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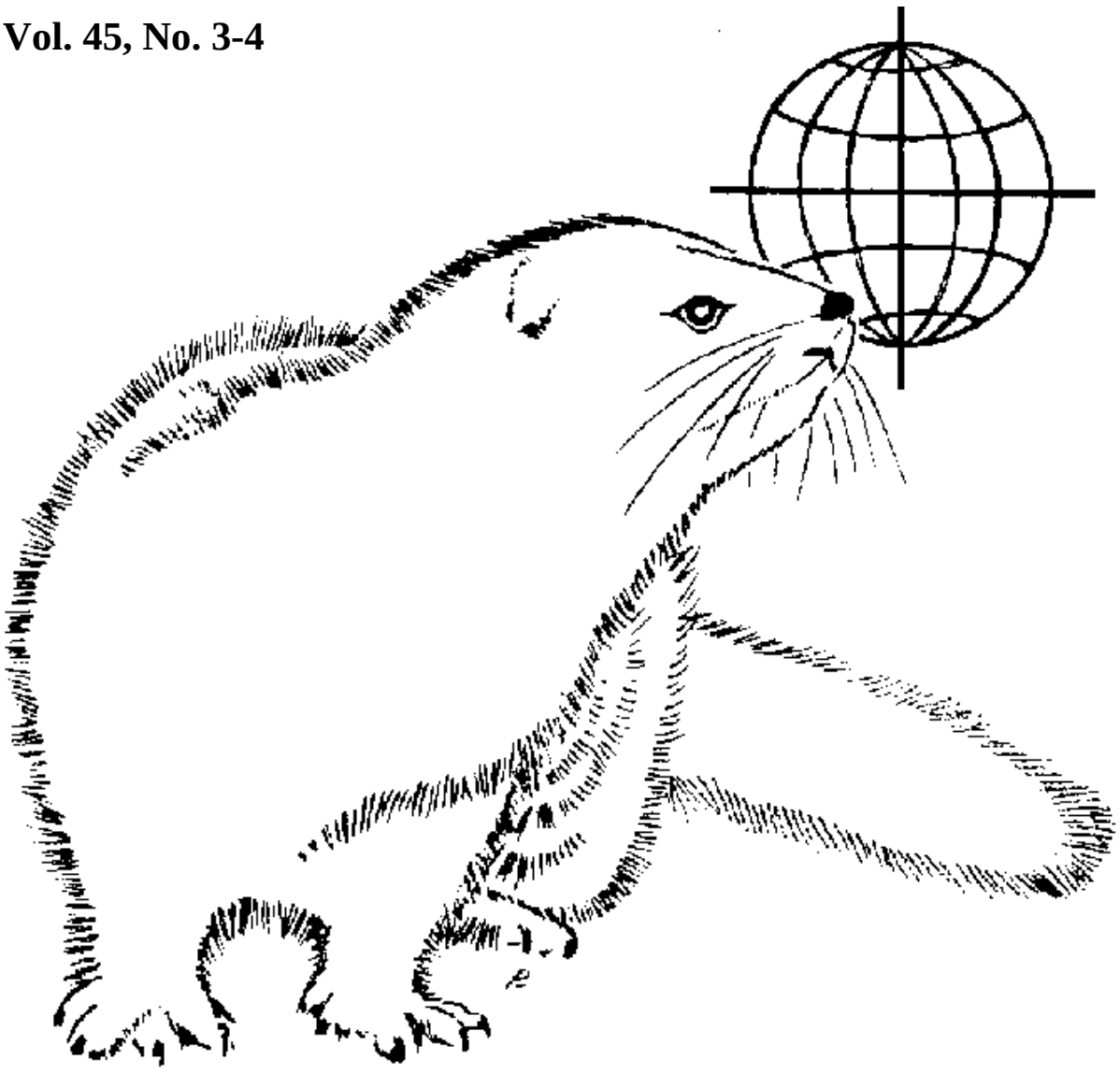

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INTERNATIONAL FUR ANIMAL SCIENTIFIC ASSOCIATION

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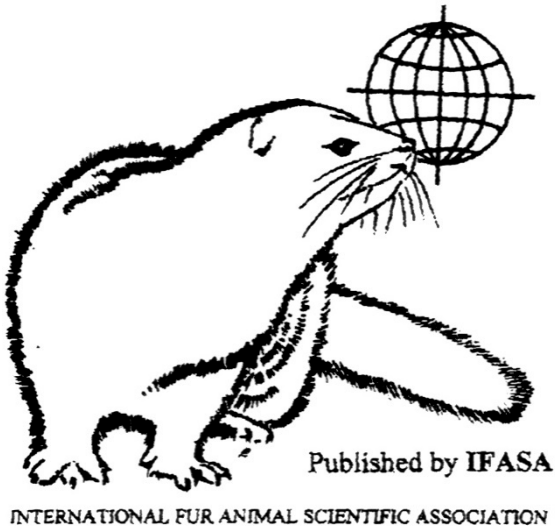
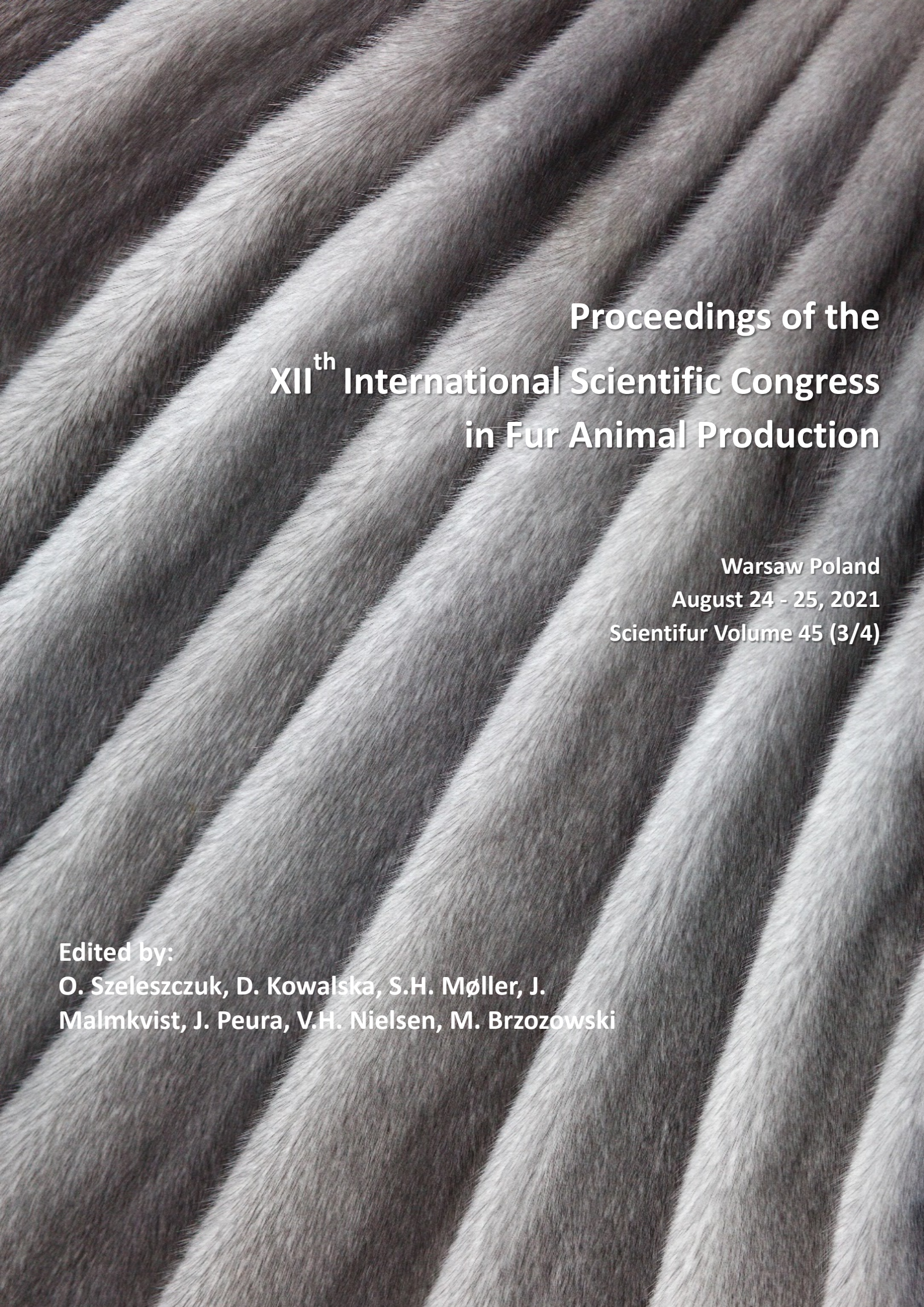


Notes from the Editor

Scientifur 45 (3/4) is directed at the publishing of the Proceedings of the XII International Scientific Congress in Fur Animal Production (the IFASA Congress). The congress was originally planned to be held in Warsaw, Poland on 25-27 August 2020. Due to the COVID-19 pandemic, the Congress was postponed to 24-25 August 2021. Participation in the IFASA Congress is possible both onsite in Warsaw and online. The link to the conference webpage is: https://ifasa2020.pl/.

