### **Abstracts book**



Scientific Committee - Professor Olga Szeleszczuk

Organizing Committee – professor Marian Brzozowski

### MAIN TOPIC: SARS-COV-2 IMPACT ON FUR FARMING

### 1. Henning Otte Hansen

Danish mink industry: Closure 2020-2021

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With the decision on 4 November 2020 to cull all mink in Denmark, a large industry was de facto closed down for the first time. It was also the first time that public health had had such a major influence on a significant political decision. Public health, and assessments and recommendations of the health authorities were decisive in the decisions.

However, because it was the first time this had occurred, it was not possible to draw on past experiences from similar situations. The decision-making process was difficult, and it resulted in a somewhat unstable and messy process:

The strategy for combating was changed several times and took place in five different phases. The estimated cost of the closure increased, and the weighing up of the pros and cons was lacking. A very important justification for the culling, the fear of COVID-19 mutations and, thus, the weakening of future vaccines, was apparently unfounded.

The process led to a number of further discussions including the extent of the cullings, the socio-economic costs, the basis for decisions, the valuation of the income losses, and a possible ban on future mink production.

2. Jenni Virtanen, Kirsi Aaltonen, Ilkka Kivistö, Tarja Sironen

### **Survival of SARS-CoV-2 on Clothing Materials**

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In order to plan and execute proper preventative measures against COVID-19, we need to understand how SARS-CoV-2 is transmitted. It has been shown to remain infectious on surfaces from hours to days depending on surface type and environmental factors. +e possibility of transmission through fur animals and contaminated pelts, along with the safety of those working with them, is a major concern. SARS-CoV-2 can infect minks and raccoon dogs and has spread to mink farms in numerous countries. Here, we studied the stability of SARS-CoV-2 on blue fox, Finn raccoon, and American mink pelt, fake fur, cotton, plastic, faux leather, and polyester and tested its inactivation by UV light and heat treatment. We detected infectious virus up to 5 days on plastic, up to 1 day on fake fur, less than a day on cotton, polyester, and faux leather, and even 10 days on mink fur. UV light failed to inactivate

SARS-CoV-2 on pelts, most likely due to the mechanical protection by the fur. Hence, it should not be used to inactivate the virus on fur products, and its use for other surfaces should also be considered carefully. Heat treatment at 60°C for 1 h inactivated the virus on all surfaces and is a promising method to be applied in practice. +is study helps prevent further spread of COVID-19 by increasing our understanding about risks of SARS-CoV-2 spread through contaminated clothing materials and giving important information needed to improve safety of those working in the production line as well as people using the products.

3. Kirsi Aaltonen, Lauri Kareinen, Ravi Kant, Essi Korhonen, Jenni Virtanen, Johanna Korpela, Jussi Peura, Heli Nordgren, Olli Ritvos, Olli Vapalahti, Tarja Sironen

### SARS-CoV-2 vaccine development for mink

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The recent SARS-CoV-2 pandemic has hit the fur industry especially hard with massive cullings of animals in both The Netherlands and Denmark and most other mink breeding countries affected. Conventional methods of bio-security, and containment have been inefficient in stopping the virus from entering new farms. As a part of Finland's pre-emptive measures, our group began the process of vaccine development. The goal was to protect mink farms from contagion and enable the pandemic to pass without adverse consequences to the fur industry. The project began by comparing immunoresponses to several vaccine candidates. The vaccine chosen for further development is based on a fusion protein which is constructed of the RBD part of the SARS-CoV-2 spike-protein and a mouse Fc protein which is a part of the antibody molecule recognized by immunecells. This acts as an inbuilt adjuvant inciting a more robust response to vaccination. Such fusion proteins are well published and provided a solid base for coronavirus vaccine development. The response was evaluated by testing the specific IgG titers against the vaccine protein by ELISA test. Additionally the neutralizing antibodies were analyzed by a microneutralization test. A follow-up study was conducted by measuring the duration of the IgG molecules in serum for five months. A virus challenge test in mink is under way and will be finalized in June 2021. Methods used to evaluate the vaccine effectiveness include PCR tests from saliva and feces, cell culture methods for infective virus identification, full necropsies with histological examinations and in situ hybridization to identify target organs, tissue types and cells. The IgG levels are extremely high in the test animals and have remained so for the duration of the follow up study. More over the levels of neutralizing antibodies compare favorably to those seen in published studies on human response to the approved SARS-CoV-2 vaccines. The animal experiments are still on going but results will be available for reporting by the date of the conference.

3. Tarja Sironen

### SARS-CoV-2 - a true One Health challenge

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Emerging infectious diseases (EIDs) are increasing due to global changes that have a fundamental impact on the dynamics of infectious diseases, challenging the existing global health infrastructure. As a major part of EIDs comes from animals, a true ability to prepare for EIDs would come from the deep understanding of the factors that drive their emergence, specifically focusing on the complex virus-host interplay. This is particularly apparent in the case of SARS-CoV-2 that is unique in its ability to cross species barriers and cause infection in a many animal species. Basic research studies have suggested that bats are likely the ancestral reservoir host, but the evolutionary history has yet remained an enigma as a multitude of animals has been proposed as potential intermediate or dead-end hosts. SARS-CoV-2 has been isolated from domestic animals, both companion and livestock, as well as in captive wildlife that were in close contact with human COVID-19 cases. However, the domestic mink is the only known animal that is susceptible to a natural infection, develop severe illness, and can also transmit SARS-CoV-2 to other minks and humans. Here we discuss the differences between animal species in their susceptibility to SARS-CoV-2 infection and propose that in order to mitigate this COVID-19 pandemic, a comprehensive evaluation of the transmission of SARS-CoV-2 at the human and animal interface is needed

### SESSION I BEHAVIOUR AND WELFARE

1. Britt I. F, Henriksen, Steen H. Møller

Is it possible to adjust female minks' body condition without compromising the welfare of the mink in the winter season?

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The present project aimed to evaluate the effect of two different feeding strategies for first-year breeding females on different welfare measures. It was hypothesized that adjusting the minks body condition from score 4 or 5 in December to 2 on 20<sup>th</sup> February compared with 31<sup>st</sup> January would reduce the number of stereotypic female mink as well as the amount of stress hormones measured as faecal cortisol metabolites. The two different feeding strategies were tested on 200 brown first-year female mink in each strategy, with half of the animals starting from body condition 4 and the other half in 5. The animals were selected at the end of November, based on weights at selection of breeding animals and body condition score, and the trial started December 10. The statistical analyses were performed in R, with the package lme4 and glmmTMB. There was a significantly lower prevalence of stereotypic behaviour and

a lower amount of faecal cortisol metabolites in the group that was slimmed down to body condition 2 in February compared with January. Besides, non-stereotypic animals in the BCS 4-group had, in January, a lower amount of stress hormones compared with the BCS 5-group. This indicates that larger changes in body condition is more stressful for the animals. We accept our hypotheses and conclude that adjusting mink to body condition 2 on 20<sup>th</sup> February is less stressful for first-year breeding females than adjusting to body condition 2 on 31<sup>st</sup> January. Even so, there are welfare challenges with both feeding strategies, and it remains to be investigated which body condition is needed for first year mink females to be able to respond to flushing. The scientific literature on mink females and flushing are mainly from the 1980. The Danish mink have almost doubled in weight since then, and the need for body condition adjustment might be different today. It is, therefore, a need for new research to find a way to feed female mink in the winter season, to respond to flushing before mating without compromising their welfare.

#### 2. Jens Malmkvist

### Early cross-fostering - dealing with the challenge of large litters in farm mink

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The early transfer of mink kits from large litters to a foster mother with fewer kits is relatively unstudied, although relevant in practice. Therefore, over two birth seasons (2018-19), we studied four factors: (1) the timing of transfer, i.e. cross-fostering D2 or D6 after birth, (2) the dam experience, i.e. first vs. older (second/third year) recipient dams, (3) prolonged early lactation period with 0, 2 or 4 days, by transferring kit to a later born litter and (4) the recipient litter size, i.e. small: 1-3 vs. medium: 4-7 kits. The 2018 study (factor 1 and 2) included 406 kits from large (9-14 kits) litters transferred to 406 foster mother (4-7 kits) litters either D2 or D6 after birth. The 2019 study (factor 3 and 4) included 572 litters (186 sender and 386 recipient litters receiving one kit). The data collection was from birth (D0) to weaning (D56): maternal retrieval of unfamiliar kit at transfer, kit mortality, growth (weight D1, Day of transfer, D28 and D56) and kit damages/wounds 7 weeks after birth. The 2018 study demonstrated that mink dams retrieve the unfamiliar kit quicker into the nest box D2 than D6 (Survival Analysis, SA; P<0.001). Further, the kit weight at weaning (ANOVA) was higher following D2 than D6 transfer to equally aged litters (in avg. +24 g, P=0.032). The 2019 study demonstrated the possibility of successfully prolonging the early lactation period, i.e. 4 > 2 > 0 days in relation to the growth of foster kits until weaning (P<0.001). The newly delivering mink mothers had the quickest accept of an older kit from a large litter, i.e. D2 was better than D4 for the recipients (SA P=0.019). Small recipient litters (1-3 kits) resulted in a reduced occurrence of damages to the kits, particularly true for the vulnerable female kits at week 7 after birth. For both years of study, the growth of kits until weaning was markedly higher in the litters nursed by the older, experienced foster dams (P<0.001). Thus, farmers are recommended to use experienced rather than inexperienced foster mothers during early crossfostering in mink.

