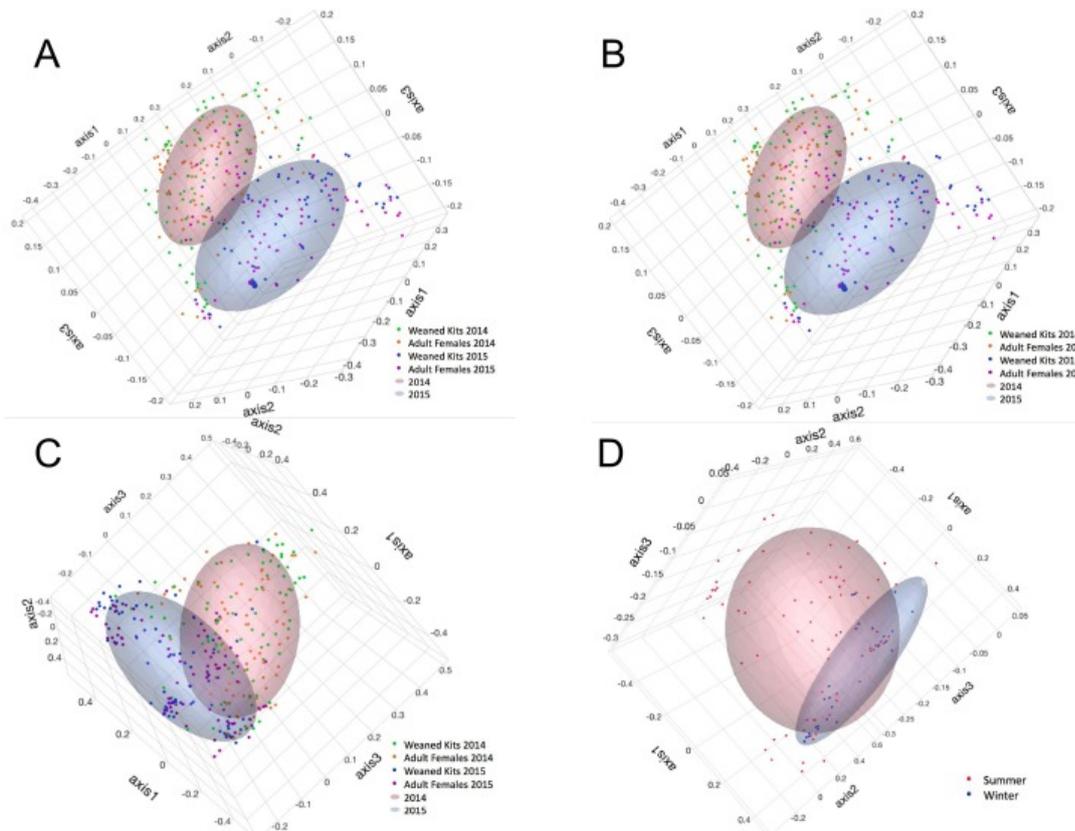


Fig. 2. Principal coordinate analysis (PCoA) of community population based on the Jaccard Index (a and b) and community structure based on the Yue-Clayton Index (c and d) of the fecal microbiota of commercial mink, by year (n = 332) (a and c) and season (n = 117) (b and d). Samples were obtained from 43, 46, and 39 farms in 2014, 2015, and 2016, respectively.

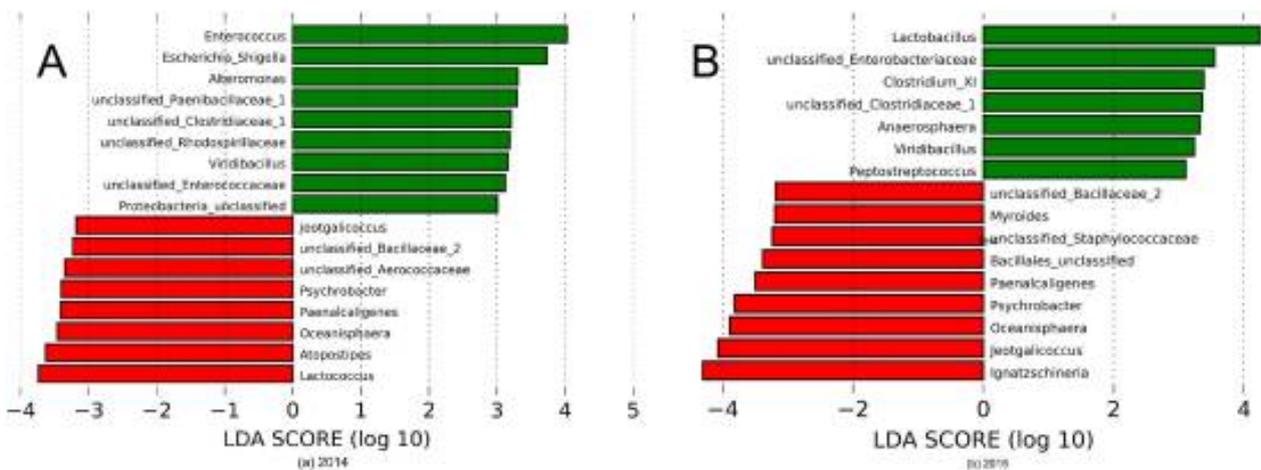


Fig. 3. Linear discriminant analysis effect size (LEfSe) indicating differentially enriched fecal bacterial communities (with LDA score >3) at the genus level between adult females (red, n = 164) and weaned kits (green, n = 168) in summer 2014 (a) and summer 2015 (b) (n = 332). Samples were obtained from 43 and 46 farms in 2014 and 2015, respectively.

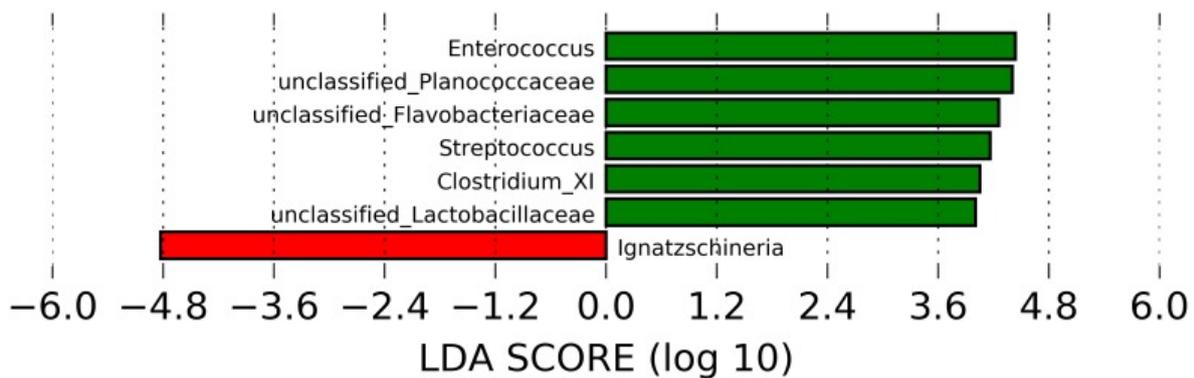


Fig. 4. Linear discriminant analysis effect size (LEfSe) indicating differentially enriched fecal bacterial communities (with LDA score >3) at the genus level of adult females by season (n = 117). Green = winter (n = 34), red = summer (n = 83). Samples were obtained from 46 and 39 farms in 2015 and 2016, respectively.

PLoS One. 2018 Nov 12; 13(11):e0207111.
 Doi: 10.1371/journal.pone.0207111.
 eCollection 2018.