Vivi Hunnicke Nielsen

Editor Scientifur



Proceedings of the XIIth International Scientific Congress in Fur Animal Production

Warsaw Poland
August 24 - 25, 2021
Scientifur Volume 45 (3/4)

Edited by:
O. Szeleszczuk, D. Kowalska, S.H. Møller, J. Malmkvist, J. Peura, V.H. Nielsen, M. Brzozowski

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Preface

Dear Reader,

Scientifur Volume 45 (3/4) contains materials of the XII IFASA 2020 Congress, which will be held in Warsaw on August 24-25, 2021 and hosted by the Polish Society of Animal Production.

In these unprecedented times, we cannot meet in person. For the first time in history, the Congress will be held online. It is a challenge for both the organizers and the participants. We are pleased that despite these difficult circumstances, so many scientists decided to attend the congress and submit their papers.

We hope that the next Congress will be organized in the traditional way.

Proceedings has papers on the following topics: Behaviour and Welfare; Breeding, Genetics and Reproduction; Environmental Impact of Fur Farms; Health and Disease. Plenary lectures focus on the most current issue: the impact of SARS-CoV-2 on fur farming.

We sincerely thank all those who made the XII International Scientific Congress of Fur Animals Production possible.

We hope you will enjoy the Congress and the Proceedings!

Anna Wójcik (President of PSAP)
Olga Szeleszczuk (Scientific Committee)
Dorota Kowalska (Scientific Committee)
Marian Brzozowski (Organising Committee)

Session I: SARS-CoV-2 impact on fur farming

SARS-CoV-2 impact on fur farming

Oral presentations

Danish mink industry: Closure 2020-2021

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Summary
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.

Keywords: Culling, COVID-19, public health, compensation

Introduction
On 4 November 2020, the Danish mink industry was de facto shut down: As a consequence of Covid-19 in mink and the threat to public health, it was decided that all mink, including breeding animals, should be culled. It is the first time that such a large industry has been closed down, and the first time that public health has had such a major impact on a major political decision.

The aim of this article is not to provide a complete assessment of the closure of the Danish mink sector as such an assessment would include several interdisciplinary problem formulations, in which public health, veterinary conditions, virology, business and socio-economic conditions as well as risk analyses would have to be assessed and weighed. Furthermore, presenting unambiguous conclusions is problematic because the alternative scenarios, i.e., situations with no or only partial closure, are difficult to describe and document.

Instead, the purpose is to describe and evaluate the overall process, the arguments, the consequences and the topics of discussion that followed. The article identifies some important lessons to learn.

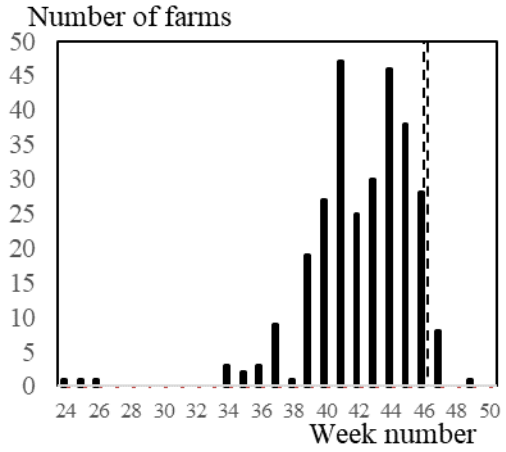
The process

On 15 June 2020, COVID-19 was detected in mink for the first time in Denmark. In the following two weeks, mink on 2 more farms were found to be infected. The mink on the infected farms were culled as a precaution. Subsequently, a nationwide COVID-19 screening of 10 per cent of all Danish mink farms was conducted. All 125 herds that participated in the study were found to be negative for COVID-19 (July 4). At the same time, the four agencies, Fødevarestyrelsen (the Danish Veterinary and Food Administration), Sundhedsstyrelsen (the Danish Health Authority), Styrelsen for Patientsikkerhed (the Danish Patient Safety Authority) and Statens Serum Institut (SSI) published a report as a basis for decision-making regarding COVID-19 in mink in Denmark. The conclusions (Fødevarestyrelsen, 2020a) were as follows:

- The risk of transmission from a COVID-19 positive mink farm to the surrounding community via humans is low.
- . . . mink animals under the conditions described above. . . with current knowledge are not expected to make a significant contagion contribution to the COVID-19 pandemic.
- There is no longer any need to cull based on a precautionary principle.

The report recommended the use of protective equipment and the introduction of a number of measures to minimise infection. Therefore, on 6 July, the government decided to change the strategy to contain the infection so that mink on infected farms would no longer have to be culled. From that point on, infected herds would be isolated and subject to restrictions, and new requirements for infection protection, control, testing, etc., were introduced. Thereafter, 7 weeks passed without any cases of infection being detected on mink farms. From mid-August and again in September, some cases of infection occurred, but it was not until the end of September that the outbreaks of infection really began, cf. Figure 1.

Figure 1. *Number of newly infected mink farms per week*



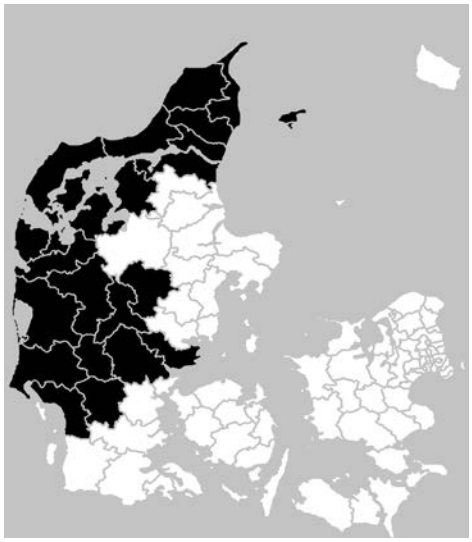
Note: The dotted line shows when the culling of the mink began
Source: Own presentation based on Fødevarestyrelsen (2021)

In mid-September, the Danish Veterinary and Food Administration concludes in a note that the risk of introducing COVID-19 to mink herds via humans was high. There was also a risk of infection spreading from

mink herds via humans to the surrounding community. It was also stated that significant genetic changes continued to occur in the mink variant, which raised concerns about future human immunity. The risk assessment could lead to further measures, including the culling of infected herds. A few days later (16 September), the Danish Veterinary Consortium (Dansk Veterinær Konsortium, 2020a) writes '*that the continued development of the virus in mink with progressively more mutations and spread by transmission to humans in Denmark may, therefore, constitute a potential danger to public health'*. Based on this, the Ministry of the Environment and Food initiated stricter control and monitoring measures as of 21 September 2020.
On 24 September, the Danish Health Authority writes in a memorandum (Fødevarestyrelsen, 2020) that overall, the recent weeks of monitoring and tracing have shown that the measures implemented at present have not been sufficient to prevent infection of new mink farms. If the efforts '*do not lead to satisfactory results and lead to further infection, it could have major consequences for the spread of infection and thus for the individual citizen as well as for society as a whole'*. This memorandum was the basis for the decision of the Ministry of the Environment and Food and the Ministry of Social Affairs and Senior Citizens on 28 September to implement a new strategy to address COVID-19 on mink farms.

On 1 October (week 40), it was clear that the measures taken had not been sufficient to contain the spread of infection. Therefore, on the basis of risk assessments conducted by SSI, the government decided that infected herds and herds within a 7.8 km radius of infected herds should be culled in the future, and that the breeders would receive economic compensation. The limit of 7.8 km was based on a study of the first 20 infected farms in North Jutland, where the distance between an infected farm and the nearest infected farm was estimated to be between 0.6 and 7.8 km. This study (Dansk Veterinær Konsortium, 2020a) emphasised that the distance between farms is the best explanation for the spread of infection, but that '*criteria for selecting which nearby herds should be culled are subject to great uncertainty due to a lack of detection of risk factors'*. On 8 October, the culling of infected herds as well as herds within the zones began. In the following weeks, the number of infected herds increased, and a total of 290 out of 1,147 fur farms became infected with COVID-19. The infection remained relatively regional, cf. Figure 2.