3. Taria Koistinen<sup>1</sup>, Hannu T Korhonen<sup>2</sup>. Jaakko Mononen<sup>1</sup>

### The farmers' view of fur chewing in farmed blue foxes (Vulpes lagopus)

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Fur chewing behaviour is not well understood in fur animals. We studied the view of fur farming experts of the factors affecting fur chewing behaviour in blue foxes. A survey questionnaire was made available (via internet or in paper form) to all Finnish fur farmers and major groups of other stakeholders (approximately 800-900 persons) in winter 2018-2019. Of the 71 respondents 93% were farmers, 87% from Ostrobothnia, and 61% had over 20 years' experience of working with blue foxes. Most of the respondents (72%) evaluated fur chewing occurring in less than 10% of blue foxes. Fur chewing was told to appear most often on the tail and hips and, less often in flanks and seldom elsewhere in the body. Fur chewing was evaluated being most serious in January-February. It was regarded as detrimental to the fur quality, but not to the health or welfare of the animal. Over 50% of the respondents evaluated fur chewing to be connected to the season, genetic predisposition, temperament and body condition of the animal. Also factors relating to feed (e.g. appetite, mineral and vitamin content of feed) were considered to be associated with fur chewing (25-50% of respondents). For example, restricted feeding/hunger (80% of respondents), earlier tendency to fur chewing (76%), stress (44%), cold weather/long periods of sub-zero temperatures (39%), agonistic behaviour in social housing units (29%), and stereotypic behaviour (28%) were seen to increase fur chewing. The respondents find it hard to recognise factors that might decrease fur chewing behaviour. The survival in cold weather conditions was evaluated to be worse in the fur chewers than in non-fur chewers, whereas the breeding result was evaluated to be better in the former than in the latter group. Two hypotheses of fur chewing can be formed based on the survey: the feeding- nutrient hypothesis and the behaviour-temperament hypothesis. The former suggests that fur chewing is related to suboptimal management of feeding, hunger or suboptimal nutritive quality of the feed. The latter suggests that fur chewing is related to the behaviour or coping style of the animal.

### 4. Jaakko Mononen, Tarja Koistinen

# A description of the WelFur Finnraccoon on-farm welfare assessment protocol score calculation system and its development

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We describe here the score calculation system of the WelFur Finnraccoon on-farm welfare assessment protocol (WelFurFR) and its development. An expert panel (three scientists, one

veterinarian and two Finnraccoon farmers facilitated by an external animal welfare scientist) was responsible for creating the data, i.e. 'expert opinion in a numerical form'. These data were required in constructing the rules and formulae that convert the original measurement data into the measurement scores (0-100, Step 1) and in aggregating the measurement scores into the criterion scores (0-100, Step 2). A 'Delphi-like method' was used resulting in one shared opinion of the expert group. A weighted sum method with a conditional penalty was selected for the aggregation of the measurement scores to the criterion scores. The aim of the penalty is to reduce the compensation of the lower scores by the higher scores in the aggregation phase. This approach differs from the WelFur fox and mink protocols (published earlier) where the less transparent Choquet integrals are employed for both the weighing the measurements and reducing their compensatory effects. The aggregation of the criterion scores to principle scores (Step 3) is done with the same Choquet integrals as in the fox and mink protocols. Unlike for the foxes and mink, the criterion and principle scores are calculated separately for each of the three data collection periods. This has two major benefits: increased transparency due to fewer layers of calculations, and better usability of the assessment results for advisory purposes. The four overall principle scores are calculated as weighted averages of the period-wise principle scores (Step 4). The winter period has a lower weight than the summer and autumn periods because of the challenges in the data collection resulting from the inactivity of the animals in the winter. The final WelFur classification is then determined from these overall principle scores with the same rule as in foxes and mink (Step 5). The lessons learned in the development of the WelFurFR scoring may help in the revision of the fox and mink scoring systems.

5. Steen H. Møller, Jens Malmkvist, Britt I. F. Henriksen

#### Weaning age and procedures in WelFur-Mink in relation to litter size

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The assessment of weaning procedures in WelFur-mink is based on the scientific knowledge on the subject of which most dates back more than 20 years. Recent studies have shown that litter size is the main risk factor for how the kits in a litter fare in the last part of the nursing period, and that the welfare effects of weaning differs with litter size for dams as well as kits. We therefore hypothesized that weaning procedures focusing also on the number of kits in the litter would more precisely reflect the welfare effects of weaning procedures, compared to the present welfare assessment protocol focusing mainly on age. In 2018 and 2019 we tested the effect of age and procedure of weaning of litters of six or more kits by weaning a number of the kits at six weeks, depending on litter size and weaning the remaining kits at eight weeks, compared to weaning all kits at eight weeks. In 2018 we found that partial weaning at six weeks gave more short-term stress responses after weaning in kits as well as dams, while this was less obvious in 2019. Both years partial weaning also had some unexpected positive long term effects around seven month *post partum*, in terms of more explorative end less fearful

temperament, and less wounds while the effect on fur chewing differed between years. The direct effects of the number of kits before and after weaning will be further investigated and presented.

#### 6. Jussi Peura

### First two years of body mass index (BMI) in Finnish blue fox certification: a case report

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This article presents a case report of the implementation of the body mass index (BMI) into the Finnish blue fox certification system. The results from the first two years of the implementation and the adjustments applied to the BMI calculations are presented. At the beginning of 2018 Fifur implemented the BMI into the Finnish fur certification system and the first certification auditions were performed during autumn 2018 and 2019 by the staff of Kannus Research Farm Luova Ltd. The calculations yielded average body weights for production animals between 14.2 and 14.4 kg for females and between 15.8 and 16.2 kg for males. The results from the breeding animals were found to be lower than those of the production animals with values between 7 and 10.2 kg and between 10.1 and 16.6 kg for females and males, respectively. On the other hand, the average body length did not vary as much between production and breeding animals. The female production animals were found to be from 68.2 to 70.2 cm long and the males between 73.5 and 75 cm. Similarly, the female breeding animals measured 67 to 68 cm while the male breeding animals were 72 to 74 cm long. Consequently, the average BMI of production animals was 20.7 for females and 21.9-22.1 for males while for breeding animals it was 14.7-22.7 for females and 14.8-25.8 for males. Although the average BMI values have not changed much during the first two years, their distribution has changed. The right tail of the distribution (highest BMI) has disappeared. The first two years of the implementation have shown that the BMI is a good measurement of the blue fox fatness and it appears to lead towards the desired direction. After the first year, the auditions were moved two weeks later and the standardization for the BMI of breeding animals was changed. For the coming autumn 2020 new benchmarking tools will be created for advising purposes and the higher limits of BMI will be slightly tightened.

7. M. Díez-León<sup>1</sup>\*, S. Decker<sup>2</sup>, N.Escribano<sup>3</sup>, D. Galicia<sup>3</sup>, R. Palme<sup>4</sup>, G. Mason<sup>2</sup>

### Cage size effect on American mink welfare

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Recent Canadian cage size recommendations for mink increased minimum floor areas since assumed to improve welfare, yet these remain smaller than those mandated in Europe. We tested whether Canadian cages (C) vs. European cages (E) compromised welfare by i) causing long-term behavioural & physiological changes (Exp.1); ii) restricting thermoregulation (Exp.2); & Deing less preferred (Exp.1&Deing). In Exp.1, we housed 64 pairs of unrelated Black females (kept on-farm longest, more at risk of experiencing chronic housing effects) from weaning in E or C. Pairs were split after 3 months & pamp; the remaining 64 females spent 3 more months singly housed in their E or C. Here, we collected data on stereotypic behaviour, fearfulness, & amp; faecal glucocorticoid metabolites. Half these females were humanely killed & amp; the degree of tail-chewing, weight of adrenal glands, thymus & pleen, & mandibular fluctuating asymmetry recorded. The remaining females were given free access to the cage they had not been raised in to assess preference for C or E. In Exp.2, we housed 64 pairs of unrelated, melatonin-implanted Pastel males (largest & growing winter fur in summer, more at risk of heat stress) from weaning in E or C. During 7 hot (>27°C) & tooler (<27°C) days, & on the cage floor, we recorded thermoregulatory behaviour, temperature of 'hot spots' via IR thermography, & the extent to which cagemates were touching. After 5 weeks, we removed half the pairs so that remaining pairs had free access to the cage they had not been raised in in order to assess preference for either type of cage. Our results suggest that singlehoused females in E experience better welfare as they prefer these larger floor areas, particularly when active, were less physiologically stressed & mp; seemed less fearful; & amp; that E allowed paired males to express more thermoregulatory behaviours (e.g. lying being apart from a cagemate adopting spread out postures), with E also being preferred for coresting on hot days. Thus current Canadian floor areas maybe poorer for welfare than European floor areas.