HEALTH AND DISEASE

Co-circulation of highly diverse Aleutian mink disease virus strains in Finland

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Aleutian mink disease virus (AMDV) is the causative agent of Aleutian disease (AD), which affects mink of all genotypes and also infects other mustelids such as ferrets, martens and badgers. Previous studies have investigated diversity in Finnish AMDV strains, but these studies have been restricted to small parts of the virus genome, and mostly from newly infected farms and free-ranging mustelids. Here, we investigated the diversity and evolution of Finnish AMDV strains by sequencing the complete coding sequences of 31 strains from mink originating from farms differing in their virus history, as well as from free-ranging mink. The data set was supplemented with partial genomes obtained from 26 strains. The sequences demonstrate that the Finnish AMDV strains have considerable diversity, and that the virus has been introduced to Finland in multiple events. Frequent recombination events were observed, as well as variation in the evolutionary rate in different parts of the genome and between different branches of the phylogenetic tree. Mink in the wild carry viruses with high intra-host diversity and are occasionally even co-infected by two different strains, suggesting that free-ranging mink tolerate chronic infections for extended periods of time. These findings highlight the need for further sampling to understand the mechanisms playing a role in the evolution and pathogenesis of AMDV.

J Gen Virol. 2018 Dec 10.

Doi: 10.1099/jgv.0.001187. Epub ahead of print.

Prevalence of *Capillaria plica* in Danish wild carnivores

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Capillaria plica is a parasitic nematode belonging to the family Capillariidae. The adult parasites reside in the urinary tract of wild and domestic canines. The infection is most often asymptomatic, but can cause a wide range of symptoms including urinary bladder inflammation, pollaciuria, dysuria and hematuria. Canines acquire the infection by ingesting the intermediate host, the earthworm (Lumbricidae). Epidemiological studies on *C. plica* infection in wildlife are few and only one previous Danish study examined the prevalence in red foxes, while studies on prevalence in other animals are limited. We examined the urine sediment or urinary bladder from 375 Raccoon dogs (*Nyctereutes procyonoides*), 247 red foxes (*Vulpes vulpes*), 20 beech martens (*Martes foina*), 16 wild mink (*Neovison vison*), 14 otters (*Lutra lutra*), nine European polecats (*Mustela putorius*), three European badgers (*Meles meles*) and one golden jackal (*Canis aureus*) received as a part of Danish wildlife surveillance. *Capillaria plica* was detected in 73.7% of red foxes, 20.0% of beech martens, 0.5% of raccoon dogs, and in the Golden jackal. Red foxes originating from all 5 regions of Denmark were infected, although with a significantly higher prevalence in the three regions in Jutland compared to Region Zealand.



Fig. 1. A typical barrel-shaped *Capillaria plica* egg in urine sediment from a red fox. The egg shows a slightly pitted shell and two opercules with polar plugs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

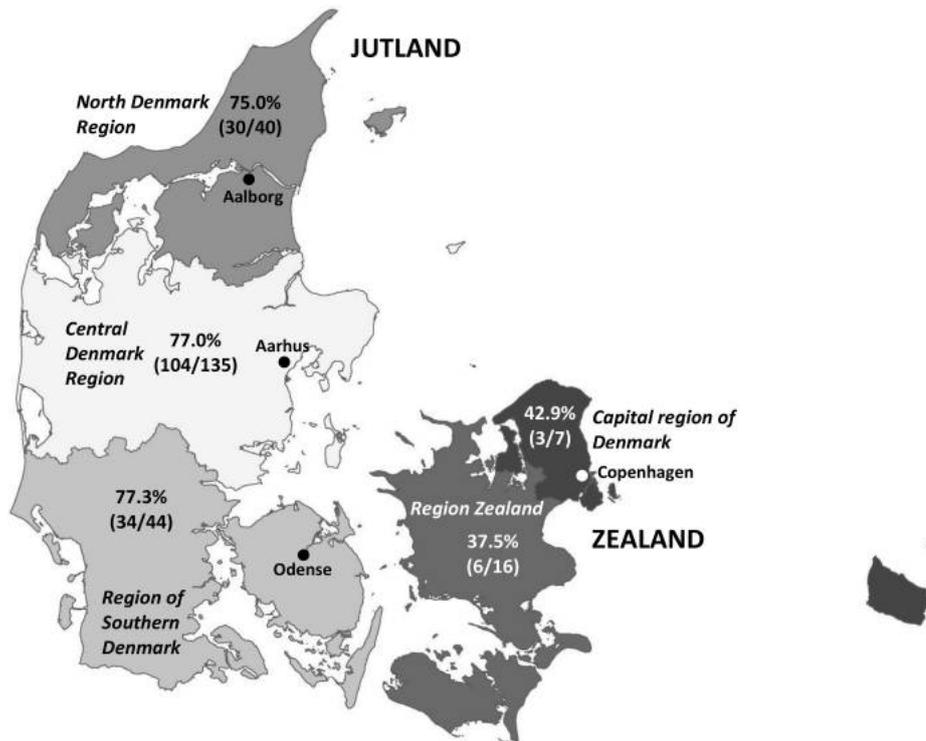
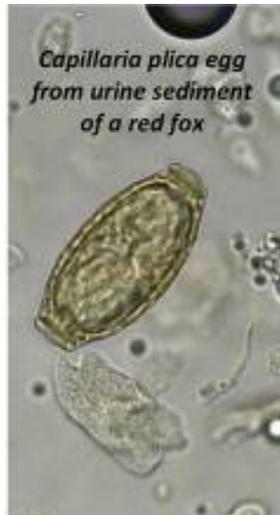
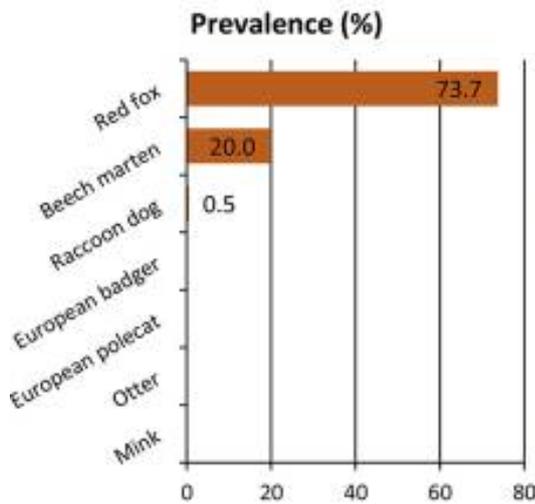


Fig. 2. The prevalence of *Capillaria plica* infections in red foxes per region of Denmark (positive red foxes/total number of red foxes examined). The origin of five red foxes was unknown.



Int J Parasitol Parasites Wildl. 2018 Sep 22; 7(3):360-363.
 Doi: 10.1016/j.ijppaw.2018.09.006.
 eCollection 2018 Dec.

Mink Circovirus Can Infect Minks, Foxes and Raccoon Dogs

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Mink circovirus (MiCV), which is clustered in the genus *Circovirus* of the family *Circoviridae*, was first described in minks from farms in Dalian, China in 2013 (Lian *et al.* 2014). The complete single-stranded circular genome of the virus is 1,753 nucleotides long and contains two major open reading frames (ORFs), designated ORF1 (*Rep* gene) and ORF2 (*Cap* gene) (Lian *et al.* 2014; Ge *et al.* 2018). Sequence analysis has shown that MiCV is most closely related to mammalian circoviruses, such as bat circovirus (BatCV), porcine circovirus (PCV), and dog circovirus (dogCV) (Ge *et al.* 2018). Epidemiological investigations have revealed that the mink circovirus is very prevalent in China, with a positive rate of up to 54.6% (101/185) on some mink farms in China (Lian *et al.* 2014; Wang *et al.* 2015; Ge *et al.* 2018). The virus is found in the liver, digestive tract, and fecal specimens of minks, with diarrhea as the main clinical sign (Lian *et al.* 2014).

Viol Sin. 2018 Dec 4.

Doi: 10.1007/s12250-018-0059-5.

Epub ahead of print.