Figure 2. *Municipalities with infected mink farms, 2020*



Source: Own presentation based on Fødevarestyrelsen (2021)

On 3 November, SSI (2020a) concluded that continued mink production in 2021 would entail the risk of a recurrence of the spread of infection among mink and humans. The institute estimated that this would represent

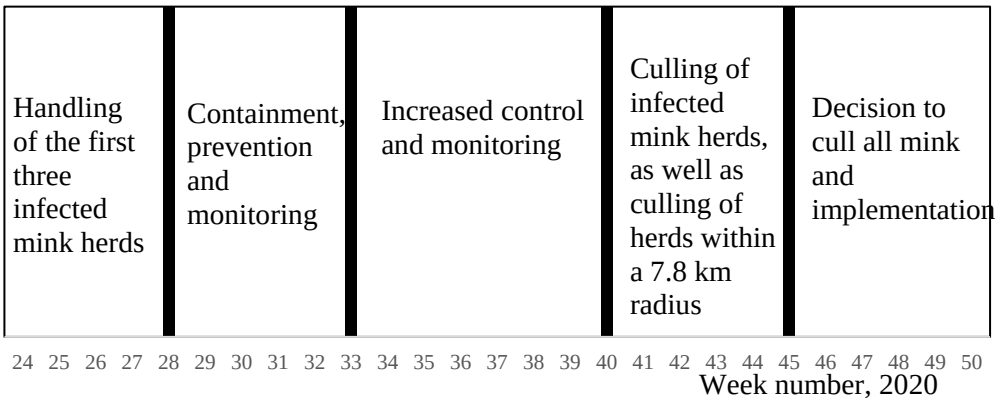
a high risk to public health through causing a greater disease burden among humans and by creating a large virus reservoir in mink, thereby increasing the risk of the emergence of new virus mutations, against which vaccines would not provide optimal protection. Therefore, the institute concluded that '*continuing to breed mink during the ongoing COVID-19 epidemic entails a significant risk to public health and the potential to prevent COVID-19 with vaccines'*. The conclusion of the SSI was based on the assumption that mink production would continue in Denmark, so that in 2021 a large population of mink would be re-established. However, no details about what constituted 'a large population' were provided. The institute stressed that the annual stock was 17 million mink, of which the breeding animals for the following year's stock made up approx. 20 percent.

By the beginning of November 2020, more than 280 mink farms had already been infected, so 2021 would definitely be an abnormal year with a significantly smaller stock and production. Since 2015, the price of mink fur had been falling, which also contributed to a reduction in the number of fur farms. In the years 2015-2019, the number decreased by 28 percent (from 1,475 to 1,060), and the production of fur skins fell by 4 percent. This development would in all probability have continued in 2020, as low prices and difficult sales conditions in the short term made mink production even less attractive.

The SSI's risk assessment was a key document, which formed the basis for the government's decision to cull all mink in Denmark, which represented the fifth and final phase in the spread and control of COVID-19 in the Danish mink sector, cf. Figure 3.

The decision to cull all mink in Denmark was made on 3 November during a meeting of the government's coordination committee, of which the prime minister and the ministers of finance, justice, foreign affairs and taxation are members. It was also decided that the possibility of rebuilding the mink industry in Denmark after the COVID-19 epidemic should not be prevented, cf. Miljø- og Fødevareministeriet (2020a).

Figure 3. *Phases in the spread and control of COVID-19 in the Danish mink industry*



Source: Own presentation based on Miljø- og Fødevareministeriet (2020a)

At a press conference on 4 November, the government announced its decision that all mink in Denmark should be culled due to the extensive spread of COVID-19 in mink and the health authorities' stricter risk assessment. At the press conference, the Prime Minister outlined the situation based on the SSI's assessments:

- Currently, COVID-19 infection on 207 mink farms.
- The increase has continued despite an intensive effort to limit the infection.
- Infection with new mutations of COVID-19 has been detected both in Danish mink herds and in the local population.
- It has not been possible to limit further spread to the surrounding areas.

The Prime Minister's conclusion was that the mutated virus - via mink - could undermine the effectiveness of the forthcoming vaccines (Prime Minister, 2020a). The assessment was that the mutated virus in mink could have devastating negative consequences for the worldwide handling of the ongoing pandemic. The situation required decisive action, which meant that all mink in Denmark - including the breeding animals - had to be culled. The action was justified by the fact that it was a matter of life and death - not only in Denmark, but in the whole world. The economy, exports and jobs were also at stake.

The academic director of SSI, Kåre Mølbak, followed up and mentioned the following two concerns (Statsministeriet, 2020a):

- A large reservoir of up to 17 million animals with COVID-19 virus, and which can infect humans and thus give rise to further epidemics in Denmark.
- Mutations, with the Cluster 5 variant of particular concern.

The conclusion was that continued mink business during an ongoing COVID-19 epidemic would pose a significant risk to public health. The risk was both national and international, and included the risk of not being able to prevent COVID-19 with vaccines (Statsministeriet, 2020a).

The breeding animals

A crucial point in the decision to cull was that the breeding animals should also be culled. The issue of breeding animals was a separate subject: if they also had to be culled, it would be very difficult to restart the industry. The Danish mink population was largely free of infectious diseases such as plasmacytosis. Furthermore, a genetic lead in fur quality, fur size, etc., led to competitive advantages over a number of other countries. For a number of years, Danish mink skins had been able to maintain a significant additional price compared to mink skins produced abroad (Hansen, 2016). At the press conference on 4 November, the Prime Minister stressed *the need to cull all mink in Denmark. Unfortunately, this also applies to breeding animals. The assessment from SSI is clear: Continued mink production during the ongoing epidemic entails a significant risk to public health, including the potential to prevent COVID-19 with vaccines* (Statsministeriet, 2020a).

However, Kåre Mølbak from SSI subsequently rejected the claim that the assessment from the SSI could be used to draw any conclusions about whether there was a great risk connected to letting the breeding animals survive as no specific questions had been asked about this. *Thus, SSI has not taken a position on various solutions such as a temporary ban on production while still allowing a small number of breeding animals and other possible solutions* (Mølbak, 2020a). *Kåre Mølbak could have imagined a model whereby only 99.5 percent of the mink were culled, and the important breeding animals were kept* (Mølbak 2020b).

The Danish Veterinary Consortium, which is a collaboration between the University of Copenhagen and SSI, and which advises the government on, e.g., mink and COVID-19, also concluded that the risk assessment could not be used to decide that the breeding animals should be culled. According to Nørrung (2020), a document

stating that the breeding animals should also be culled could not be presented. On the contrary, The Danish Veterinary Consortium wrote that the genetic material from mink could only be preserved by maintaining a population of live animals. It was considered that it would be possible to store such a number of animals in a safe manner (Dansk Veterinær Konsortium, 2020b).

The cost of closure

The possible cost of closure was, of course, of great interest both before, during and after the decision to close was made. As early as 10 October, a Member of Parliament asked the Minister of Agriculture how much culling all mink herds would cost the state. The Danish Veterinary and Food Administration's response to Parliament (Miljø- og Fødevareministeriet, 2020b) was based on a scenario that involved the culling of all Danish mink herds (approx. 1,100) as part of infection control and not on a scenario that involved the phasing out of the mink industry. The following costs and cost items were stated (1 EUR = 7.45 DKK):

- 2.3 – 2.8 billion DKK in immediate compensation to the fur farmers for loss of animals and operating losses.
- 2.4 billion DKK for cleaning and disinfection of infected herds - if all herds are infected.
- Culling, destruction, administration, etc. (no indication of amount).
- Other compensation claims against the state as a result of culling mink (no indication of amount).

The total amount was estimated at 5 billion DKK, of which approx. half was earmarked for the mink breeders and half for cleaning. On 5 November, the loss from just lost investments, lost earnings and lost employment in the industry was estimated at 8-10 billion DKK (Hansen, 2020a). On 10 November, the loss was estimated at 10-12 billion DKK, which included the up- and downstream industry (Hansen 2020b). The bill that was presented on 10 November (Folketinget, 2020) was to list the consequences for the economy and the consequences of implementation for the public sector. *It was stated that it was not possible to arrive at a reliable estimate of the total costs for the public sector, but the total costs for the state as a result of the bill was estimated to amount to at least 5 billion DKK.* On 22 September, the Minister of Food, Agriculture and Fisheries approved a memorandum on corona infection in mink (Miljø- og Fødevareministeriet, 2020a). The assessment was: *From a precautionary point of view, all mink herds can also be culled. However, it will be very far-reaching and it requires an assessment of the compensation to be given to the affected mink farm owners, which is estimated to amount to 3 billion DKK (culling before pelting) and 1.13 billion DKK (culling after pelting). Costs related to the loss of buildings and equipment as well as the loss of approx. 6.000 jobs in the mink industry must be added.* The final - but only estimated and preliminary - loss was determined according to both economic and political considerations in the final agreement, which was agreed on 25 January 2021 between the government and most other parties in the Parliament. The expected loss was estimated at 16-19 billion DKK.