8. <u>Eeva Ojala<sup>1</sup></u>, Maarit Mohaibes<sup>1</sup>, Jaakko Mononen<sup>2</sup>

# The effects of concealment screens and enrichments on behavioural test performance of blue foxes (Vulpes lagopus)

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The blue foxes are typically housed in wire mesh cages furnished with a platform and gnawing object. It has been suggested that the foxes should have also an opportunity to hide. A hiding place may impair the development of an appropriate human-animal relationship (HAR), but this effect might be mitigated by enrichments increasing the predictability and controllability of the environment. We studied the effects of concealment screens (S) and extra enrichments (E) on the behavioural test performance in female blue foxes. In August, 40 adult and 40 juvenile female blue foxes were allocated into treatment groups BN, EN, BS and ES in a two (basic E only = B; basic + extra E = E) times two (no S = N; S = S) experimental

setup. The E groups were provided with two extra enrichments that were changed every second week and received also an edible 'super enrichment' on every Monday. In December, the foxes' behaviour was measured in three dichotomous behavioural tests: the subjective evaluation of HAR (SEH), the stick test (ST) and a catch test (CT). The effects of the treatment and age on the behavioural test performance were analysed with logistic regression. The foxes with the screens explored the stick in the ST and approached the assessor in the SEH less frequently than the foxes without the screens: 73.0% (n = 37) vs. 97.4% (n = 38) (P = 0.011) and 51.4% (n = 37) vs. 78.9% (n = 38) of the foxes (P = 0.015), respectively. For the ST, the effect was confounded by the age and enrichments (S\*E\*Age: P = 0.031): 100% of the adults explored the stick in all the groups (n = 8-10 in a group) except the BS (44.4%) exploring, n = 9), whereas all the juvenile groups responded similarly (70.0-100% exploring, n = 8-10). In the SEH, the BS foxes were the least (36.8%, n = 19) and the BN foxes the most (85.0%, n = 20) probable to approach the assessor (S\*E, P = 0.044). The juveniles in the S groups were more difficult to catch (difficulties with 90.0% in the BS and 70% in the ES foxes, n = 10 and 10) than the juveniles in the N and adults in the S and N groups (0-25%, n = 10) than the juveniles in the N and adults in the S and N groups (0-25%, n = 10). 8-10) (S\*Age: P = 0.001). The foxes with the screens avoided humans, hindering their daily care. The enrichments mitigated only slightly this negative effect.

9. Tarja Koistinen, Jaakko Mononen

#### The structure of the WelFur Finnraccoon on-farm welfare assessment protocol

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Here we describe the on-farm welfare assessment protocol for Finnraccoons (WelFurFR) and its development. The protocol follows the same structure of four welfare principles and 12 criteria as the WelFurFox and WelFurMink protocols. First, the potential welfare measures were selected based on a literature review. The implementation of the early version of the protocol on farms enabled an iterative process in the refinement of the protocol. Absence of prolonged hunger is measured by Body condition and Availability of nutritional fibre, the former evaluating quantitative and the latter the qualitative dimension of hunger. Absence of prolonged thirst is covered by an input based measure of the watering system. Comfort around resting is measured by Opportunity for allohuddling and Resting shelter, since studies show a strong preference for allohuddling and the use of shelters. Protection from exceptional hot weather, Protection from wind and Cleanliness of the fur describe Thermal comfort. Ease of movement is measured by Possibility for horizontal and vertical movement, where the behaviours enabled by these dimensions are evaluated. Absence of injuries is described by Difficulties in moving and Skin lesions. Absence of diseases is measured by the diseases occasionally observed in Finnraccoons, i.e. Bent feet and Diarrhoea, and Other diseases and Mortality (including quality of the data and humanely killed animals out of total mortality). Emergency killing and Killing at farm level describe Absence of pain induced by management procedures. Expression of social behaviour is described by Social housing of juveniles. Expression of other behaviours is measured by Stereotypic behaviour, Fur chewing, Availability of straw, Opportunity to use activity object and Quality of the available area. The

first two are abnormal behaviours, whereas the other three measure opportunities for species specific behaviours. Not all measures are taken in all of the three seasons and not in all types of animals. The categorization, data collection and sampling methods are adapted to the activity level of the animals; no detailed inspection of the animal is done in winter due to the natural lethargy of the Finnraccoons.

10. Jaakko Mononen<sup>1</sup>, Eeva Ojala<sup>2</sup>, Tarja Koistinen<sup>1</sup>

# Blue fox vixens with a proactive coping style have a slightly increased tendency for fur chewing

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Chewing one's own fur is a form of self-injurious behaviour that can be regarded as an indicator of impaired welfare in farmed fur animals. We studied the association between fur chewing and coping style in blue fox vixens. The measurements were carried out in February-March on a private Finnish fox farm in 2017 (n = 660), 2018 (n = 156) and 2019 (n = 577). Each fox was included in the data only once, i.e. in the year when it appeared first time on the farm. The age of the foxes ranged from 1 to 7 years, but the foxes were classified to four age categories (AGE): 1 yr (n = 498), 2 yr (n = 247), 3 yr (n = 307) and 4 yr or older (n = 341). The fur chewing status (FCS) of each fox was measured with the WelFur scale: no (0) or clear (1) signs of fur chewing. The coping style of the foxes was assessed with a 'coping style index' (CSI) based on three temperament tests: exploring (0) or not (1) a stick in the Stick Test, approaching (0) or avoiding (1) the assessor in the Subjective Evaluation of Human Animal Relationship, and eating (0) or not (1) in the Feeding Test. The sum of the three test results was calculated, and finally a dichotomous CSI was used in the statistical analyses. Animals with the CSI sums 0 and 1 were interpreted to have an 'active coping style' (ACS) and animals with the CSI sums 2 and 3 a 'non-active coping style' (NACS). The results were analysed with a logistic regression model where FCS was the dependent variable and CSI, AGE and CSI x AGE the independent variables. CSI x AGE was non-significant (P = 0.31), and it was dropped from the final model. There was a higher percentage of fur-chewers among the ACS (33.9%, 171/504) than the NACS (26.5%, 265/889) blue foxes (P = 0.0012, odds ratio = 1.484). In addition, fur chewing was most frequent in 2 yr animals (35.6%) and least frequent in the 4 yr. or older animals (20.8 %), the two other age groups being intermediate (1 yr. 31.3% and 3 yr. 30.0%) (P = 0.00018). Our results demonstrate a paradox: an active coping style can be interpreted as a positive feature in terms of animal welfare, but it may be associated with an increased tendency for fur chewing.

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11. Marian Brzozowski, Anna Grzeszczak-Pytlak

### Evaluation of human impact on the behavior of the farmed chinchilla

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Chinchillas behaviour is still poorly understood and appears to have a significant impact on breeding results. One of the behavioral issues to be explored is determining the level of confidence of chinchillas in humans. The aim of the study was to establish whether human contact (handling) will influence the behaviour of the chinchilla estimated by using an elevated plus-maze test. The investigations were performed on juvenile chinchillas divided randomly into two groups: the control group (11 animals: 4 females and 7 males) and the experimental group (10 animals: 5 females and 5 males).

The differentiating factor was handling process. This term refers to the direct, regular contact of the animal with the human hand. The behaviour of the animals was evaluated with the use of an elevated plus-maze test. In order to account for the impact of the age of the chinchillas on the test results, the study was performed twice: when the animals were 30 and 240 days old, respectively. The obtained results were subjected to statistical analysis with the use of the Statistica 12. The 30-day animals were more active than 240-day-old animals. There are no relevant data in literature on chinchilla ontogeny, but by analogy to other mammals, it can be surmised that the process of postnatal development of juveniles is similar, which means that differences in physical activity among younger and older chinchillas may be the result of subsequent stages in development. Lower physical activity was found among handled chinchillas, both among 30-day-old and 240-day-old animals. This may be the result of their higher degree of trust toward humans, which would indicate why they did not jump off from the maze during observation, as has been the case with animals from the control group.

In conclusion, it can be said that the process of handling has resulted in changes in physical activity among chinchillas: animals from the experimental group were calmer and more trustful toward humans. A larger degree of physical activity among younger animals may be the result of their stage of development; however, confirmation of this thesis would require further studies.