Protease inhibitors broadly effective against feline, ferret and mink coronaviruses

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Ferret and mink coronaviruses typically cause catarrhal diarrhea in ferrets and minks, respectively. In recent years, however, systemic fatal coronavirus infection has emerged in ferrets, which resembles feline infectious peritonitis (FIP) in cats. FIP is a highly fatal systemic disease caused by a virulent feline coronavirus infection in cats. Despite the importance of coronavirus infections in these animals, there are no effective commercial vaccines or antiviral drugs available for these infections. We have previously reported the efficacy of a protease inhibitor in cats with FIP, demonstrating that a virally encoded 3C-like protease (3CLpro) is a valid target for antiviral drug development for coronavirus infections. In this study, we extended our previous work on coronavirus inhibitors and investigated the structure-activity relationships of a focused library of protease inhibitors for ferret and mink 3CLpro. Using the fluorescence resonance energy transfer assay, we identified potent inhibitors broadly effective against feline, ferret and mink coronavirus 3CLpro. Multiple amino acid sequence analysis and modelling of 3CLpro of ferret and mink coronaviruses were conducted to probe the structural basis for these findings. The results of this study provide support for further research to develop broad-spectrum antiviral agents for multiple coronavirus infections. To the best of our knowledge, this is the first report on small molecule inhibitors of ferret and mink coronaviruses.

Antiviral Res. 2018 Dec; 160:79-86.

Doi: 10.1016/j.antiviral.2018.10.015.

Epub 2018 Oct 19.

Dam characteristics associated with pre-weaning diarrhea in mink (*Neovison vison*)

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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.



Fig. 1. Scoring of rectal contents/feces in mink kits. The numbers refer to the consistency of the feces and the letter to the color. **a** Score 1; Firm to normal soft, log-shaped and moist with smooth surface. **b** Score 2; Soft without shape, very moist, cow-pat like consistency. **c** Score 3; Runny, loose, no defined shape with some texture. Also notice external signs: a sticky exudation on the skin, red swollen anus and black claws. **d** Score 4; Liquid, not containing any particular matter, no texture and may be foamy. **e** Score a; Undigested, white or beige color. **f** A mink litter affected with PWD and cutaneous exudation located to the neck, legs and paws.

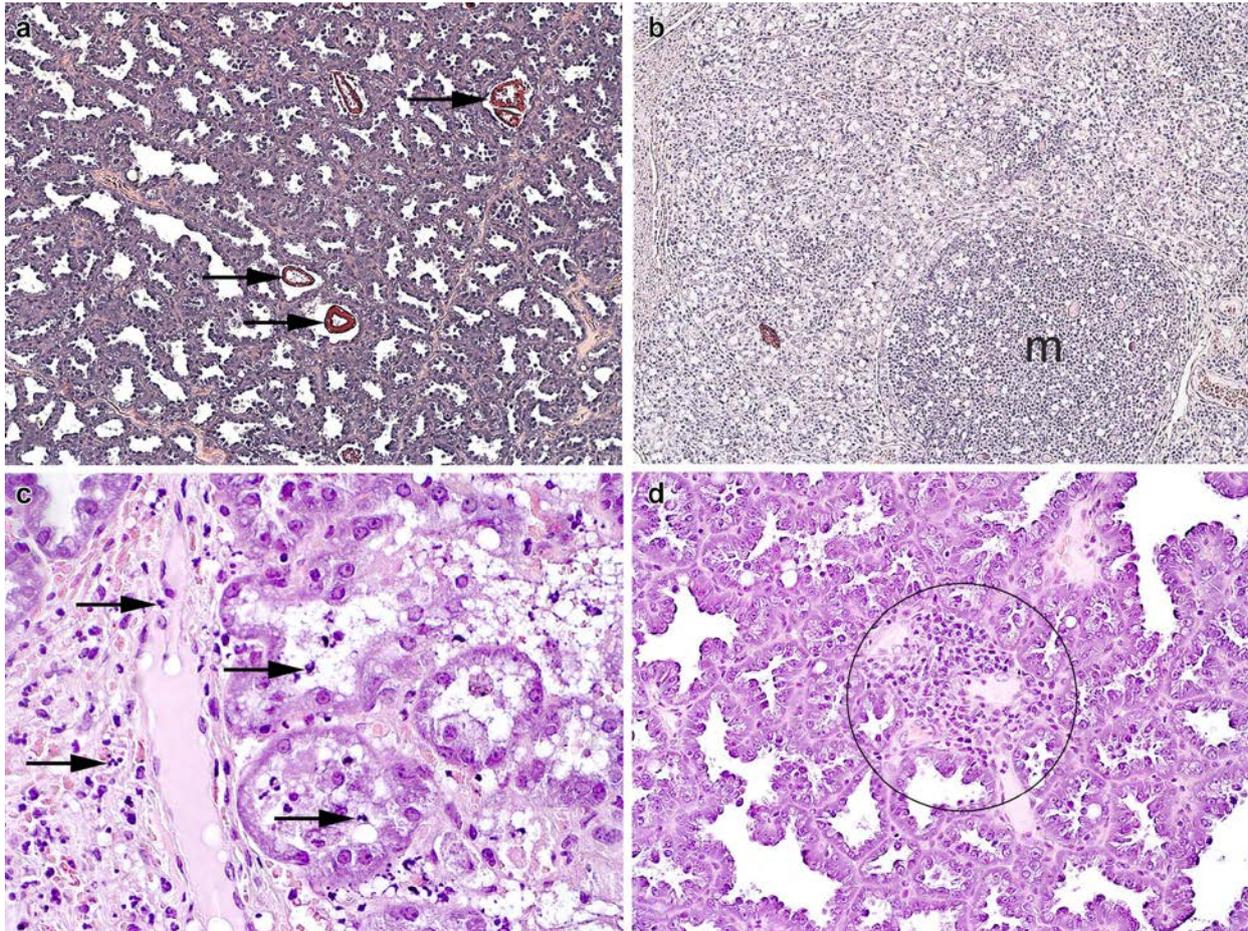


Fig. 2. Examples of histopathological findings in mammary gland tissue from mink dams. **a** Non-infiltrated gland tissue with several corpora amylacea (milkstones) (arrows). **b** Disseminated infiltration of polymorphonuclear leucocytes with microabscess formation (m). **c** Infiltration with polymorphonuclear leucocytes (arrows) in the connective tissue and lumen of acini with disruption of the normal architecture. **d** Focal intralobular infiltration of mononuclear leucocytes (circle).

Acta Vet Scand. 2018 Nov 12; 60(1):73.

Doi: 10.1186/s13028-018-0427-z.

Investigation of the viral and bacterial microbiota in intestinal samples from mink (*Neovison vison*) with pre-weaning diarrhea syndrome using next generation sequencing

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Pre-weaning diarrhea (PWD) in mink kits is a common multifactorial syndrome on commercial mink farms. Several potential pathogens such as astroviruses, caliciviruses, *Escherichia coli* and *Staphylococcus delphini* have been studied, but the etiology of the syndrome seems complex. In pooled samples from 38 diarrheic and 42 non-diarrheic litters, each comprising of intestinal contents from 2-3 mink kits from the same litter, the bacterial populations were studied using Illumina Next Generation Sequencing technology and targeted 16S amplicon sequencing. In addition, we used deep sequencing to determine and compare the viral intestinal content in 31 healthy non-diarrheic and 30 diarrheic pooled samples (2-3 mink kits from the same litter per pool). The results

showed high variations in composition of the bacterial species between the pools. Enterococci, staphylococci and streptococci dominated in both diarrheic and non-diarrheic pools. However, enterococci accounted for 70% of the reads in the diarrheic group compared to 50% in the non-diarrheic group and this increase was at the expense of staphylococci and streptococci which together accounted for 45% and 17% of the reads in the non-diarrheic and diarrheic group, respectively. Moreover, in the diarrheic pools there were more reads assigned to Clostridia, Escherichia-Shigella and Enterobacter compared to the

non-diarrheic pools. The taxonomically categorized sequences from the virome showed that the most prevalent viruses in all pools were caliciviruses and mamastroviruses (almost exclusively type 10). However, the numbers of reads assigned to caliciviruses were almost 3 times higher in the diarrheic pools compared the non-diarrheic pools and Sapporo-like caliciviruses were more abundant than the Norwalk-like caliciviruses. The results from this study have contributed to the insight into the changes in the intestinal microbiota associated with the PWD syndrome of mink.