The model for compensation for the mink farmers consists of the following three main elements (Erhvervsministeriet og Ministeriet for Fødevarer, Landbrug og Fiskeri, 2021):

1. Compensation for mink culled and not pelted in 2020, which, therefore, cannot be sold in 2021. The compensation is based on the number of mink culled and not pelted in 2020. The mink farmers receive compensation per mink and can choose whether the compensation is calculated on the basis of the average of the 2019 and 2020 price or based on the market price at Kopenhagen Fur's auctions in 2021, with a maximum of 250 DKK per skin. All expenses saved including for feed, veterinarians,

fur, etc., in 2020 will be deducted from the calculated compensation.

2. Compensation for loss of future income in 2022-2030. The expected future earnings for the individual breeder are based on an expected price of mink skin calculated as the average of the last ten years' prices, excluding the highest and lowest value. The price for the individual grower will be adjusted for the quality of the farmer's skins measured by the prices received in 2017-2019 compared to the average prices in the year period. A ceiling is added to the calculation so that the adjustment for quality can only give a maximum price of 5 per cent higher than average.

3. The expected residual value of the mink farmers' assets in 2030 including stables, cages, etc. The value of the assets in 2030 is discounted back to 2021, and this amount is paid to the farmers together with the other compensation.

Table 1 shows the exact but estimated cost items. The largest item is "Compensation for mink farmers 2022-2030", which includes both compensation for loss of future income base and compensation for the residual value of the production assets that no longer have a value for the mink farmers.

Table 1. *Estimated and expected costs for the State resulting from the closing down of the mink industry*

	Million DKK
Compensation to the mink farmers	
Compensation to mink farmers, 2022-2030	8,900
Compensation in 2021 for culled, non-pelted mink	1,800 - 2,800
Dormancy scheme	60
Shut-down costs	80
Resources for mink farmers close to bankruptcy	30
Direct compensation, in total	10,870 - 11,870
To the industry	
Compensation to up- and downstream industry	3,000 - 4,000
Resources for transformation of the industry	100
To the industry, in total	3,100 - 4,100
Other	
Demolition of production facilities, etc.	1,500 - 2,700
Valuation commissions	100
Other, in total	1,600 - 2,800
Total	15,570 - 18,770

Source: Own presentation based on Erhvervsministeriet og Ministeriet for Fødevarer, Landbrug og Fiskeri (2021).

The second largest item is "Compensation to up- and downstream industry", which includes a number of industries that are economically directly dependent on the mink farmers' production including feed mills, skin processing, auction houses and transport companies connected to the Danish mink industry. The agreement on

compensation for the mink industry was concluded 2½ months after the decision to close down had been made. This delay has several explanations:

A number of political motives for focusing on the case, including a lack of legal authority, delayed the process.

The delay was also due to difficulties in reaching a consensus on the size of the compensation. The value of the lost fur production was difficult to calculate as the market price had been very unstable over time. Furthermore, there are price cycles on the market and clear correlations between supply and prices, which also had to be taken into account (Hansen, 2020d). The decision was finally based on the prices of the previous ten years.

Another point of discussion was the length of time for which compensation should be paid. The result was that the compensation for loss of future income should cover the period 2022-2030, i.e., 9 years. No explanation as to why 9 years was selected was given. On the one hand, many mink farmers have developed a special competence over the years, and this competence has been largely lost forever. On the other hand, mink farmers are often regarded as a stable labour force that can relatively easily find employment in other industries. In general, the lost earnings will vary greatly between mink farmers, and it is impossible to calculate the loss on an individual basis.

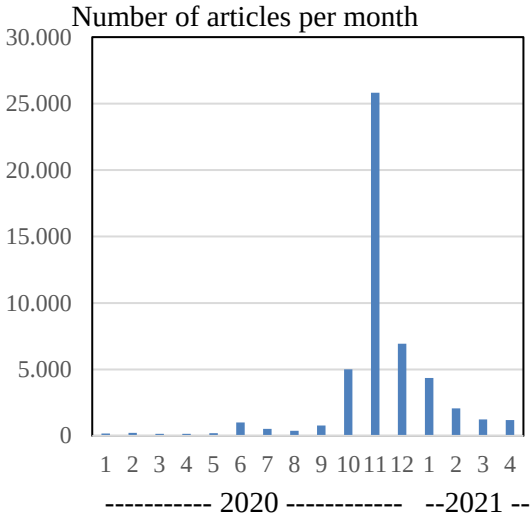
Finally, there was great disagreement about the so-called dormancy scheme, which also delayed reaching a consensus. The dormancy scheme should make it possible for the mink farmers who wanted to stay in the mink industry to temporarily cease production until the end of 2021, when mink production will again be possible and justifiable in terms of public health. These farmers would receive compensation for their costs during the dormant period, just as they would receive compensation to cover operating losses connected to breeding animals, although they would not receive any compensation for buildings, machinery, etc.

The Danish Fur Breeders' Association advised its members against using the scheme because all the associated companies - Kopenhagen Fur, the feed factories, and the entire fur infrastructure - would disappear forever (Pedersen, 2021). The dormancy scheme was also criticised for not being attractive enough for the mink farmers, which is why two right-wing parties were not included in the agreement. Two left-wing parties were also not included in the agreement because they thought the level of compensation was too high (AgriWatch 2021).

The ensuing discussions due to the shutdown

The culling of all mink and thus the closure of the Danish mink industry naturally led to a heated debated and many different reactions: It is rare to hear the government and the authorities talk about a serious threat to global health, and about the closure of a relatively large industry. It is also rare for such a far-reaching decision to be made so quickly. The significant interest in the case - both politically and in the media - is illustrated in Figure 4, which shows the number of articles in Danish media in which the word 'mink' is included.

Figure 4. *Number of articles in Danish media in which the word 'mink' is included*



Note: Articles, news, etc., in all kinds of media
Source: Own calculations based on Infomedia.

The figure shows that in November 2020, especially, media interest in the mink case was very high. These opinions must, of course, be assessed against a background of a worldwide pandemic, for which the cause, process, consequences and suitable interventions were completely unknown or very difficult to assess. Several interdisciplinary issues connected to public health, veterinary conditions, virology, business economics and socio-economic conditions as well as risk analyses had to - or should - be assessed and weighed. This made both the basis for decision-making and the political decisions even more complicated. A number of questions will probably be answered in future studies. However, all alternatives and scenarios, by their nature, can never be fully analysed. Nevertheless, the discussions can be grouped into a number of themes, cf. below:

- The decision to cull all mink in Denmark including breeding animals and production animals was both tangible and significant. The Prime Minister justified the decision as follows, *'on the basis of the clear recommendation from the health authorities' (*Statsministeriet, 2020a). However, the risk assessment to which she referred did not include any recommendations. In particular, the culling of all breeding animals was not part of the recommendations by the authorities at the time of the decision.

- Immediately afterwards, SSI emphasised that they had not proposed culling the mink, '*SSI has only prepared a risk assessment in relation to human health if mink farmers continue mink production into 2021 as normal after culling infected animals or after pelting in the 2020 season. SSI has thus not provided any recommendations for possible solutions'* (Statens Serum Institut, 2020b).

- It was also argued that a nationwide culling was an overreaction. For example, no infected mink were found outside Jutland, and the five mink farms on the island of Bornholm - far away from Jutland - also had to cull all mink.

- Professor Lars Erik Larsen from the University of Copenhagen assessed that continued production in large mink herds in Denmark would have been completely justified as long as it had taken place under stricter conditions.

- Another important discussion centred on the balance between, on the one hand, the risk assessment made by the health authorities and, on the other, the derived and associated costs and consequences for the industry and for the economy.

- The health authorities concluded several times - under different conditions - that continued mink production in 2021 would pose a potential risk to public health and, at some point, even a risk to global public health.

Risk assessments are extremely important, and they should, of course, be based on scientific rigour and include indications of probabilities as well as short- and long-term effects. Risk assessments document the possible disadvantages or the associated advantages of avoiding disadvantages (benefits). These risks and benefits must, of course, be weighed against the possible costs of preventing the risks - or of obtaining the desired benefits. This balance between 'cost and benefits' has largely been absent in this case:

- *If it had been known that it would cost 19 billion DKK, I don't think the decision to shut down would have been made. But they said that it threatened public health, and then how much it would actually cost was irrelevant.*

- *When you play the trump card of public health to justify a decision, no one dares to question it. Perhaps the health authorities have become the legislative authority here in Denmark. If they say something, it becomes law.*

- *As a citizen, one must assume that the government assesses the costs when making a decision. So the government shouldn't be surprised that the bill is 19 billion DKK. The government should have made a realistic estimate of the costs instead of simply referring to the threat to public health (Hansen, 2021).*

- The 19 billion DKK corresponds very closely to the average price of four super hospitals, which are currently being built in Denmark. Therefore, the question is whether the culling of all Danish mink had the same - or greater - value for Denmark than four new Danish super hospitals. Regardless of the answer to this question, it underlines the need for interdisciplinary assessments the next time decisions have to be made about major interventions and investments of importance to both public health and the economy.