### 12. Markku Lätti<sup>1</sup>, Tea Elstob<sup>1</sup>, Veli-Matti Tuure<sup>1</sup>, Jussi Peura<sup>2</sup>

### **Fox Farmers in Focus**

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Concerns about the economy and own welfare are common stress factors among fur farmers currently. The aim of the project "Fox farmers in focus" is to improve both profitability and

the welfare of people and animals on fox farms by developing work methods. The project aims to find out restrictive factors of work productivity and of farmers welfare on fox farms as well as to discover targets in work processes that need to be developed. The most essential topics of the project are closely related to the work of entrepreneurs and employees workflow, know-how and workload – as well as welfare of animals. The target is to improve the operating ability of fox farms and to produce practical operation models for developing work. To compile information of fox farming, interviews of advisors, questionnaires for fox farmers as well as work studies are required to be carried out. Identified needs for developing will be discussed in workshops to concretize the topics and to find out practical solutions. The solutions will be reported as information cards and videos as well as introduced in the result seminar. Electronic information channels available for fox farmers will be used to deliver the information of cost-effective and animal friendly operation models. According to the interviews of advisors, essential topics to be developed are time-consuming tasks and tasks that have problems in workflow like catching foxes, cleaning instruments used in insemination, weaning pups and handling of ID-cards. There are also tasks in which the physical workload is high due to lifting of mature animals (for example in insemination), repetitive movements (weaning and skinning) and poor working postures (feeding). The project is carried out in the strong fur farming district of Pohjanmaa in Finland. Information cards and videos will also be available for other fur farmers, industries connected to fox farming and other stakeholders. The project is supported financially by the Rural Development Programme for Mainland Finland 2014–2020 and by the Finnish Fur Breeders' Association (FIFUR) and carried out by FIFUR and TTS Work Efficiency Institute in 2021-2022.

13. Jens Malmkvist<sup>1</sup>, María Díez-León<sup>2</sup>, Janne Winther Christensen<sup>1</sup>

### Running wheel activity in mink with different forms of abnormal behavior

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Understanding animal motivation, stress responses and abnormal behaviour is important for pursuing good welfare in husbandry. In farm mink, fur chewing and stereotypies are main types of abnormal behaviour. Stereotypies can take many different forms – such as repeated pacing, licking, and head-twirls – but many studies and the WelFur protocol pool these together. However, it is currently unknown if these different forms of stereotypic behaviour share a similar motivational background and have equal welf

are significance for the mink. As part of a larger study addressing this issue, we investigated whether free access to a running wheel would substitute, i.e. reduce the prevalence of, the various forms of stereotypic behaviour. We screened in 2019 c. 1100 Palomino and Brown mink dams (born 2016, 2017, 2018), individually housed at the AU-farm, into six groups based on their behavioural phenotype: 1. Fur-chewing (n=14), 2. Pacing stereotypic behaviour

(SB) (n=16), 3. Stationary SB (n=15), 4. Licking SB (n=11), 5. Mixed SB (n=14) and 6. Control, free from abnormal behaviour (n=18). In total, 88 mink were transferred to cages with running wheel access until day 10. We analysed automatically recorded running wheel activity as rounds per days (rpd, i.e. per 24h) using repeated measures mixed ANOVA. The running-wheel naïve mink dams started more or less immediately to use the running wheels (avg. 960 rpd on the first day, 1025 rpd on day 10). There was a considerable variation in running wheel activity between mink, with the younger dams being most active (P<0.001). The major finding was that running wheel activity differed between groups with pacers, the stationary and mixed SB groups using the running wheel significantly more (1474, 1404 and 1753 rpd, respectively) than the other groups (P<0.001; avg. 336-467 rpd). Thus, different forms of abnormal behaviour influence the running wheel activity in mink. Results on the effects on stereotypic behaviour will be presented.

### **SESSION II: BREEDING, GENETICS & REPRODUCTION**

<u>1. Andrey Manakhov<sup>1</sup></u> Tatiana Andreeva<sup>1</sup>, Maria Mintseva<sup>1</sup>, Igor Andreev<sup>2</sup>, Lev Uralsky<sup>1</sup>, Oleg Trapezov<sup>3</sup>, Evgeny Rogaev<sup>1</sup>

# Identification of molecular genetic factors linked to phenotypic diversity fur colours in American mink (Neovison vison) by whole genome sequencing analysis

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American mink is the most numerous member of Mustelidae family, it has an important role in human economic activity, and characterized by several unique biological features perspective for fundamental studies. Extremely wide range of fur colour variations made mink the most popular species in the world fur industry, accounting for 80% of international trade in unprocessed fur. To date, at least 35 mutations affecting fur colour have been reported. However, only a few of mink's fur colour phenotypes were characterized at molecular-genetic level. Our study, to the best of our knowledge, is the first to perform whole genome sequencing of American minks with three distinct four colours: Silverblue, which was the first colour mutation, described in mink, Hedlund white that is a commercially valuable phenotype that can be dyed easily, Moyle that is member of wide range brown phenotypes and standard dark brown. All phenotypes are inherited as Mendelian autosomal recessive trait. Using whole genome comparison analysis, we identified mutations in splice donor sites of MLPH (c.901+1G>A) and MITF-M (c.33+1G>A) genes in Silverblue and Hedlund white minks respectively. On the mRNA level, we confirmed that these mutations lead to splicing impairments and shift in open reading frames of MLPH and MITF proteins. In Moyle minks we identified 2 frame shift mutations in RAB38 gene (c.20-21dup, c.574-589del). The MLPH

gene, especially its melanocyte-specific isoform MITF-M, is well known to be critical for the development of neural-crest-derived melanocytes. RAB38 gene encode protein which participates in the transport of newly synthesized tyrosinase and TYRP1 in maturing melanosomes. Taking all data together we suggest that mutations MLPH c.901+1G>A, MITF-M c.33+1G>A and RAB38 c.20-21dup / c.574-589del are causative for the commercially valuable Silverblue, Hedlund white and Moyle fur colour phenotypes respectively. Results of our study provide novel data and pipeline for molecular genetics characterization of American mink fur colours diversity. Moreover, our data can contribute to improving world mink fur production through selective breeding.

### 2. Bruce D. Murphy:

### The Contribution of Molecular Biology to Improving Mink Fertility

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Successful reproduction is an essential element for the success of husbandry of the mink. Physiological studies over the last century have provided information about the processes of both male and female reproduction in this species that have been valuable and pertinent to increasing productivity. The emergent molecular technology that has the potential to greatly amplify our knowledge of all aspects of reproduction has not yet been much exploited in the mink. The recent completion of the draft mink genome sequence will allow for valuable new studies. Further, transcriptomic methods applied to discovering the role of the uterus in regulating embryo development have revealed significant new information about early gestation. Similar transcriptomic analysis has provided novel information about developmental arrest of the mink embryo this is pertinent to reducing prenatal mortality. In overview, application of the molecular tools that have become available in recent years has unprecedented potential to understand and improve mink fertility.

### 3. Riitta Kempe<sup>1</sup>, Ismo Strandén<sup>1</sup>, Jussi Peura<sup>2</sup>

### Early selection of leg conformation in Finnish blue fox (Vulpes lagopus)

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It is recommended that blue foxes should be selected for breeding early in life based on their estimated breeding values. The main aim of this research was to investigate the early selection, genetic associations and suitability of two leg conformation traits, carpal hyperextension (LEG) and valgus deformity (VALGUS), for introduction into the breeding

scheme. Studied traits were assessed twice: at weaning (July-Aug) and grading time (Oct-Nov). Foreleg carpal joint angle was used as an indicator of carpal hyperextension. Valgus deformity (VALGUS) was a new studied leg conformation trait. The experiment was carried out on five commercial fur farms in Finland. Data were collected from altogether 3299 blue foxes in 2018. Variance components were estimated with DMU program, which relies on the restricted maximum likelihood (REML) method. Both single and multiple trait animal models were used. Results showed that carpal hyperextension (LEG1) occurred at an early age (5-14 weeks) in 44% of slim Finnish blue fox puppies. LEG1 had moderate heritability (0.20) and fairly high genetic correlation of 0.40 with LEG2, which was assessed at the time of grading. Heritability estimate of LEG2 (0.06) was lower than in LEG1. The frequency of VALGUS was quite low, 6 to 7%, in the data. Also, the heritability was low, close to zero in VALGUS1 and 0.11 in VALGUS2. Genetic correlation between the two VALGUS traits was moderate (0.27). Obtained genetic parameters indicated that genetic improvement of LEG through early selection has potential to improve carpal hyperlaxity of blue foxes. The evaluation and selection of valgus deformity is more complex. Yet, VALGUS2 has some genetic variance, suggesting that ideal evaluation and selection time point is at later growth phase. Selection against VALGUS and LEG is highly recommended in order to improve animal welfare. Animals with poor leg conformation should not be used for breeding. If more efficient selection is needed, the selection index and multiple-trait animal models can be applied in breeding for better leg conformation

### 4. Sonja Gerke:

### WebSampo, a breeding program for Finnish fur farmers

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Finland has a long tradition as a fur farming country. In the 80's there were over 5500 fur farms. Most of the farms are nowadays located in Ostrobothnia in the Western part of Finland. The first old breeding programs were introduced in the 1970's. A program called Visual Sampo was introduced in the 1990's.