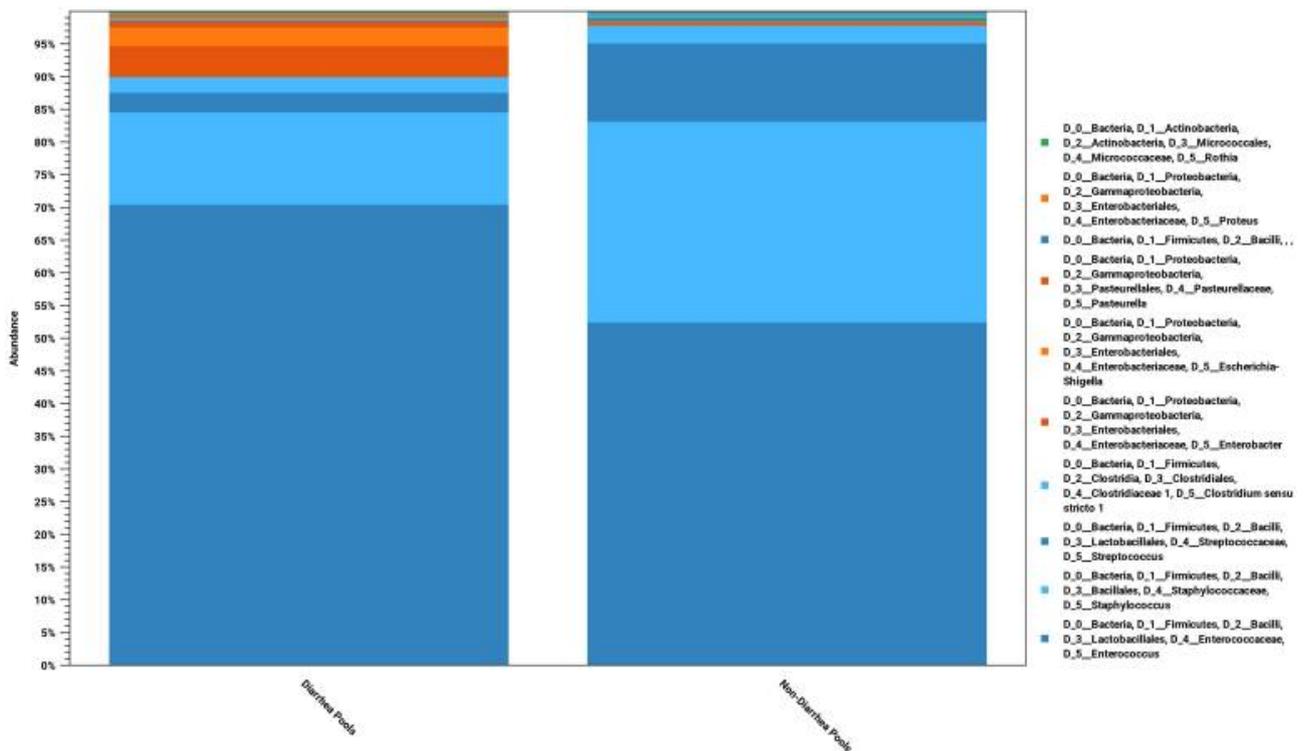


Fig. 1. The bacterial populations derived from 16S rRNA sequence classification for the sum of all mink kit feces pools grouped as diarrheic and non-diarrheic. Of the 83 pools included in the study, 80 (38 diarrheic and 42 non-diarrheic) passed the set low coverage limit of 40 000 reads and contribute to the data shown. Two of the diarrhea pools and two of the non-diarrhea pools were run in duplicate and in total 84 pool samples are included. The populations are displayed aggregated and colored at the taxonomic genus and phylum levels, respectively.

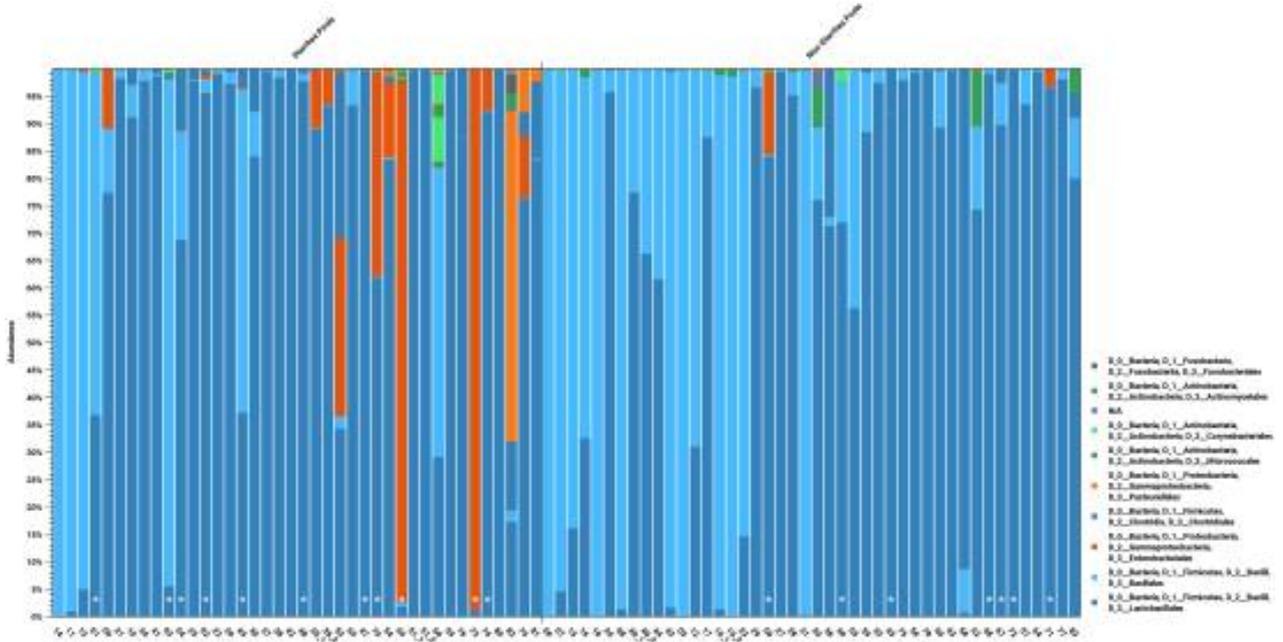


Fig. 2. Individual bacterial populations derived from 16S rRNA sequence classification for each of the 84 mink kit feces pool samples grouped as diarrheic and non-diarrheic and ordered according to litter age. Two each of the diarrheic pools and non-diarrheic pools were run in duplicate. The stars (*) indicate pools from litters where the female was treated with the antibiotic amoxicillin. The populations are displayed aggregated and colored at the taxonomic order and phylum levels, respectively. The duplicates are indicated. The litter age of the pools is not show in this figure.