- An intermediate solution whereby, for example, a larger number of breeding animals was allowed, or regionalisation was possible, would have reduced the costs considerably. Protecting public health would probably have been possible at a much lower cost.

The question of the scientific basis for culling also led to much discussion:

- The risk of weakening or completing nullifying the effect of a future vaccine was an important argument at the time when the question of whether to shut down was being considered. *But with the mutation now found, we have an even greater responsibility including to the rest of the world. . . We have seen several different types of mutations. And what has particularly worried us is this Cluster 5* (Statsministeriet, 2020a).

- However, as it transpired, the Cluster 5 mutation had probably already disappeared at this point (Berlingske, 2020a).

- Furthermore, the value and weighing up of the various inputs to the crucial risk assessments was also discussed.

- Finally, whether there was a reasonable connection between the analyses made by the health authorities and the political actions was discussed. The analyses conducted by the health authorities did not include recommendations for culling, and conclusions of the analyses were based on some assumptions that could hardly justify the culling of all the mink in Denmark.

- SSI did not recommend culling all the mink. Nevertheless, the political decision to cull all the mink has been justified by referring to the recommendations of the health authorities many times.

The discussions also concerned the valuation of the mink skins that were to be destroyed or would not be produced. Ideally, the mink breeders should receive compensation based on a market price that they would have received in the subsequent 9-year period if intervention had not occurred. The sales price of mink fur was very low at the end of 2020 and the beginning of 2021, and prices had been falling since 2015. The question was whether prices in 2020/21 reflected either a new equilibrium price created by declining demand, or a minimum level in a normal price cycle, which would be followed by a price increase created by a sharp decline in supply. The result was an average of the previous ten years' prices, albeit with the exclusion of the highest and lowest value.

The discussion also included the question of a final ban on mink production in Denmark. A number of countries had already banned mink production or had made mink production very difficult including the UK, the Netherlands and Norway. However, the law stipulates that the ban on mink in Denmark applies until 31 December 2021. Subsequently, the Prime Minister also emphasised: '*the government has not made a decision to close down the mink industry in Denmark. We have not decided that'* (Frederiksen, 2020).

Conversely, two political parties in the Danish parliament argued that the outbreaks were another argument for phasing out Danish mink production (DR, 2020). The two supporting parties to the government requested a formal consultation with the minister. At the same time, they proposed the banning of mink production in Denmark. However, the proposal was rejected by the government on the grounds that a profession cannot be banned overnight: '*the industry must be treated properly and decently. It is not fair to want to close a legal industry in Denmark where many people are employed'* (DR, 2020).

Both the animal protection organisation, Anima, and the Danish Society for Nature Conservation (Danmarks Naturfredningsforening) opposed the possible continuation of the mink industry. Anima) argued that the compensation agreement should include a complete halt to mink production, while the Danish Society for Nature Conservation argued that the mink industry has a detrimental effect on nature, the climate and the environment (Berlingske, 2021a).

Lessons to learn?

Hopefully, a similar pandemic will not recur, and developing preventive measures based on this mink case will be unnecessary. However, realistically, pandemics occur again. Therefore, the question is when and where will a future pandemic occur? What form will it take? How extensive will it be and what consequences will it have? Such questions are difficult to answer as they concern an uncertain future scenario. Furthermore, next time a balance will again have to be struck between, on the one hand, acting quickly and decisively and, on the other, reaching a consensus about the socio-economic foundation for decision-making. However, such a balance can

only be reached when the extent of the pandemic is known, by which time it may well be too late. This experience also demonstrates that the costs of both closure and continued operation are difficult to calculate. Even the politicians who implemented the closure were surprised by the size of the compensation. This indicates that there was an absence of clarity regarding the associated costs and benefits when it was decided to close down the industry. The lesson is that the most important advantages and disadvantages should be identified and quantified in a transparent way before major decisions that cannot be reversed are made.

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Survival of SARS-CoV-2 on Clothing Materials

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Abstract

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. The 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 mink 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. This 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.

Keywords: SARS-CoV-2, virus culture, stability

SARS-CoV-2 vaccine development for mink

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Abstract

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.

Keywords: SARS-CoV-2, vaccine, mink

SARS-CoV-2 - a true One Health challenge

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Abstract

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 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 mink 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.

Keywords: SARS-CoV-2, mink, One Health, emerging infectious diseases

Session II: Behaviour and welfare

Behaviour and welfare

Oral presentations

What is the best feeding strategy to adjust female minks' body condition without compromising the welfare during the winter season?

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Abstract

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 20th February compared with 31st 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 (BCS 4-group) and the other half in 5 (BCS 5-group). 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 20th February is less stressful for first-year breeding females than adjusting to body condition 2 on 31st January. Even so, there are welfare challenges with both feeding strategies, and it remains to be investigated which feeding strategy during the winter best prepares mink females to be able to respond to flushing, without compromising their welfare.

Introduction

Restrictive feeding is one of the main welfare challenges for commercial mink in the winter period as it increases the risk of prolonged hunger and stereotypy. Female mink should not be too fat to respond to flush feeding, and a common recommendation is to adjust the female minks' body condition from selection of breeders in November until the unset of flushing in late February to body condition score (BCS) 2. The challenge is that the selected animals usually have been fed to become very heavy in order to produce a large skin for pelting. The selected breeding females are, therefore, often in a high BCS of 4 or 5 and need to reduce their weight with around 40 % to be fit for flushing and mating. There are different practices and recommendations for how much, over how long time and at what time the animals should be slimmed down, and in Denmark many farmers aim at BCS 2 already by the end of January (Nielsen, 2018).

The aim of the present project was to evaluate the mink welfare in different strategies for body condition adjustment in the winter period. In order to achieve this we tested 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 November to 2 by either the 20th of February or the 31st of January will reduce the number of stereotypic female mink and result in less amount of stress hormones measured as faecal cortisol metabolites.

Material and method

There were 400 first-year brown female mink included in the study. The study was conducted in Denmark at Aarhus University's research farm, in the winter season 2018 to 2019. The animals were divided into two groups, of 200 animals of which half were in BCS 4 and half in BCS 5 at live animal grading. The animals were selected for the project in the end of November, based on weight at and BCS at live animal grading, and the trial started 10th December. The animals in the two groups were slimmed down to BCS 2 either by 31st January or by 20th February. The welfare measures were body condition score, stereotypic behaviour, and faecal cortisol metabolites (FCM).

The statistical analyses were performed in R, with the package lme4 and glmmTMB. The effect of the two feeding strategies on stereotypic behaviour was analysed using binomial mixed models with feeding group, body condition group and days from first assessment as explanatory variables. Animals' id were included as random effect, and the timely correlation between observations within animals was accounted for using the Ornstein-Uhlenbeck autocorrelation structure. The effect of the two feeding strategies on FCM was analysed by a general linear model, with feeding group, BCS group and stereotypy level as explanatory variables.

Results and discussion

There were significantly lower prevalence of stereotypic behaviour (A=14.3%, B=17.2%, $p<0.05$) and lower amount of faecal cortisol metabolites (FCM) in the group that were slimmed down to body condition 2 in February compared with January (A=166 ng/g and R=254 ng/g FCM the 30th January, $p<0.05$). We, therefore, accept our hypotheses that adjusting mink to body condition 2 in 20th February is less stressful for first year breeding females than adjusting to body condition 2 in 31st January.

Even so, there are some welfare challenges with both feeding strategies. One reason could be that the slimming did not begin until 10th December shortening the period for a gradual weight loss compared to starting immediately after live animal grading. Animals in BCS 4 in our project though could theoretically be comparable with animals in BCS 5 at grading. Another question is if it is necessary to adjust the BCS down to 2 or if the females would be able to respond to flushing with a BCS of 2,5 or even 3. The minks' subcutaneous body fat layer plays an important role in the minks' need for energy in the winter period (Mustonen, 2005). The layer gives some isolation of the mink in cold periods but is also their main energy reservoir in longer periods with negative energy balance. Animals in BCS 2 have, by definition, no subcutaneous body fat layer (NFACC, 2013) and will, therefore, have been in a negative energy balance over a longer period, and thereby probably felt prolonged hunger. The knowledge about the females' body condition and flushing are mainly from the work of Tauson between 1980 and 1990 (Tauson, 1985; 1988; 1993; Tauson and Alden, 1985). The animals have become much bigger since then, and the need for body conditioning might also have changed. Huge changes in body condition is probably the main challenge for the mink in the winter season (Boudreau, 2014), and a more moderate reduction of breeding females to BCS 2,5 or 3 instead of BCS 2, and a longer time span allowing for a more moderate daily reduction will probably be beneficial for the animals' welfare. The question is to what extent females in BCS 2,5 or 3 will respond to flushing and how to balance litter size and welfare in the best possible way. There is, therefore, a need for more research to find the best way to feed female mink in the winter season, to respond to flushing before mating, without compromising the minks' welfare.