In 2013 Saga Furs introduced a new web-based application called WebSampo which works with foxes, minks and finnraccons. In 2014 the possibility to count national indexes for foxes with the MIX99 was implemented. The indices are standardized and there is multiple trait evaluations of the fertility traits. There is also estimated breeding value accuracies. The national value calculation tells the genetic level of the animal and makes is possible to compare indexes directly between the fox farms. The value calculating program MIX99 is a Finnish program developed and owned by Luke Natural Resources Institute Finland. MIX99 can also be used for genomic evaluation and is used for counting indexes for several other animal species also. The value calculation is counting several different indexes for fertility traits, like mating success, whelping success and litter size. Grading traits of live animals and pelt traits like skin size, quality, colour and even behaviour of the animal. Animal cards for the breeding animals and puppies can be ordered through the program and also puppy cards that have a barcode ticket. The barcode ticket allows follow-up of every individual skin with

quality information and skin prices. The most important reason for the farmers to use the program is to follow-up the development of the skin quality. Especially during times with a lot of challenges the program is a big help, because it is possible to follow which breeding animals have produced high class offspring and pelts. There is also a new application called Sampo Feeder for individual feeding. An interface to WebSampo is available whereby feeding data is transmitted to the WebSampo database. The WebSampo program has a lot of different farm statistics available. The farmer can also compare his own results with the whole country. Data from WebSampo has been utilized in several Master science projects (University of Helsinki).

#### 5. Zhang

# Epidermal growth factor promotes proliferation of mink's dermal papilla cells via Notch signaling pathway

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The effect of epidermal growth factor (EGF) on the development and growth of hair follicle is contro-versial. In the present study, 2-20 ng/ml EGF promoted the growth of mink hair follicles in vitro, whereas 200 ng/ml EGF inhibited follicle growth. Further, dermal papilla (DP) cells, a group of mesenchymal cells that govern hair follicle development and growth, were isolated and cultured in vitro. Treatment with or forced expression of EGF accelerated proliferation and induced G 1 /S transition in DP cells. Moreover, EGF upregulated the expression of DP mesenchymal genes, such as alkaline phosphatase (ALP) and insulin-like growth factor (IGF-1), as well as the Notch pathway molecules including Notch1, Jagged1, Hes1 and Hes5. In addition, inhibition of Notch signaling pathway by DAPT significantly reduced the basal and EGF- enhanced proliferation rate, and also suppressed cell cycle progression. We also show that the expression of several follicle-regulatory genes, such as Survivin and Msx2, were upregulated by EGF, and wasinhibited by DAPT. In summary, our study demonstrates that the concentration of EGF is critical for the switch between hair follicle growth and inhibition, and EGF promotes DP cell proliferation via Notch signaling pathway.

#### 6. Younes Miar

## Enhancing production and Aleutian disease resilience in mink through advanced genomics

### Dalhousie University

The fur industry is one of the oldest and the most historically significant industries in Canada. The industry has used American mink (Neovison vison) as the major source of fur for decades because of their high-quality fur and wide range of colours. This project will seek to (1) create the first accurate whole-genome sequence assembly of mink using next-generation sequencing technology to help understanding the biology and evolution of the order Carnivora, (2) design a robust and informative SNP assay (50K) for genomics discovery in mink, (3) discover genome structure and signature of selection as well as identify new genetic

variants explaining variation in economically important traits, and (4) identify the genetic relationships among these economically important traits including feed efficiency, Aleutian disease resilience, fur quality, reproductive performance, growth rate and pelt size. One hundred mink DNA samples from the Canadian Centre for Fur Animal Research at Dalhousie Agriculture Campus (Truro, Nova Scotia), and one breeding population (Millbank Fur Farm Limited, Rockwood, Ontario) were sequenced using next-generation whole-genome sequencing to high coverage (>30x) to create the first SNP assay for American mink. A DNA panel composed of 100 American mink from 5 color-types were assembled to identify the most homozygous individual as the reference animal for whole-genome sequence assembly development. The phenotypic data and DNA samples from 3,323 animals were collected and these mink will be genotyped using the customized assay for designing a marker assisted selection (MAS) approach and assessing the potential of genomic selection (GS) in mink production systems. The ultimate objective is to develop the new tools for implementation of MAS or GS in mink breeding programs for development of superior, highly efficient, and healthy animals. This approach will help improve the overall performance of the North American mink industry, which is now in difficulty due to several economic factors such as the high price of feed, declining price of fur and prevalence of diseases.

7. Cepica  $A^{1*\S}$ , Anistoroaei  $R^{2\S}$ , Iwamoto  $T^{I}$ , Christensen  $KA^{2\S}$ .

# Genes enhancing disease resistance in Aleutian mink disease virus (AMDV) infected American mink (Neovison vison)

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Two groups of serologically confirmed AMDV infected mink were used to analyze the association of Single Nucleotide Polymorphisms with Aleutian disease (AD) resistance. Group I (n=97) comprised diseased animals (AD susceptible /ADS/). These animals exhibited disease indicated by hyper-gammaglobulinemia with the lowered albumin: IgG ratio (A: IgG), Group II (n=97) included healthy, disease resistant animals (AD resistant /ADR/) with A: IgG ratio indicative of undisturbed homeostasis. The phenotypic assignment into the groups was done according to the MALDI-TOF A: IgG ratio in AMDV infected mink. Illumina Hiseq 2500 sequencing was used to produce sequence libraries, which were biocomputationally analyzed. The biocomputational analyses led to identification of genome-wide spread SNPs and their association with the ADS and ADR groups of animals. There was a clear overdominance for the GATOR complex protein NPRL3 isoform X6, with a very strong effect on the differences between the animals of ADS and the ADR groups. A minor effect, scattered over more genes in this area around the HLA complex, was also observed. The Protocadherin Fat 3 (FAT3), or its vicinity, could also be regarded as the candidate area influencing the

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outcome of the infection. In the light of the virus eradication and immunization failures, the results provide the basis for the development of the genomic test for the AD resistance, as the novel selection tool in the breeding for the disease resistance program. Additionally, the results could potentially be useful in elucidation of the genetic basis of resistance to the animal viral diseases where hypergammaglobulinemia is similarly a significant component of

### 8. Zongyue Liu:

# Effect of FSH $\boldsymbol{\beta}$ and NCOA1 gene polymorphisms and expression on pink eyed white mink reproductive traits

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The present study was designed to investigate comparative expression of follicle-stimulating hormone beta subunit (FSHβ) and nuclear receptor coactivator 1 (NCOA1) genes by real-time PCR (RT-PCR to detect the polymorphisms in FSHβ and NCOA1 genes, using Polymerase Chain Reaction- Single -Strand Conformation Polymorphism (PCR-SSCP) methods to investigate the effects of gene polymorphisms on reproductive traits including total number of kits born (TNB) and number of born alive (NBA) in pink eyed white mink. Four SNPs were identified RT-PCR to detect the polymorphisms in FSHB and NCOA1 genes, using PCR-SSCP methods to investigate the effects of gene polymorphisms on reproductive traits including total number of kits born (TNB) and number of born alive (NBA) in pink eyed white mink. Four SNPs were identified in the FSHB and NCOA1 genes, including g.1228G>A, g.1866T>C, g.151536T>C, and g.185162C>T. The g.1228G>A polymorphism of FSHB was associated with NBA and TNB (P<0.01). The g.151536T>C polymorphism of NCOA1 was associated with NBA and TNB (P<0.01). RT-PCR analysis indicated that the FSHβ and NCOA1 genes are expressed in the hypothalamus, pituitary, uterus, and ovary over different periods. NCOA1 mRNA levels in hypothalamus, ovary, and uterus during the first half of gestation were higher than during the middle term and last half of gestation (P<0.01). FSHB mRNA levels in the hypothalamus and uterus were higher during the first half of gestation than during the middle term and last half of gestation (P<0.05). In conclusion, the g.1866T>C polymorphism of FSHβ and the g.151536T>C polymorphism of NCOA1 could be molecular markers for reproductive traits, and expression of FSHB and NCOA1 might be involved in the regulation of embryo attachment mechanisms in pink eyed white mink breeding.

9. Olga Szeleszczuk, Magdalena Denysenko, Piotr Niedbała, Dorota Maj, Marta Kuchta – Gładysz, Anna Grzesiakowska

# Polymorphism of milk proteins in the silver fox (Vulpes vulpes) and the finraccoon (Nyctereutes procyonoides) – preliminary research

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Milk proteins comprise a non-uniform group of compounds different in terms of composition and properties. Protein compounds of milk are divided into two basic groups: casein and whey proteins. Each milk fraction has a different content of basic elements, a specific composition of amino acids, molecular mass and properties. Polymorphism of casein and whey milk proteins is fully understood in most animals species, however, there is lack of information about polymorphism of proteins in carnivorous fur animals. 12 milk samples of both species were selected for the separation. Milk was collected in various phases of lactation. To separate the milk proteins, poliacrylamide gel electrophoresis (PAGE) in the presence of denaturating SDS, was used. The SDS-PAGE electrophoresis in a Laemmli system (1970) (following: Gallagher 2000) was conducted with our modification.