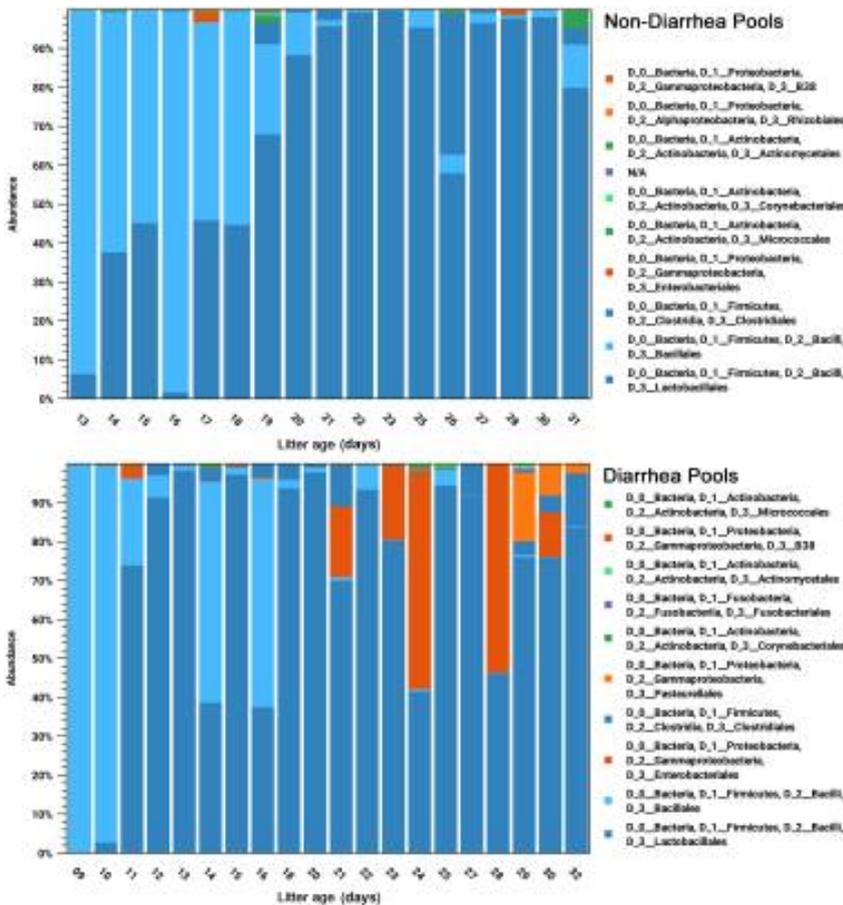


Fig. 3. The bacterial populations of the 83 mink kit feces pool samples derived from 16S rRNA classification and grouped according to the litter age. Pools were grouped according the non-diarrheic (top panel) diarrheic (bottom panel) status. Populations are displayed aggregated and colored on the taxonomic order and phylum levels, respectively.

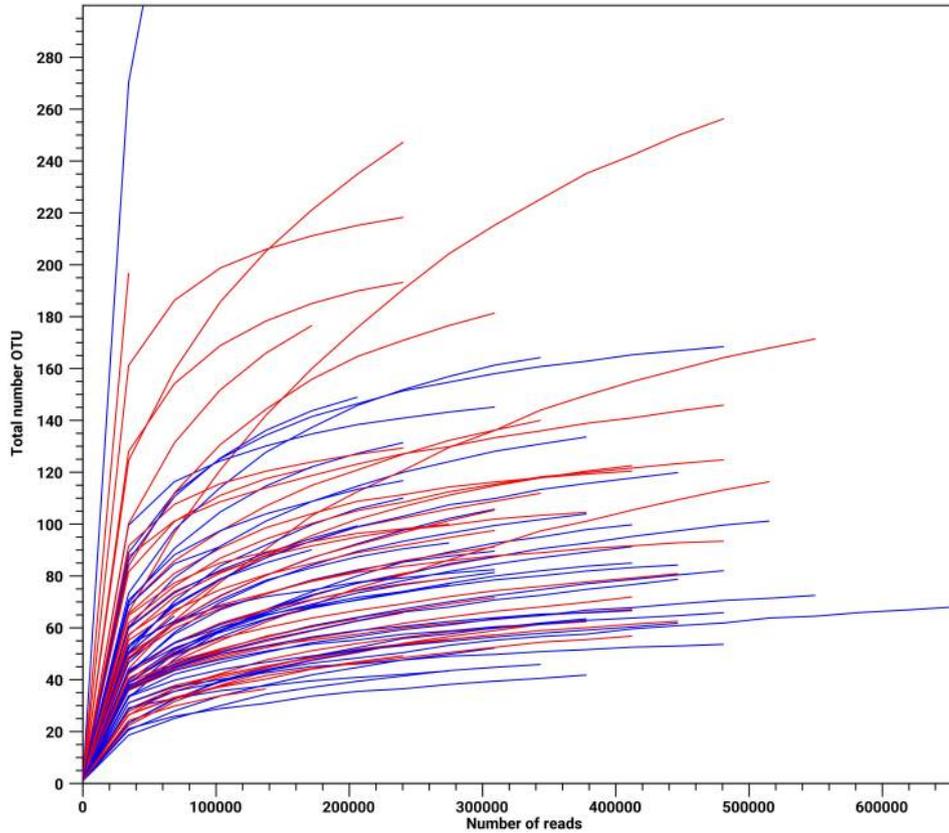


Fig. 4. The α -diversity of the bacterial populations of the 83 mink kit feces pool samples displayed as the total number of operational taxonomic units at different numbers of sequence reads. Sampling was carried out without replacement and with 100 replicates. Pools classified as non-diarrheic and diarrheic are shown as blue and red curves, respectively.

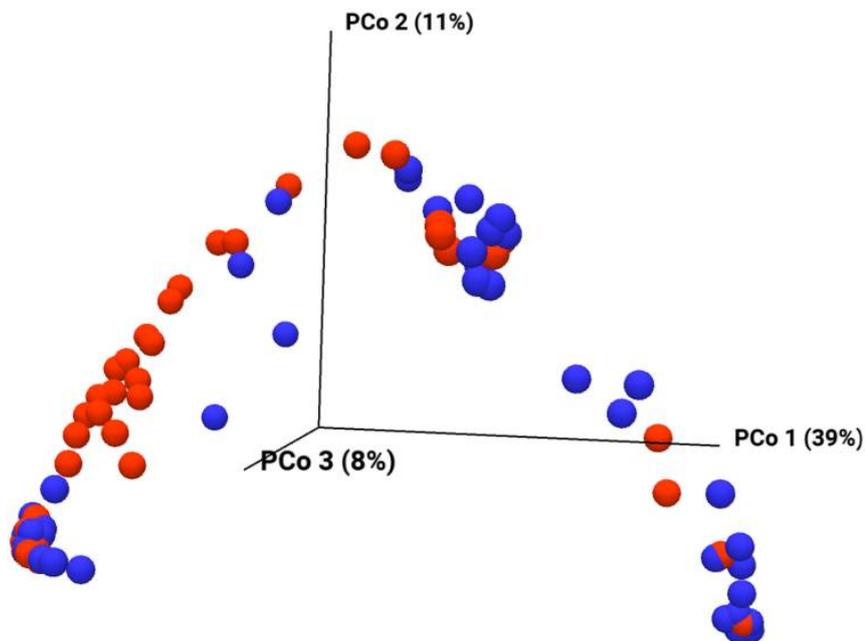


Fig. 5. The β -diversity of the bacterial populations of the 83 mink kit feces pool samples. The β -diversity is shown as a three-dimensional principal component plot using the Jaccard diversity measure. Pools classified as non-diarrheic and diarrheic are shown as blue and red spheres, respectively.