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

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Abstract

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 experienced by the foster kit (not the dam), i.e. 4 > 2 > 0 days, for increased growth in foster kits ($P<0.001$). 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 around 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 cross-fostering in mink.

Keywords: growth, litter size, maternal behaviour, mortality, *Neovison vison*.

Introduction

The birth and the preparation prior to the delivery is critical for the reproduction result in farm mink. For example whether the dams are well mated, in a good feeding condition and had access to material for nest-building behaviour prior to delivery (Malmkvist et al., 2007; Malmkvist and Palme, 2008; Schou and Malmkvist, 2018). After birth, the early management vary considerable as revealed in studies on commercial farms. This is true also for the management of large litters with more than 8 kits early after birth. However, nearly no studies exists on the cross-fostering management in mink. 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 focus was on maternal acceptance/rejection of a foster kit, kit growth, wounds/damages and dam welfare until weaning at 8 weeks.

Methods and materials

The study design consisted of four factors, as presented in the introduction. The mink was brown delivering dams housed in standard Danish commercial conditions at the research farm AU-Foulum. 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 foster kits originated from litters with on average 10.8 living kits day 1 (D1) after birth. The 2019 study (factor 3 and 4) included 572 litters (186 sender and 386 recipient litters receiving one kit). One kit of the same sex as the transferred kit from the same litter was also chip marked and weighed, but remained in the sender litter, now reduced to 8 kits. The foster kits originated from litters with on average 10.1 living kits day 1 (D1) after birth. 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. Each transferred kit was chip-marked to make individual identification possible.

Results

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 ($P<0.001$).

Discussion

One potential argument against cross-fostering at farms is the risk of disturbance on the maternal care and on the kits. A recent study found – surprisingly – that the mink mother can differentiate between own and strange kit within few days after birth, based on kit vocalisations (Malmkvist, 2018). A smaller study reported signs of a higher occurrence of kits being bitten to death among foster than among own kits, following transfer to young dams of another colour type (Skovgaard, 1998); this result was not confirmed in a study of transfer of kits within the same colourtype of both young and older dams (Clausen & Larsen, 2018). Therefore, it is a relevant question, whether the foster dams ability to recognize own from unfamilar kits induce rejection of unfamiliar kits. The present study found no damaging by the dam or other signs of rejection (throwing kits out of the nest, reduced growth, increased mortality) of foster kits. On the contrary, mink mothers are eager to take care of an unfamilar kit, but early transfer after birth to a smaller litter resulted both in a quicker accept as well as better growth for the foster kit until weaning. For both years of study, the growth of kits until weaning was markedly higher in the litters nursed by the older, experienced foster dams. Thus, farmers are recommended to use experienced rather than inexperienced foster mothers during early cross-fostering in mink.

Overall, the kit weight decrease and the mortality increase with the number of kits in the litter at birth (Schou et al., 2017). Based on the 2019-study, the acute maternal retrieval behaviour was impaired in dams with 7 kits compared to in dams with 4-5 kits. This may indicate a lower motivation for maternal care in mink, even when only 7 kits in the litter. There was no statistical difference in the risk of kit mortality between (1) transfer to a small litter with 1-3 kits, (2) transfer to a medium litter with 4-7 kits, or (3) staying at home with the biological mother with 8 kits. Thus, cross-fostering had no effect on kit mortality. It should also be noted that the majority of mortality is early in mink – peaking during the delivery and within the first 24h after (Malmkvist et al..

2007), i.e. before the transfer of kits. The main gains of cross-fostering from large to smaller litters are increased kit weight at weaning and fewer damages around 7 weeks after birth – a critical period in particulary for the female kits in larger litters.

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The farmers' view to the fur chewing in farmed blue foxes (*Vulpes lagopus*)

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Summary
We surveyed the Finnish fur farmers' and other farming experts' view on the possible factors affecting fur chewing behaviour in blue foxes. The respondents evaluated fur chewing to occur in less than 10% of blue foxes, appear most often on the tail and hips, and be most serious in January-February. It was regarded as detrimental to the fur quality, but not to the health or welfare of the animal. Fur chewing was conceived to be connected to the season, genetic predisposition, and 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. For example, hunger, earlier tendency to fur chewing, stress, cold weather, agonistic behaviour in social housing unit and stereotypic behaviour were considered to increase fur chewing in a fox. The survival in cold weather conditions was evaluated to be worse and breeding result better in the fur-chewers than in non-fur-chewers. Two hypotheses of fur chewing were formed: 1) the fur chewing is associated to feed and nutrient availability/quality or 2) the behaviour and/or temperament of the fox.

Keywords: behavioural disorder, fur quality, welfare, questionnaire

Introduction

Fur-chewing is a self-injurious behaviour (SIB) observed in several animal species (e.g. dog, cat, fox, mink, rat) kept in confinement (Devine, 2012). Various other forms of SIB have been observed in captive animals and in humans with neurodevelopmental disorders, e.g. hair pulling (Devine, 2012, Muehlmann and Lewis, 2012). Muehlmann and Lewis (2012) have suggested that the phenomenology and pathophysiology of SIB resemble that of abnormal repetitive behaviours (ARB): tics, stereotypies and compulsions. The common inducing conditions for these four are genetic mutations, behavioural deprivation and environmental restriction.

Despite the fact that fur chewing is found on up to 90% of Finnish fox farms (Ahola et al., 2014), and the farmers and other stakeholders have communicated various hypotheses of this behaviour, the causes of this abnormal behaviour are not understood in detail in blue foxes. We surveyed the fur farmers' and other farming experts' understanding of the phenomenon and the possible factors affecting the fur chewing behaviour in blue foxes.

Material and methods

The factors possibly relating to fur chewing behaviour communicated by farmers and other stakeholders, and those found from the literature were identified and formulated to a questionnaire. The initial questionnaire was tested by seven board members of the Finnish Fur Breeders' Association. The final version of the questionnaire consisted of 23 questions, with most of them including sub-questions. The questions covered the background of the respondent, and detailed questions of the respondent's view of the prevalence and aetiology of the fur chewing behaviour. The questions included dichotomous (yes/no) questions, multiple choice questions (e.g.

"do not affect", "increase" or "decrease" fur chewing) and open questions. It took 10-15 mins to complete the questionnaire. The questionnaire, in Finnish or Swedish, is available upon request from the authors.

The Webropol questionnaire, including a short cover letter explaining the purpose and scope of the study, was advertised in the farmer letters of the Finnish Fur Breeders' Association three times in the winter of 2018-2019 (October-April). The authors spread information of the questionnaire also directly (e.g. in meetings or via email) to other stakeholders. It was ascertained that the Webropol questionnaire could be filled in also by using mobile devices. It was also possible to answer in paper form; the questionnaires with a freepost envelope were made available at the meetings of the farmers in autumn 2018. The total numbers of farmers and stakeholders getting the information of the survey was approximately 900 persons.

The percentages of various responses were calculated, and reported, without differentiating the type of the respondent. Not all respondents answered all questions or sub-questions, and therefore the percentages presented below were calculated out of the responses to the question/sub-question, and not from the total number of respondents. The 23 questions of the questionnaire were re-arranged to larger units for presenting the results.

Results

A total of 71 persons (response rate less than 10%) responded to the questionnaire. A majority of the respondents (93%) were fur farmers and the rest were other stakeholders. Up to 87% of the respondents were from the Ostrobothnia, which is the main fur farming area in Finland. Up to 89% of the respondents raised blue foxes currently and up to 61% of the respondents had an experience longer than 20 years of working with blue foxes. A total of 53% of the respondents raised (also) silver foxes (*Vulpes vulpes*), 70% crossbreed foxes (a cross between blue fox and silver fox), 35% mink (*Neovison vison*) and 18% Finnraccoons (*Nyctereutes procyonoides*).

The respondents evaluated that fur chewing most typically occurs in 0-10% of blue foxes (72% of respondents), the less frequent options being "does not exist" (14%), "exists in 10-20% of foxes" (9%) and "I do not know" (4%). A majority of the respondents considered that silver foxes (63%) and crossbreeds (61%) do not perform fur chewing at all. However, 23 and 33%, respectively, of the respondents held the view that a maximum of 10% of these two fox types perform fur-chewing, and 2% of the respondents evaluated that fur chewing occurs even in 20-30% of these types of foxes. Fur chewing was evaluated to occur most typically in less than 10% of mink (57% of respondents). Also higher and lower percentages got support: in 10-20% of mink 4%, up to 20-30% of mink 4% and no fur chewing in mink 6%. Majority of the respondents could not evaluate the level of fur chewing in Finnraccoons, but those who did (47 %), responded that it does not exists (26%) or at the maximum of 10% of Finnraccoons (21%).