In comparison to the other species, milk of red fox females is characterized by the profile of casein proteins. The fraction of  $\alpha$ -casein was not revealed in fox females milk, whereas, in raccoon dogs, this fraction was stated in trace amounts in the  $1^{st}$  and  $2^{nd}$  phase of lactation. In the milk of both canids,  $\beta$ -casein prevails between casein proteins. There are more whey proteins in the tested species which induces occurring a greater amount of functional substances of immunoglobulins as well as lisozyme and lactoferrin. Higher content of particular fractions of whey proteins can point to the presence of inflammation taking place in a milk gland. The content of lisozyme in milk of foxes and raccoon dogs remains on a relatively high level in contrast to milk of the other females of domestic animals.

10. Duy Ngoc Do, Guoyu Hu, Siavash Salek Ardestani, Younes Miar

### Estimation of genetic parameters of growth curve parameters in mink

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Understanding the genetics underlying growth curve is important for selection of animals with better growth potential. Non-linear model approaches have succeeded to describe the growth curve and estimated growth parameters, which are useful in genetic selection. However, little is known about the genetics of growth curve parameters in mink. This study compared the

performance of six common-used growth models (Gompertz, Logistic, Brody, Richards, Brigets and Janoschek) and then estimated the heritability and genetic correlations for parameters derived from the best-fitted model. For this purpose, individual body weights (BW) of 1,026 mink measured seven times in three-week interval (from week 13 to week 31 of life) were used for assessment of model performance. Based on the Akaike's information criterion (AIC), Richards was the best model to describe the growth curve in mink. Variance components and heritability of traits were estimated using univariate models while phenotypic and genetic correlations were estimated from the results of a series of bivariate models using the REML method implemented in the DMU package. Univariate models indicated that sex, color-types and birth year had significant effect (P < 0.05) on asymptotic weight ( $\alpha$ ), constant growth rate (β), growth rate at mature (k), shape parameter (m), weight at inflection point (WIP), age at inflection points (AIP) and absolute growth rate (AGR). The estimated heritabilities ( $\pm$ SE) for  $\alpha$ ,  $\beta$ , k, m, WIP, AIP and AGR were 0.21 $\pm$ 0.08, 0.15 $\pm$ 0.08, 0.07 $\pm$ 0.07,  $0.20\pm0.08$ ,  $0.33\pm0.09$ ,  $0.10\pm0.08$  and  $0.15\pm0.07$ , respectively. Significant positive genetic correlations (P < 0.05) were estimated between α, k, m, WIP and AIP. Positive genetic correlations were also observed between the estimated growth parameters and different measures of BW. Strong positive genetic correlations between α and different measures of  $BW(r_g = 0.65-0.98)$  indicated that asymptotic weight can be used in genetic selection of BW in mink. Overall, the results suggested that growth curve parameters are heritable and can respond to the genetic/genomic selection for optimizing the growth performance in mink.

11. Anna Grzesiakowska, Marta Kuchta-Gładysz, Olga Szeleszczuk, Katarzyna Jarguz

Skeletons morphometrics of the farmed types of blue fox (Alopex lagopus); preliminary study.

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Breeding work carried out over the years in the Polish breeding of the blue fox, involving the introduction of new types of the blue fox from the Polish type, through Norwegian, to Finnish, has contributed to a significant increase in the body weight of animals. This increase automatically caused anatomical changes in the fox organism, especially in the bone system. Determination of changes in skeletal structure and comparison of the structure of examined animals with each other is possible by zoometric measurements. The aim of the study was to determine the morphometry of the skeletal system in the blue fox, Polish, Norwegian and Finnish type. The preliminary study was carried out on 8 animals from Polish farms, and the material was obtained from 2007, during fur slaughter. The bodies were cleaned of muscle and treated with 3% water solution of NaHCO3. After complete bone cleaning, they were bleached in perhydrol solution and then dried. Morphometric measurements were taken using a slide caliper with an accuracy of 0.1 mm. Among other things, the height and width of the

skull, body length, length of the front and hind legs and the length of individual sections of the spine were subjected to zoometric analysis. In almost every analyzed parameter mainly bone length and spinal segments, the highest average measurements were of Finnish type and the lowest of Polish type. Intermediate values, often similar to the Finnish type, were recorded in the case of the fox of the Norwegian type, which coincided with the variation in body weight. The greatest morphometric changes of the skeletal system were found in foxes of the Finnish type, where, despite the largest bone sizes, they were the most delicate. The measurements of skeletons of Polish and Norwegian foxes did not differ significantly from each other and were more compact. Morphometric tests of the skeletal system of various types of the blue fox may allow to assess the correctness of breeding work and animal health.

12. Knud Christensen, Mette Jull Jakobsen, Merete Fredholm

### DNA specific repeats in mink and some related species

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Repeats are found in all types of mammals. Some occur in all mammals as for instance SINE and LINE. Some occur only in closely related species. The repeats are more widely spread among species the older they are. In this study, the occurrence of 3 repeats specific for the mink and closely related species is investigated. Two come from the NCBI seq. KU186801.1, which can be divided in a first part "m-repeat" of 374 bp and a last-part "virus" of 274 bp related to rat sarcoma virus, and finally a b-locus repeat found in an insert in the mink b locus responsible for brown cote colour. The material investigated are genomic DNA from 6 domestic mink, 2 trapped mink from North Wisconsin, a ferret an otter and an ermine. DNA from all some of the animals was sequenced on the Illumina HighSeq 2000 platform to the depth of XXX. The sequence data was investigated using the freeware BOWTIE, and BLAST was used to investigate the NCBI sequences. While the m-repeat and the b-locus repeat were never completely linked, the m-repeat and the virus repeat were found to be linked in several loci. The results showed that one of the trapped mink had had contact with domestic mink, whereas the other had an extremely high number of the virus repeat compared with the domestic mink. Only the m-repeat was occurring very seldom in the ferret and the ermine, none of the repeats was found in the otter. There were a substantial variation in the occurrence of the 3 types of repeats between the 6 domestic mink. All the 3 repeats have existed in the wild North American population. New studies are needed to determine the insertion rate and the selection pressure on the new insertions. No doubt, these new insertions create new genetic variation, which enhance selection response.

13. Stanisław Łapiński, Sylwia Pałka, Weronika Pudło

## Effects of supplementation with tyrosine and phenylalanine on the colour type and behaviour of chinchillas

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Most chinchillas have a beautiful blue-grey colour of fur, shaded darkest on the back with a lighter grey on the sides and white on the belly. However, dark coloured chinchillas are usually more appreciated than lighter ones. This is due to the methods of assessing animals, when they get a better rating for the darker colour type and thus have a higher value as breeding animals. The aim of the study was to verify whether increasing the content of tyrosine (Tyr) and phenylalanine (Phe) in granulated feed will affect the more intense colouring of chinchilla fur and also (as a noradrenaline precursor) will affect the chinchilla reaction to the hand test. The four-month-old chinchillas were divided into 3 groups (n=18 each): G-1 - control, fed with a complete commercial fodder (Tyr - 6. 91 mg/g, Phe - 7. 45 mg/g); G-2 - received a feed consisting of commercial and experimental fodder (50% to 50%); G-3 - was fed with experimental fodder (Tyr - 9. 31 mg/g, Phe - 16. 65 mg/g). During the experiment the colour type of fur was assessed by two techniques: objective, using the CR-410 colorimeter (CIE L\*a\*b\* colour space) and subjective, by a qualified chinchilla judge. A behavioral hand test was used in categorizing responses of chinchila towards human intrusion into their cage. The results of the colorimetric evaluation showed statistically significant differences only in the a\* value (green "-" to red "+") between the G-3 (0. 15  $\pm$  0. 10) and the other two groups (G-1: 0. 28  $\pm$  0. 16; G-2: 0. 27  $\pm$  0. 11). However, subjective evaluation did not show statistically significant differences in the color type of chinchilla from particular groups. In contrast, animals from the experimental group were better rated in trait of "size and structure" compared to the control group. The study using a hand test showed no differences in chinchilla behaviour. Animals fed with experimental food with higher Tyr and Phe content were larger, they differed less from each other, in the colorimetric evaluation their fur color was "less red" than in the others, but this was not noticeable in the organoleptic assessment.