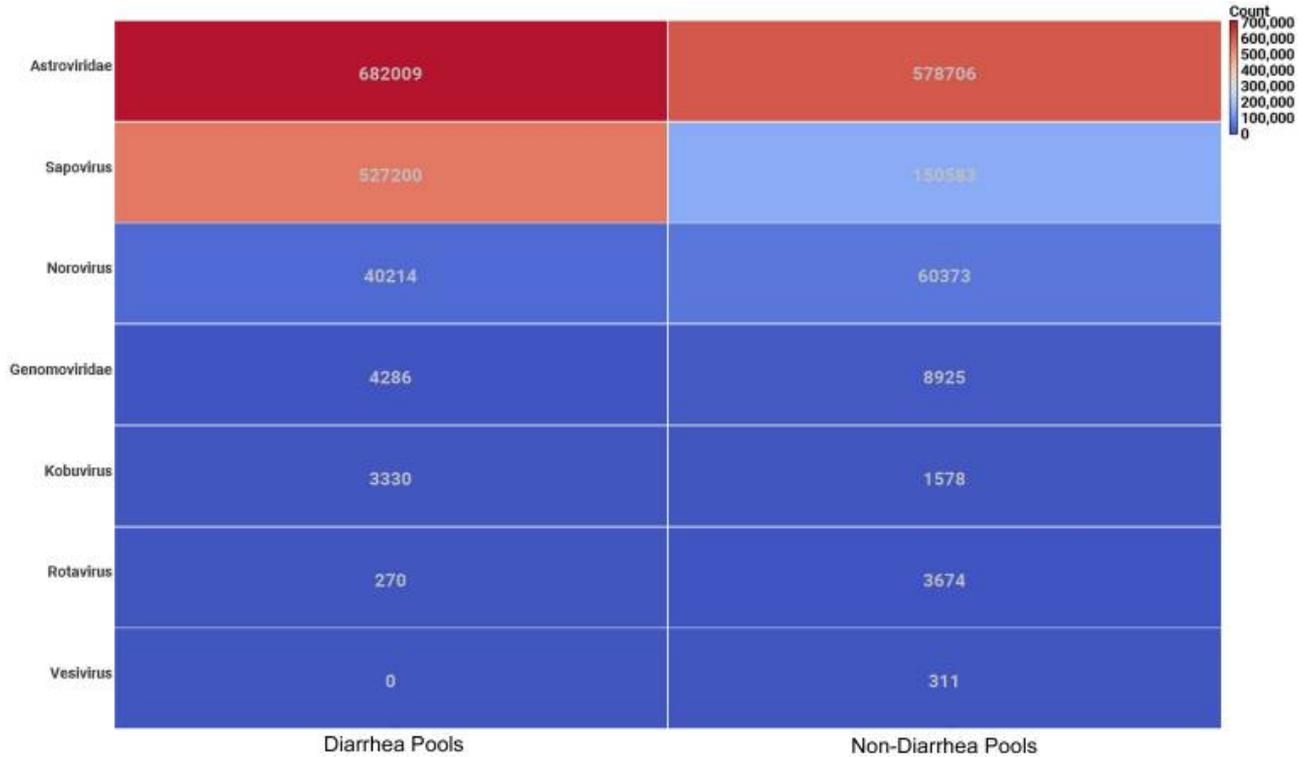


Fig. 6. The number of sequence reads assigned to viruses for 61 mink kit feces pool samples. Of the 83 mink kit feces pool samples 61 (30 diarrheic and 31 non-diarrheic) produced sufficient data (more than 95 000 reads) for comparative study. Pools classified as non-diarrheic and diarrheic are shown to the left and right, respectively. A cut-off has been employed where viruses with less than 1000 reads assigned are not shown except vesiviruses that belong to the *Caliciviridae* family.

PLoS One. 2018 Oct 18; 13(10):e0205890.

Doi: 10.1371/journal.pone.0205890.

eCollection 2018.

Hemorrhagic pneumonia in neonatal minks in Greece concomitant with *Leishmania infantum* detection

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In the present study, a severe outbreak of hemorrhagic pneumonia (HP) in neonatal minks concomitant with *Leishmania infantum* (*L. infantum*) detection is reported. The outbreak took place on a Greek mink farm and affected 1,362 mink kits, with 524 dying. Macroscopic lesions of 14 necropsied affected kits were confined to the respiratory system with dark red, consolidated lung lobes and to the small intestine with severe, acute, hemorrhagic and necrotic enteri-

tis. Microscopic examination of lung sections revealed severe hemorrhagic pyogranulomatous pneumonia. Bacteria were obtained in pure culture from the lungs of all necropsied animals and were confirmed as *Pseudomonas aeruginosa* (*P. aeruginosa*). Three out of 14 (21.4%) animals were positive for the presence of *L. infantum* DNA. The outbreak was attributed to the infection of minks with *P. aeruginosa*, possibly as a consequence of being immuno-suppressed by *L. infantum*. Further research is necessary, especially on the pathogenesis of *P. aeruginosa*/*L. infantum* co-infection and the implications of this interaction on HP disease outcome.

Pol J Vet Sci. 2018 Jun; 21(2):405-408.
Doi: 10.24425/122608.

Molecular epidemiology, antimicrobial susceptibility, and pulsed-field gel electrophoresis genotyping of *Pseudomonas aeruginosa* isolates from mink

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Pseudomonas aeruginosa is an important animal pathogen and contributes to hemorrhagic pneumonia in mink. Between April 2011 and December 2016, samples of lung, liver, and spleen were collected from mink with this disease on 11 mink farms in 5 Chinese provinces. From these samples, we obtained 98 isolates of *P. aeruginosa* that belonged to 5 serotypes: G ($n = 58$), I ($n = 15$), C ($n = 8$), M ($n = 5$), and B ($n = 2$); 10 isolates were not typeable (10/98). More than 90% of the isolates formed biofilms, and 85% produced slime. All 98 isolates were resistant to 10 antibiotics (oxacillin, ampicillin, penicillin G, amoxicillin, ceftriaxone, cefazolin, cefaclor, tilmicosin, tildipirosin, and sulfonamide). However, almost all were susceptible to gentamicin, polymyxin B, and amikacin. We identified 56 unique genotypes by pulsed-field gel electrophoresis. These findings have revealed genetic diversity and high antimicrobial resistance in *P. aeruginosa* isolated from mink with

hemorrhagic pneumonia and will facilitate the prevention and control of this disease.

Can J Vet Res. 2018 Oct; 82(4):256-263.

Transfer of amoxicillin to suckling mink (*Neovison vison*) kits via the milk from dams treated orally or intra-muscularly

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Treatment of mink kits with pre-weaning diarrhea (PWD) can be time-consuming and expensive for the farmer, and the efficacy of the treatment procedure may be questioned. Evidence-based treatment protocols for application on affected animals at farms with outbreaks of PWD are lacking. In Denmark, the dams are sometimes treated with amoxicillin, however, it is unknown if it is passed on to the mink kits via the milk. The aim of the present study was to investigate if amoxicillin is transferred via the milk to the kits after oral (PO) and intramuscular (IM) treatment, respectively, of the dam. Moreover, we estimated the concentrations of amoxicillin continuously in serum from the kits up to 8 h after administration. The concentration of amoxicillin was not affected by the route of administration ($P = .64$) and serum reached the highest level after 8 h (34 ng/mL, $CI_{95\%} = [24.3-47.7]$). The serum concentrations of amoxicillin in the mink kits achieved within 8 h were judged too low to exert antimicrobial impact on relevant bacterial species.

Res Vet Sci. 2018 Dec 19; 123:47-50.
Doi: 10.1016/j.rvsc.2018.12.008.
Epub ahead of print.

Induction of Maxillary and Mandibular Squamous Epithelial Cell Proliferation in Mink (*Neovison vison*) by β -Naphthoflavone

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Environ Toxicol Chem. 2018 Dec 7.
Doi: 10.1002/etc.4329. Epub ahead of print.