Fur chewing is found most often in the tail, hips and flanks in blue foxes (Figure 1). Fur chewing was evaluated to be most severe in the tip of the tail (93% of the respondents). In the open comments it was reported several times, that fur chewing starts from the tip of the tail, may continue to the rest of the tail and thereafter to the flanks/back. In the open comments, it was stated both, that fur chewing gets serious within a few days and that it takes a longer period (even months) to become serious. The respondents evaluated that the fur chewing behaviour steadily increases as the autumn proceeds and it is most intense in winter (January-February), decreasing thereafter with advancing spring. In the open comments, it was often mentioned that fur chewing starts when restricted feeding starts, i.e. conditioning of breeding females for breeding.

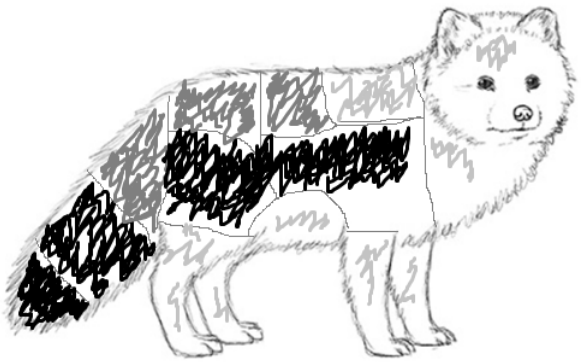
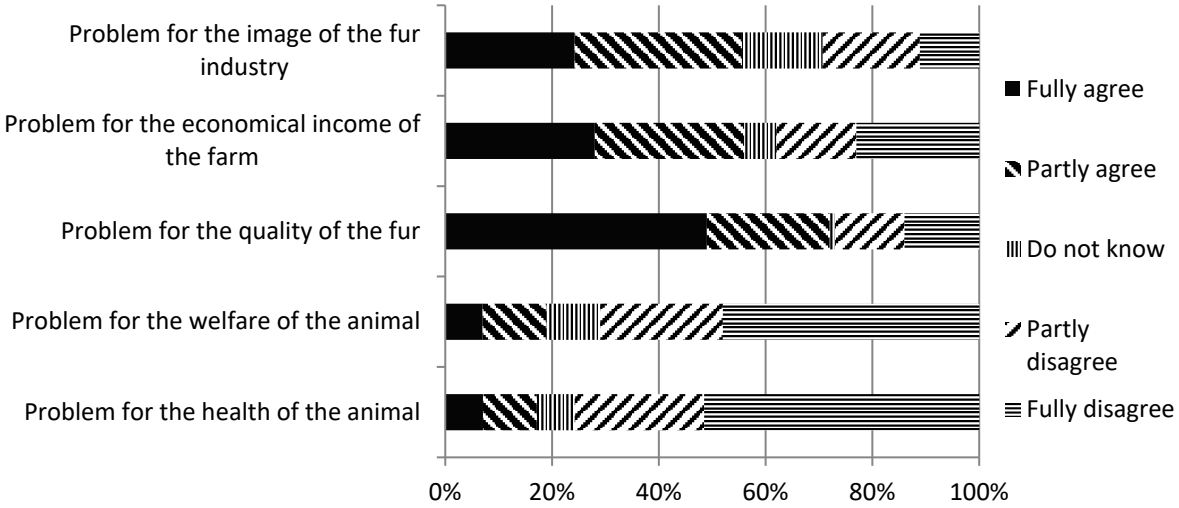


Figure 1: *The fur chewing areas in blue foxes. More than 75% of the respondents reported fur chewing in the areas marked in black, from 50 to 75% of the respondents reported fur chewing in areas marked in dark grey; and less than 50% of the respondents reported some fur chewing in areas marked in light grey. The painted area further indicates the severity of the fur chewing in each area, the larger the area, the more severe fur chewing.*

The respondents did not consider fur chewing as a problem for the health or welfare of the blue fox, but they considered fur chewing as a problem for the quality of the fur (Figure 2). The respondents had somewhat dichotomous attitudes to the effect of fur chewing into the image of the industry and economical result of the farm. However, in both cases, more of the respondents agreed (at least partly) with the statement than disagreed.

Figure 2: *The respondents' evaluation whether fur chewing is a problem for the health of the animal, welfare of the animal, fur quality, economical income of the farm or for the image of the fur industry.*



The respondents were asked to evaluate whether 37 separate factors are somehow connected to fur chewing behaviour in blue foxes. Up to 76% of the respondents thought that the fur chewing behaviour is connected to the season. Over 50% of respondents connected also genetic disposition (heritability), temperament of the animal and body condition to fur chewing behaviour. From 40 to 50% of the respondents connected the gender (in open comments fur chewing is reported more common in females than in males), animal stock, hormone levels and vitamin levels in the feed to fur chewing. From 30 to 40% of the respondents connected appetite of the animal, whelping and mineral content of feed to fur chewing. From 20 to 30% of respondents connected weather, year, activity level of the animal, gut microbiota, feeding frequency, and hygienic quality, fat, carbohydrate and protein content of the feed to the fur chewing behaviour. Only less than 20% of the respondents connected the following issues into the fur chewing behaviour: quality of the fur, colour type, poor peripheral circulation (very much "I don't know" – answers), unusual vocalization of the animal,

behaviour of the cage mate, behaviour of the stockperson, size of the cage, number of animals in the cage, location of the cage in the shed, shed itself, dry matter content in the feed, quality of the drinking water, availability of drinking water, general management of the farm, hygiene of the environment, purchasing new animals to the farm and removal of the manure.

The respondents were very unsure about the factors that may decrease fur chewing in blue foxes (Figure 3). These factors were availability of numerous activity objects, availability of straw/hay, confident temperament of the animal, obesity of the animal and availability of a platform, but only 10-20% of the respondents found these factors decreasing fur chewing. Instead, many factors were considered increasing fur chewing. These included e.g. restricted feeding/hunger, earlier tendency to fur chewing, stress, cold weather, agonistic behaviour in social housing units, other stereotypic behaviour and feeding less often than daily. However, most of the respondents found no significant connection between the listed factors and occurrence of fur chewing. In the open comments, for example poor feed efficiency, lack of some nutrients (not specified what nutrients), lack of B vitamin, lack of occupation/boredom during mid-winter, start of the heat and need for nesting behaviour (in spring) were mentioned increasing fur chewing. Also some farmers state that they eliminate the fur-chewers from breeding animals stock, and can this way decrease occurrence of fur chewing behaviour on the farm. These farmers also typically mentioned their worry about the effect of fur chewing to the image of the fur industry.

The respondents were asked to compare the fur-chewers against the non-fur chewers. Most of the respondents did not state any difference between these two types of blue foxes (Figure 4). Only, the survival in various weather conditions was evaluated more often worse in the fur-chewers than in non-fur-chewers. In contrast, the success in cub nursing, cub result and tendency to begin gestation were evaluated more often better in the fur-chewers than in non-fur-chewers.

Figure 3: *The respondents' opinion on whether the listed factors decrease, do not affect or increase fur chewing.*