14. Guoyu Hu, Duy Ngoc Do, Janine Gray, Karim Karimi, Younes Miar

### Genetic and phenotypic parameters for Aleutian disease tests and their correlations with reproduction and fur quality traits in American mink

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Aleutian disease (AD) brings huge financial losses to the mink industry. Several AD tests have the potential to be used for genetic selection of AD resilience, but little is known about their genetic and phenotypic parameters. Data on 1,979 individuals from the Canadian Center for Fur Animal Research at Dalhousie Faculty of Agriculture were used to estimate the

genetic and phenotypic parameters for AD specific tests of quantitative enzyme-linked immunosorbent assay (qELISA, n=2,162) and counterimmunoelectrophoresis (CIEP, n=1,127), non-specific AD test of iodine agglutination test (IAT, n=1,497), health test of packed cell volume (PCV, n=1,501), reproductive performance of number of live kits at birth (LB, n=1,073), live grading of fur quality (LFQ, n=930), and live grading of fur nap (LFN, n=929). Significance (P<0.05) of fixed effects (sex, year, age, colour-type, and test month), and random additive and permanent environment effects were determined by univariate analyses in ASREML 4.1. The genetic and phenotypic parameters for all traits were estimated under bivariate analyses using ASREML 4.1. Estimated heritabilities (±SE) were 0.43±0.05 for gELISA, 0.22±0.06 for CIEP, 0.19±0.05 for IAT, 0.22±0.05 for PCV, 0.08±0.02 for LB, 0.35±0.07 for LFQ, and 0.52±0.06 for LFN. The qELISA had significant positive genetic correlations with CIEP (0.41±0.11) and IAT (0.61±0.08), and a significant negative genetic correlation with PCV (-0.49±0.08). The qELISA showed low negative genetic correlations with LB (-0.23±0.13), LFQ (-0.05±0.10), and LFN (-0.18±0.09). The CIEP had low negative genetic correlations with PCV (-0.03±0.17), IAT (0.28±0.15), LFG (-0.09±0.17), LFN (-0.19±0.14), and LB (-0.21±0.22). The high heritability of qELISA and its significant genetic correlations with LB, PCV, and LFN suggested that qELISA can be used as an indicator of AD resilience for implementing genetic/genomic selection in mink populations.

15. Marta Kuchta-Gładysz<sup>1</sup>, Anna Grzesiakowska<sup>1</sup>, Olga Szeleszczuk<sup>1</sup>, Katarzyna Rymuza<sup>2</sup> Ewa Wójcik<sup>3</sup>

# Identification of chromosome instability in interspecific hybrids (Alopex lagopus x Vulpes vulpes)

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As a result of inter-species crossbreeding, new genetic variability is created, but also aberrations occurring in parental species are mixed or accumulated. In the case of farm foxes, such variability of the karyotype is caused by a centric fusion (chromosome aberration) commonly referred to robertsonian translocation in blue fox, and in the case of the silver fox, the presence of chromosomes B. One of the methods used to determine the influence of such endogenous factors is the fragile site test (FS), which belongs to the group of diagnostic instability tests with chromosome marker. The aim of the study was to determine how the presence of centric fusion and B chromosomes affects the stability of the karyotype of crossbreeding blue and silver fox using the fragile site test. The study was performed with 12 farmed interspecific hybrids (Alopex lagopus x Vulpes vulpes), including 6 males and 6 females in the same age. Twenty complete and well-spread metaphase plates were analysed per animal for karyotype control. For mutagenesis assay ffty complete metaphases per animal

was analysis. The parameter tested was the number of identified chromosomal damage as Fragile Sites (FS) in the form of chromatin gaps, breaks and deletions in the chromosomes. Cytogenetic analysis showed that the number of chromosomes A in the hybrid karyotype ranged from 33-45, while the number of chromosomes B ranged from 1-3. The total number of fragile site (FS) was 131.67±66.09, with the number per cell equal to 3.26±0.89. The most common form of fragile site was the number of breaks in the study group of 110.33±55.40 and the least number of deletions 12.25±14.29.

### SESSION III: ENVIRONMENTAL IMPACT OF FUR FARMS

1. Henning Otte Hansen:

#### Changes and megatrends in global fur business

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The fur industry is a part of very global value chains: The production of raw fur skin and the fur farms are typically located in Europe and North America. The fur processing (tanning, dressing etc.) as well as the fur garment industry (sewing etc.) are typically located in lowwage countries. The international trade and specialization is very large and advanced, which makes the whole industry and the integrated fur value chain competitive In recent decades, the international trade pattern in both raw fur skin and fur garment has changed. Central and eastern European countries have become increasingly important as producers of raw fur skin. Fur garment industry has to a high degree moved from Europe to Asia – in particular China. The picture of Asia as very important importer of raw fur skin, and as manufacturers of fur garment is also changing. Until few years ago, Hong Kong imported a significant share of world raw fur skins. Typically, Hong Kong was a transit country, where China was the final destination market. Subsequently, China became the direct market for export of raw fur, while Hong Kong lost importance. In the most recent years - from 2015 - an increasing share of raw fur skin exports to Asia has been directed to a number of countries, which can be called "New Asia". This group consists of countries such as Cambodia, Vietnam, Malaysia and Thailand Cambodia in particular has become a large market and is now the second largest export market for Danish raw mink skins. In most cases, the skins are purchased by Chinese, who ship the skins directly to their processing companies, which now increasingly have moved from China to these new Asian countries. Thus, from an overall and historical perspective, three waves can be seen: In the first period, the fur garment industry was located in Europe. In the next period, it moved to China. For an upcoming period, it seems to move to other Asian countries Apparently, the fur clothing industry follows the same pattern as the textile industry, which has also moved to countries with lower labor costs. The new countries will typically

only be processing countries and will not significantly demand fur garments.

2. Minna Sarvi, Tapio Salo, Kimmo Rasa, Sari Luostarinen

### Pyrolysis of fur animal manure into fertilizer product

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Finnish fur animal production is concentrated to the area with other intense animal production leading to regional phosphorus (P) surplus and need to manure P transfer. Fur animal manure is dry and rich in nutrients enabling various processing technologies to produce transportable P fertilizer product. One option could be pyrolysis where manure is heated in low oxygen or oxygen free atmosphere and char, liquid and gas fractions are produced. Thus slow pyrolysis of fox (FM) and mink manure (MM) was studied and suitability of char fraction as P fertilizer and liquid fraction for biogas production was evaluated. Also energy content of liquid and gas fractions were studied. Proper utilization of gas and liquid fractions is pre-requisite for process sustainability. Pre-dried FM and MM was pyrolysed in a lab at 340 and 470°C. Total nutrient and ash contents, P solubility, pH and bulk density were analyzed from fresh and predried manures and char fractions. P plant availability was assessed with pot experiment (completely randomized block design, n=3) using ryegrass (3 harvests), P deficient sandy soil and superphosphate (SP) as reference. The energy content of pyrolysis gas was calculated (lower heating value, LHV) based on detected gas main components. LHV was analyzed for unseparated pyrolysis liquids (470°C), whereas methane production potential (37 °C, 37 d, n=3) was tested after tar separation. About half of the pre-dried manure ended up to the alkaline (pH 8-10) char fraction with high ash.(45-70%) and P (64-96 g P/kg DM) content. N ended up to gas and liquids. P plant availability in fresh and pre-dried manures was almost comparable to SP. Although pyrolysis decreased P solubility in both manures, P plant availability decreased only with pyrolysed FM. The energy contents of pyrolysis gas from MM were higher than from FM. The pyrolysis liquid from MM had also higher LHV and methane production potential (36 MJ/kg, 306-320 L  $CH_4/kg$   $VS_{added}$ ) than from FM (28 MJ/kg, 170-212 L CH<sub>4</sub>/kg VS<sub>added</sub>). Results are promising for the suitability of pyrolysis for fur animal manures although further research is needed.

### 3. Steen Henrik Møller:

## Calculation of and development in nutrient content of mink waste in Denmark since 1999

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Since 1999 the Danish regulations of mink waste have been based on calculations of the content of nutrients in the urine and manure from one year of production from a mink dam. This means that a mink farmer only have to know the number of dams mated and kept for gestation in order to calculate the content in the total mink waste from the production. It also means that the key number is the concept of a 'Year dam production' including the kits delivered and breeder males needed for mating. As litter size and feed consumption may vary between farms, each farmer may adjust his calculations based on actual litter size and feed consumption.

The calculations are based on the total input in the form of annual food production on each feed kitchen in Denmark, the number of dams on the farms under each feed kitchen and the analysed content of N, P and K in the feed throughout the year. From this the nutrients deposited in the bodies of the animals produced is subtracted in order to calculate the amount of nutrients in the urine and manure to be used as fertilizers in the field. Since 1999 the amount of N, P and K per Year dam production have increased gradually by 30,0% while P and K have decreased by 8,3% and 2,4% to now 5965 g N, 943 g P and 531 g K excreted per year dam in production.