Diversity of *Anaplasma* and *Ehrlichia*/*Neoehrlichia* Agents in Terrestrial Wild Carnivores Worldwide: Implications for Human and Domestic Animal Health and Wildlife Conservation

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Recently, the incidence and awareness of tick-borne diseases in humans and animals have increased due to several factors, which in association favor the chances of contact among wild animals and their ectoparasites, domestic animals and humans. Wild and domestic carnivores are considered the primary source of tick-borne zoonotic agents to humans. Among emergent tick-borne pathogens, agents belonging to family Anaplasmataceae (Order Rickettsiales) agents stand out due their worldwide distribution and zoonotic potential. In this review we aimed

to review the genetic diversity of the tick-transmitted genera *Ehrlichia*, *Anaplasma* and "*Candidatus Neoehrlichia* sp." in wild carnivores Caniformia (Canidae, Mustelidae and Ursidae) and Feliformia (Felidae, Hyanidae, Procyonidae and Viverridae) worldwide, discussing the implications for human and domestic animal health and wildlife conservation. Red foxes (*Vulpes vulpes*) have been identified as hosts for *Anaplasma* spp. (*A. phagocytophilum*, *Anaplasma ovis*, *A. platys*), *Ehrlichia canis* and "*Candidatus Neoehrlichia* sp." (FU98 strain) and may contribute to the maintenance of *A. phagocytophilum* in Europe. Raccoons (*Procyon lotor*) have been reported as hosts for *E. canis*, *A. bovis*, "*Candidatus Neoehrlichia lotoris*" and *A. phagocytophilum*, and play a role in the maintenance of *A. phagocytophilum* in the USA. Raccoon dogs (*Nyctereutes procyonoides*) may play a role as hosts for *A. bovis* and *A. phagocytophilum*. New *Ehrlichia* and/or *Anaplasma* genotypes circulate in wild canids and felids from South America and Africa. While *Ehrlichia* sp. closely related to *E. canis* has been reported in wild felids from Brazil and Japan, *Anaplasma* sp. closely related to *A. phagocytophilum* has been detected in wild felids from Brazil and Africa. Red foxes and mustelids (otters) are exposed to *E. canis* in countries located in the Mediterranean basin, probably as a consequence of spillover from domestic dogs. Similarly, *E. canis* occurs in procyonids in North (raccoons in USA, Spain) and South (*Nasua nasua* in Brazil) Hemispheres, in areas where *E. canis* is frequent in dogs. While "*Candidatus Neoehrlichia lotoris*" seems to be a common and specific agent of raccoons in the USA, "*Candidatus Neoehrlichia* sp." (FU98 strain) seems to show a broader range of hosts, since it has been detected in red fox, golden jackal (*Canis aureus*) and badger (*Meles meles*) in Europe so far. Brown (*Ursus arctos*) and black (*Ursus americanus*) bears seem to play a role as hosts for *A. phagocytophilum* in the North Hemisphere. *Anaplasma bovis* has been detected in wild Procyonidae, Canidae and Felidae in Asia and Brazil. In order to assess the real identity of the involved agents, future works should benefit from the application of MLST (Multi Locus Sequence Typing), WGS (Whole Genome Sequencing) and NGS (Next Generation Sequencing) technologies aiming at shedding some light on the role of wild carnivores in the epidemiology of Anaplasmataceae agents.

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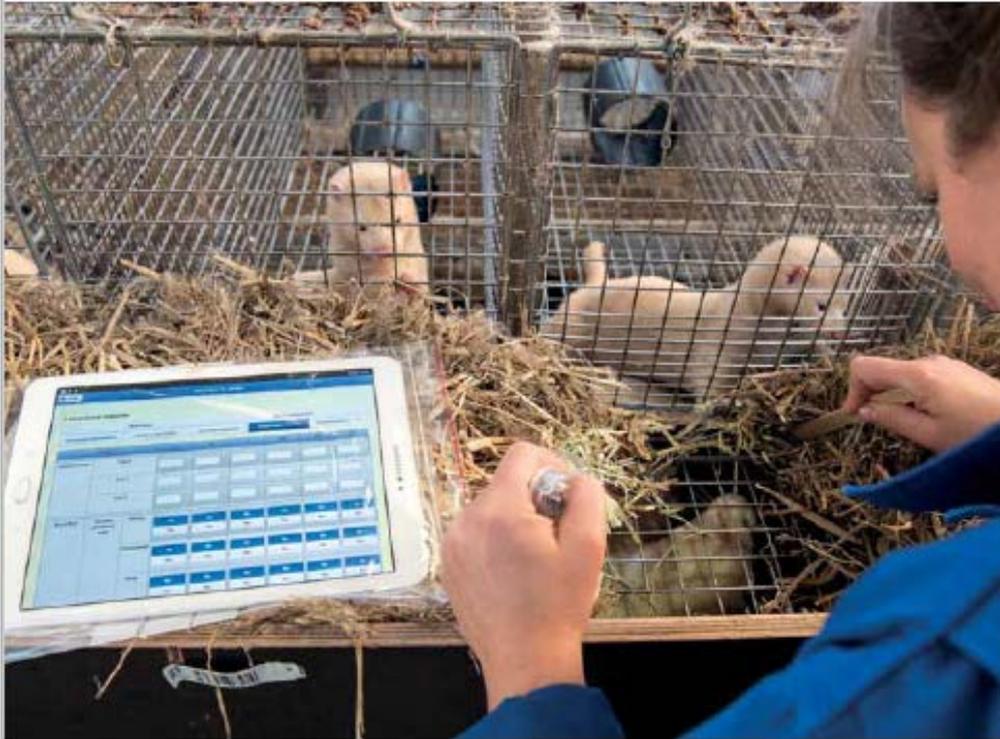
**ON-FARM ANIMAL WELFARE ASSESSMENTS – HANDLING
CHALLENGES OF IMPLEMENTING THE WELFUR-MINK
PROTOCOL ON A LARGE SCALE**

ANNA FELDBERG MARSBØLL

PHD THESIS · SCIENCE AND TECHNOLOGY · 2018



AARHUS UNIVERSITY



PhD thesis by Anna Feldberg Marsbøll

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2018

WelFur-Mink is an on-farm animal welfare assessment protocol for farmed mink. This protocol, and a similar for foxes (WelFur-Fox), is included in an animal welfare assurance scheme for mink and fox pelts produced in Europe. In this scheme, only pelts from farms with an assessed welfare above a defined minimum, which also live up to the industry standards regarding cage size and number of animals per cage and the national legislation, will be sold at the European and North American auction houses.

The WelFur protocols are based on the concept of the animal welfare assessment system Welfare Quality®. In WelFur, the welfare is assessed at farm level based on a range of measurements taken on the farm. In order to take the seasonality of mink and fox reproduction into account, the overall assessment of animal welfare at farm level is based on three assessments, one in each of the three assessment periods. The assessment periods are 1) Winter, 2) Reproduction and nursing and 3) Growth. The measurements are both animal- and resource-based, and most measurements are taken on a sample of the cages with animals on the farm. Each measurement is relevant to some aspect of the welfare according to the four welfare principles with the 12 underlying welfare criteria that were defined in Welfare Quality®. The results of the measurements taken in each assessment period are transformed into scores. These scores are aggregated across the three assessment periods into 12 criteria scores, which are further aggregated into four principle scores. The overall assessment of welfare at farm level is then categorised based on the four principle scores.

The overall aim of this PhD study was to address central challenges regarding the validity, reliability and feasibility of the WelFur-Mink assessment protocol, which became apparent when the protocol was developed and tested. The challenges were addressed in three different parts of this study. The first part had to do with the sampling of cages with mink for the on-farm assessment, and the hypothesis was: *A feasible and unbiased method for taking a representative sample of mink for welfare assessments according to WelFur-Mink can be developed. We expect that samples selected with this method will reflect, but not necessarily be an exact representation of, the farm on which they are taken.* The second part had to do with the possible change in the welfare assessment with date of assessment in the winter and growth assessment periods, and the hypothesis was: *Date of assessment in the winter and growth assessment periods affect the result of some measurements in WelFur-*

Mink. These changes do not necessarily affect the welfare scores or the overall categorisation. The third part had to do with the timing within the day of the observation of stereotypic behaviour in the winter assessment period, and the hypothesis was: The prevalence of stereotypic behaviour in the winter assessment period in WelFur-Mink depends on the time of observation, but it is possible to derive a conversion factor to adjust for the differences.

In the first part, a new sampling method was developed and tested by simulated sampling on a model farm which mimicked a quite large and complicated commercial Danish mink farm. This new method is considered semi-random as it is based on a systematic distribution between sheds followed by a random selection of cages within the respective sheds. This method was developed to replace the original sampling method in WelFur-Mink which was not feasible when used in practice. In addition, there was a risk of assessor bias. The new sampling method was found to be sufficiently feasible. Moreover, assessor bias was avoided as the selection of cages is independent of the individual assessor. The results of the simulated sampling, where the representativeness of the simulated samples was evaluated in regard to factors related to the minks' characteristics and housing environment, showed that the method has no systematic skewness. The results also showed that individual samples selected with this new method cannot be expected to be representative according to all factors. However, the selected samples can be expected to be representative according to some or most factors. Therefore, this method is suggested to balance feasible, unbiased and representative sampling in WelFur-Mink.

In the second part, the possible changes in the WelFur-Mink assessment with date of assessment during the winter and growth assessment periods were investigated based on repeated welfare assessments on eight Danish mink farms. There was a change with date of assessment in the results of some measurements and the related welfare scores, which may in some cases affect the overall categorisation of welfare at farm level. Some changes were positive in regard to the animal welfare while others were negative. Conversion factors may be derived to adjust for these changes at measurement level to ensure the most correct assessment independent of date of assessment. However, the possible variation at European level needs to be taken into account to ensure that the conversion factors can be applied all across Europe.

In the third part, the difference in the prevalence of stereotypic behaviour observed before feeding and before sunset was investigated based on behavioural observations on five Danish mink farms, and the results were compared to the results from all European WelFur-Mink assessments in the winter assessment period 2018. The results showed a large variation in the prevalence of stereotypic behaviour during winter on mink farms in Europe. The prevalence of stereotypic behaviour was found to vary with the timing of the observation and was higher when the observations were carried out before feeding than before sunset. The results also indicated that the relationship between the prevalence of stereotypic behaviour observed before feeding and before sunset varies between farms, thereby making it challenging to derive a reliable conversion factor. Restricting observations

of stereotypic behaviour in WelFur-Mink to be carried out before feeding only (or when feeding is postponed up to 1.5 hours) may be the most feasible and reliable solution until more knowledge about the variation within the day in the prevalence of stereotypic behaviour in the winter assessment period is available.

This PhD study provides examples of how validity, reliability and feasibility may have to be re-evaluated and balanced in order to facilitate a large-scale implementation of an animal welfare assessment system. The challenges addressed in this study can be considered examples of more general challenges of implementing on-farm animal welfare assessment systems on a large scale as there may be similar challenges when assessing animal welfare in other animal production systems.

The effect of 'Easy-strø complete' or straw as bedding material for mink and the use of a netting insert on the quality of the nest and early kit mortality

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Effekten af easy strø complete eller halm som redemateriale til mink samt netruse eller ingen netruse på redekvalitet og tidlig hvalpedødelighed



Åbent projektarbejde

5 ECTS

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Aarhus Universitet

Agrobiologi (Husdyrsundhed – og Velfærd)

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The effect of 'Easy-strø complete' or straw as bedding material for mink and the use of a netting insert on the quality of the nest and early kit mortality

An Aarhus University open project by student Malene Thusgaard Refsgaard, from June 2017

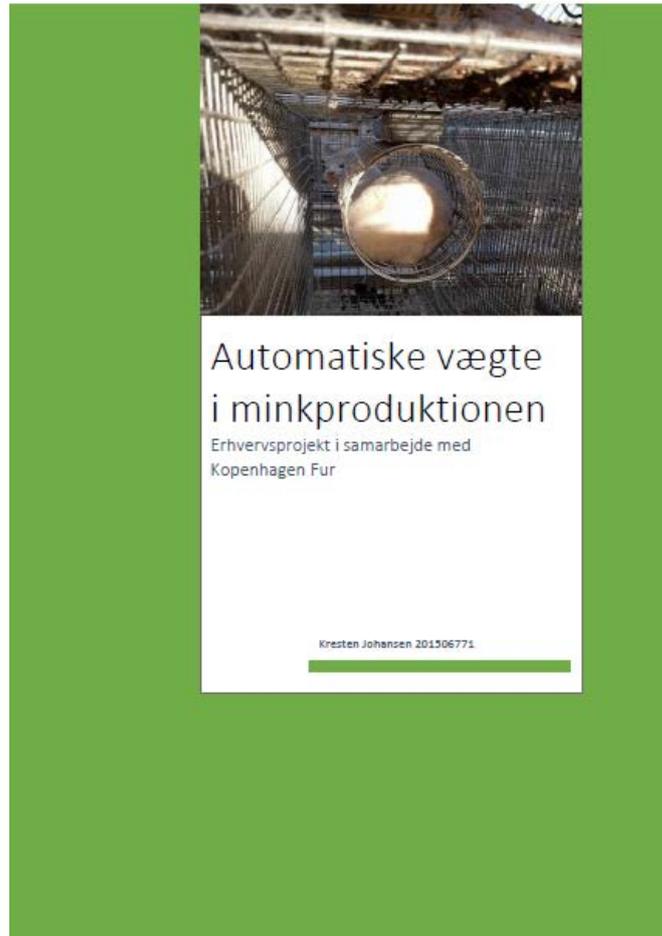
Abstract

This student project set out to test three hypotheses; 1) 'Easy-strø complete' gives the dam a possibility to build a solid and closed nest than straw and this will increase the number of live kits one and seven days after birth. 2) A netting insert can reduce the thermal problems in nest boxes made of plywood. 3) A good nest quality defined as a high score for 'solid and closed nest' before and after birth, will increase the number of live kits. The hypothesis were tested on two private farms with 250-300 mated dams with each type of bedding material and 250-300 with and without netting insert in a cross-over design. The results are based on 1028 dams that delivered a litter. It is concluded that the management conditions (nesting environments) examined do not contribute to an improvement of the dam's ability to carry out early parental care under different Danish management conditions, and thus help

increase kit survival and general welfare seven days after birth. We did not find significant differences between the number of surviving kits from day 1 to seven days after birth, or in kit mortality day 1 to seven days after birth in nest boxes with a netting insert compared to nest boxes without netting inserts. Across the farms, the nest boxes with Easy-strø complete and straw, respectively, demonstrated identical numbers of living kits day 1 after birth, the same kit mortality rate and the same number of living kits day 7 after birth. No significant differences were identified. The major part of all nest boxes had been given a medium nest score, both prior to and after birth, irrespective of type of bedding material and use of netting insert. Nest evaluations demonstrated that the dams were generally good at nest building, which may explain the lack of effect of the factors bedding material netting insert on kit survival and kit mortality. The project illustrated that a nest score of good quality (nest with sides higher than 5 cm) may be defined as an advisable nest. This project further illustrates the obvious need for further studies to support breeders in achieving a reduced kit mortality in mink farms in the future.

Automatic weights in mink production

Kresten Johansen



Automatic weights in mink production

**An Aarhus University business internship project,
in collaboration with Kopenhagen Fur**

By student Kresten Johansen, from December 2017.

Abstract

The project goal was to evaluate the automatic weighings from WEIGHTlog in relation to the timing and amount of activity. Furthermore it was a goal to assess the how the automatic weights were used by the mink in relation to their sex, total weight and individual cages. Finally the weight development was evaluated in relation to the output from WEIGHTlog. The study was based on the full output from WEIGHTlog on September 29th, October 7th and October 30th 2017 from 6 cages with male-female juveniles, and the average daily weights from the whole growth season from July 12th to October 30th. The project has demonstrated that the use of WEIGHTLog provides a wide range of op-

portunities for on-farm monitoring of mink. Evaluations of the weighings registered during a 24-hour period, revealed various activity patterns demonstrating that the mink is primarily active during twilight and has a very active period around feeding. In addition, significant activity at night was identified and, in some cases, during the day. The weight readings were analyzed with a view to determining whether there was any variation in activity on the different weighing dates. No such differences were identified; however, a certain difference in the number of active periods between males and females was demonstrated. Female mink used the weight more than male mink, and a correlation between this use and mink weight was demonstrated. Weight output has been described, but due to the limited data amount, it was not possible to describe general mink weight development. We consider WEIGHTLog to possess a broad range of utilization opportunities that will ease manual work tasks for mink breeders and ensure calmer mink. In addition, it will increase the information on mink that can be used to select breeding animals.

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