#### 4. Henning Otte Hansen

## Estimation of the amount of fur skins on the European consumer market – closing the knowledge gap for fur environmental footprint calculation

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The amount of fur skins on the European consumer market must be estimated as input to calculate the fur environmental footprint in Europe. However, data to quantify the consumer market on country levels are not directly available, and a new way or model to collect data and to estimate the consumer market volume is presented and used. The model starts with raw fur skin, and based on a.o. mark-ups, import and export the total final amount of fur skins on the European consumer market is estimated. Production of raw fur skins, auction prices and international trade are three important factors in the estimation, and they are volatile and significant for the European consumer market. It is calculated, that Europe accounts for 20-25 per cent of world fur retail (consumer) sale, and a correction for different mark-ups means, that Europe will consume 16-20 per cent of all fur skins produced in the world. Number of fur

skins are converted to area and weight using average figures reported from business organisations.

### **SESSION IV: HEALTH & DISEASE**

1. Karin Mundbjerg<sup>1</sup>, Ida Sebbelov<sup>1</sup>, Tove Clausen<sup>2</sup>, Anders Tolver, Anne Sofie Hammer<sup>1</sup>

## Preliminary results of an investigation of familial occurrence of urolithiasis and cystitis in farm mink (Neovison vison)

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A study of four generations of mink on a Danish research farm (n=62932) was conducted with the objective to examine occurrence of urolithiasis and cystitis in relation to familial disease history. 421 mink were diagnosed postmortem with urinary tract disease resulting in a prevalence of 0.7 %. Using Chi square test with 2000 simulations we found association between familial history of urolithiasis and cystitis in breeding animals and urolithiasis and cystitis in offspring (p=0.0015). The results indicate that selective breeding (exclusion of animals with a family history of urinary tract disease) may be applied as a preventive measure for urolithiasis and cystitis in mink.

2. J. Virtanen<sup>a,b,\*</sup>, K. Aaltonen<sup>a,b</sup>, A-M. Moisander-Jylhä<sup>a</sup>, H. Nordgren<sup>a</sup>, R. Kant<sup>a</sup>, T. Smura<sup>b</sup>, J. Peura<sup>c</sup>, O. Vapalahti<sup>a,b</sup>, T. Sironen<sup>a,b</sup>

### Study on mink tolerant to Aleutian mink disease virus

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Aleutian disease (AD), caused by Aleutian mink disease virus (AMDV), is one of the most important infectious diseases of farmed mink, causing significant welfare problems for the animals and financial losses to the farmers. In addition to mink, AMDV also infects several other mustelids such as ferrets, martens and badgers. Some mink tolerate this disease better than others but the reasons to that are unclear. In this project, we investigated the mechanisms

behind disease tolerance by performing a two and a half year follow-up for asymptomatic AMDV-positive mink in the farm that has been controlling the disease for decades by breeding disease-tolerant mink. As a comparison, we studied symptomatic mink from a different farm and mink from an AMDV-clean farm. We have compared gross — and histopathology, antibody profiles, and viral loads in spleen and kidneys between each group. In the future, we will also compare transcriptomes to study differences in gene expression between symptomatic and asymptomatic mink as well as AMDV-negative mink. We will discuss these results in the presentation.

3. Anna-Maria Moisander-Jylhä, Moisander-Jylhä A-M., Aaltonen K., Sironen T.

# Fight against antibiotic resistance requires better understanding and diagnostic methods in fur animal digestive tract disorders

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Digestive tract disorders, especially diarrhea, are the most common reason to prescribe antibiotic therapy to whole animal stock in a fur farm in Finland. Diarrhea may be a sign of unbalanced intestinal microbiome or infection and feed quality and environmental factors may influence animal intestinal health. Diarrhea reduces growth and animal welfare and nutritional requirements may not be met due to impaired nutrient absorption. Lack of nutrients may lead to lower skin quality and size thus farmers income decreases. Fear of monetary losses due to diarrhea and desire to cure animals results to unnecessary antibiotic use. We did a survey in 2014 in which we sampled 21 voluntary fur farms in Finland. Farms had mink (Mustela vison) and blue fox (Vulpes lagopus). Fecal samples were analyzed with same method as diagnostic fecal samples are analyzed in National Food Safety authority's laboratory in Seinäjoki. Results of the fecal analyzes differs between fox and mink. Campylobacter and coccidia were common finding in mink but mink enteritis virus was not detected. In blue fox parvovirus was common and spectrum of possible pathogens was broader. Unnecessary treatment with antibiotics is harmful to normal microbiome and creates possibilities for bacteria to become resistant to one or many different antimicrobial substances. Lack of knowledge of disease mechanism and possible viral pathogens increases antibiotic treatments because it is the one thing veterinarian could try to treat diarrheic animals. With more knowledge of mink and blue fox pathogens antibiotic may be reserved only for those cases where it is obligatory.

#### 4. Pauliina Isohanni

### Autumn diarrhea project at Fin FurLab

Fin FurLab Oy Ab

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Autumn diarrhea is observed rather often in Finnish fur animals during the warm and moist late summer months (August, September, early October). The causes of autumn diarrhea are still uncertain. The objective of the autumn diarrhea project to be conducted at Fin FurLab is to introduce new analysis methods to the laboratory and thus improve diarrhea diagnostics. Simultaneously, more information about the possible causes of diarrhea will be gained. The pathogens for which new analysis methods will e developed are: Campylobacter spp., Coccidia spp., Salmonella spp., Parvovirus and Lawsonia intracellularis (from fox feces). They were chosen into the project based on earlier studies conducted at the Finnish Food Authority. During the project fecal samples will be collected from diarrheic fur animals. Samples will be transported immediately to the laboratory, kept cool and analysed within 24 h of purchase. For the detection of Campylobacter spp., ISO 10272-1:2017 method will be used. Shortly, a loopful of the fecal sample is streaked directly onto a Campylobacter Selective Agar and presumptive colonies are further identified by biochemical tests, Gram staining and by their ability to grow aerobically. The counts of Coccidia spp. in the fecal samples will be determined by using a quantitative McMaster flotation method. For that, feces are suspended into a flotation solution with specific gravity, and the total counts of Coccidia spp. per g feces are counted in a McMaster counting chamber.

For the detection of Salmonella spp. in fecal samples, ISO 6579-1:2017 method will be used. Shortly, the fecal samples are cultivated into an enrichment media, which is further subcultured onto Salmonella Selective Agar. Presumptive colonies are sub-cultured onto another selective agar and typical colonies are further identified by biochemical tests. Parvovirus and Lawsonia intracellularis from the fecal samples will be analysed by PCR-methods. The prevalence of each pathogen in the fecal samples will be determined at the end of the autumn diarrhea season. Conclusions are made after data collection. In the future, this new autumn diarrhea surveillance program will be conducted at Fin FurLab annually.

5. Maciej Klockiewicz<sup>1</sup>, Tadeusz Jakubowski<sup>1</sup>, Ewa Długosz<sup>1</sup>, Małgorzata Sobczak-Filipiak<sup>2</sup>

### Pathomorphological pattern of farm mink kidneys – preliminary study

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Chronic kidney disease affects health and productivity in animals. There are several kidney syndromes recognised in companion, farm and laboratory animals. The aim of the study was

pathomorphological evaluation of kidneys in farm mink. Samples were obtained from 25 randomly selected American mink (Neovison vison) at harvesting time. Routinely collected material was processed by haematoxylin-eosin (HE), Masson's (connective tissue collagen fibres) and Köss (calcium salts) methods. Microscopy revealed a list of major glomerular lesions as focal, proliferative or membranoproliferative inflammation in 17 mink; chronic inflammation of interstitial connective tissue in 13 and calcification in 10 mink (exclusively localized in the medullary part of kidneys of 9 mink), respectively. There were also parenchymatous and lipid degeneration with necrosis of the epithelial cells noted in the renal tubules of 17 mink (in 4 cases that noted as macrovesicular lipid degeneration-like lesions). It was only parenchymatous degeneration and/or necrosis discriminated in 5 mink. Conclusion: Histopathological monitoring of the kidneys pattern in farm mink might be a useful tool for health and welfare status as well as feed quality assessment.

6. Maciej Klockiewicz<sup>1</sup>, Tadeusz Jakubowski<sup>2</sup>, Małgorzata Sobczak-Filipiak<sup>3</sup>, Justyna Winiarska<sup>4</sup>, Ewa Długosz<sup>1</sup>

## Preliminary report on parasitological investigations in wild American mink (Neovison vison) from Narwiański National Park (Poland).

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Naturally living American mink (Neovison vison) are considered as an invasive in wild natural environment in Poland. Wild mink as predators are particularly exposed to different parasite infections. The aim of the study was to discriminate eventual parasitic infections of wild mink captured in Narwiański National Park. There were gastrointestinal tracts of 79 individuals examined macroscopically and with intestinal contents by flotation method. The intestinal flukes were noted in 7 f 79 mink (- prevalence 9%; intensity from 1 to 6 specimens per animal). The eggs of Capilaria sp. nematodes were found in 6 mink (- prevalence 8%). Similarly, coccidian oocysts were detected in 6 mink (- prevalence 8%). But the mixed infection of flukes together with coccidia and Capilaria sp. with coccidia were noticed in 1 mink, respectively. It was concluded that gastrointestinal parasitic infections remained at low level and occurred in less than 10% of examined population.