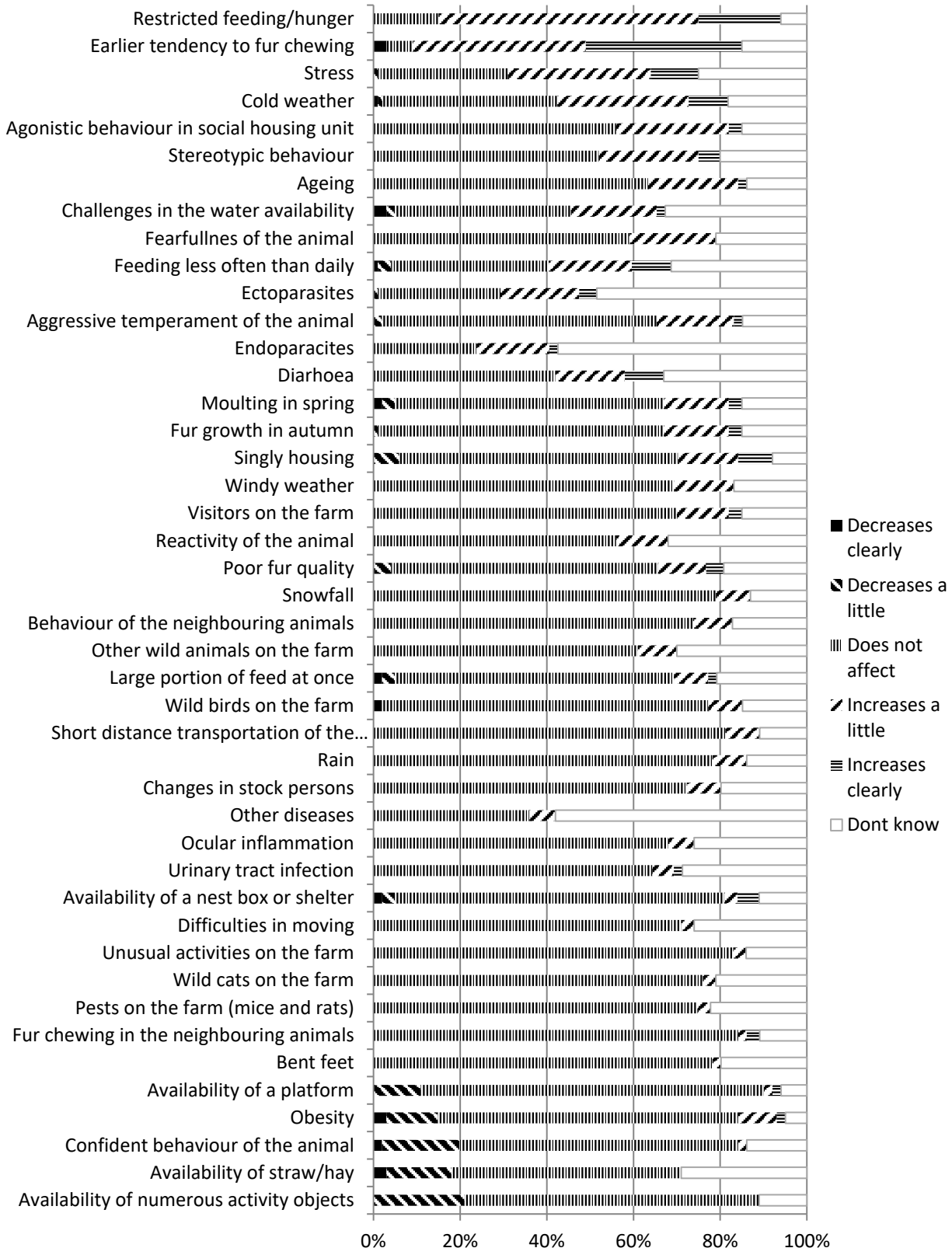
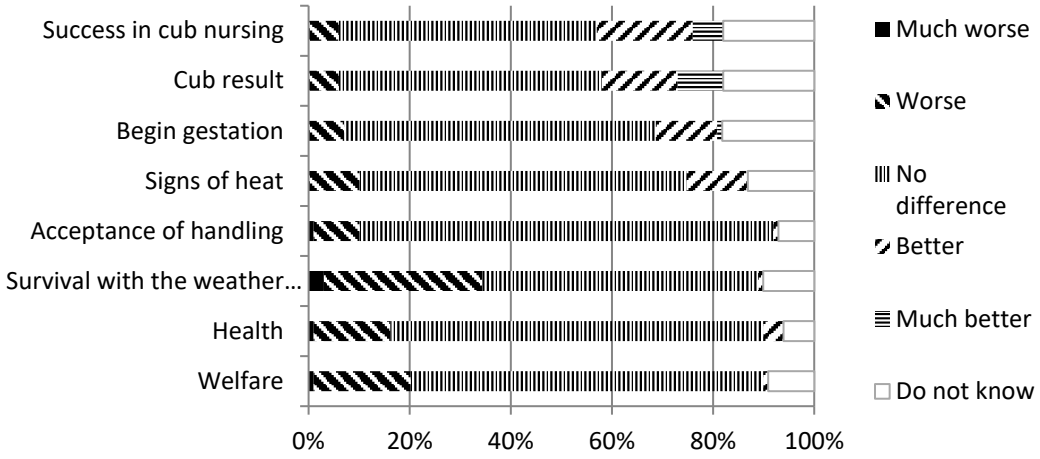


Figure 4: *The respondents' comparison of the fur chewers against the blue foxes not performing fur chewing.*



Discussion

The response rate of the questionnaire was relatively low, but we consider that 71 replies can be used to increase the information of the causation of fur chewing behaviour in blue foxes.

According to the present data, two main hypothesis of fur chewing behaviour can be drawn: the feeding-nutrient hypothesis and the behaviour-temperament hypothesis. According to the feeding-nutrient hypothesis, the animal tries to compensate the suboptimal feeding regimen and/or composition of the feed by performing fur chewing behaviour (chewing or eating fur). The conditioning of females to reach an optimal body weight for gestation means changes to both, composition of the feed and management of feeding (feeding frequency and portion size). This indicates that hungry animals, with empty stomach, and without source of fibre or oral manipulation are prone to fur chewing behaviour. Other option is that the nutritional quality of the feed is suboptimal, i.e. lack or surplus of vitamins and minerals, like many responders suggested. In the behaviour-temperament hypothesis, it is suggested that certain types of animals are prone to fur chewing behaviour as a response to e.g. fear of human or boredom. This is supported by the fact that many of the responders reported earlier fur chewing being a risk factor for future fur chewing. Stress and agonistic behaviour were reported to increase fur chewing, whereas there was some indication that confident behaviour might decrease fur chewing. Furthermore, small proportion of the respondents evaluated that access to activity objects, straw/hay and platform decrease fur chewing. This indicates that the animals benefit from the increasing complexity of the housing condition, which provides more occupation and choice.

Since the farmers and stakeholders do not see fur chewing as a problem to the health or welfare of blue foxes (except for the survival in cold temperatures), but instead the fur chewer females are considered succeeding better in reproduction, the elimination of the fur chewing behaviour from the blue fox stock is challenging. Furthermore, since the most severe fur chewing was evaluated to occur and has been earlier observed to occur in mid-winter (Ahola et al., 2014) and not before or at the pelting season, the effect to the income of the farm is not straightforward. This may explain the dichotomous attitude to the questions about the effects to the economic income of the farm.

As a conclusion, fur chewing behaviour is well known phenomenon and fur farmers' and other farming experts' have various opinions of the reasons behind it. The fur chewing behaviour in blue foxes may indicate

sub-optimal welfare through challenges in feeding or through a mismatch between the temperament/behaviour of blue fox and the current housing conditions and management practices. However, not all farmers considered fur chewing as a serious threat to the welfare of blue foxes, which may be due to the image of the better breeding result in fur-chewers than in non-fur-chewers. This may also make it hard to eliminate the fur chewing behaviour from the whole blue fox population, although some farmers report eliminating fur-chewers from their stock of breeding animals. The gathered information has been used for designing experimental hypotheses for the studies exploring development and prevalence of the fur chewing in blue foxes.

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

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Summary
The score calculation system of the WelFur Finnraccoon on-farm welfare assessment protocol (WelFurFR) differs slightly from those of the WelFur fox and mink protocols. We describe here the WelFurFR scoring system and its development. An expert panel was responsible for creating the data, i.e. the 'expert opinion in a numerical form'. These data were required in constructing the rules and formulae that convert the original measurement data into measurement scores and to aggregate the measurement scores into the criterion scores. The aggregation of the criterion scores to principle scores is done in WelFur FR 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. The four overall principle scores are calculated as weighted averages of the period-wise principle scores, the weights being: winter 0.2, summer 0.4 and autumn 0.4. The final WelFur classification is then determined from these scores with the same set of rules as in foxes and mink. The lessons learned while developing the WelFurFR scoring system may help in the revision of the fox and mink scoring systems.

Keywords: *Nyctereutes procyonoides*, fur farming, animal welfare

Introduction

The on-farm welfare assessment protocols for foxes (WelFur, 2015a), mink (WelFur, 2015b) and Finnraccoons (WelFur 2020) use the Welfare Quality® approach (Mononen et al., 2012, Blokhuis et al., 2013). The scoring systems are an essential part of the welfare assessment protocols. The general structure of the scoring system is similar in the WelFur (WelFur, 2015a,b, 2020) and WQ (Botreau et al., 2013) protocols. The original measurement data are first interpreted into 12 welfare criterion scores (0 - 100), then criterion scores are aggregated into the four welfare principle scores (0 - 100), and finally the principle scores determine the overall category (one out of four) of the farm (Table 1).

Table 1. *The general structure of the WelFur (2015a,b 2020) and Welfare Quality® (WQ; Botreau et al., 2013, Welfare Quality®, 2009) scoring systems.*

Level	***Type of information***	***Additional information***
Original measurements	Typically, percentages of animals in various 'welfare situations' on a farm, or the situation prevailing for all the animals on the farm.	The measurements and their numbers vary between the species: e.g. mink 22, foxes 25 and dairy cattle 30. See WelFur (2020) for the 25 measurements in the WelFur Finnraccoon protocol.
Criterion scores	Score from 0 - 100: 0 - 20 = Not acceptable 20 - 50 = Acceptable 50 - 80 = Enhanced or Good 80 - 100 = Excellent or Best	The same 12 criteria (see e.g. WelFur, 2020) for all species, but the number of measurements determining each of the 12 criterion scores varies between the species.
Principle scores	Score from 0 - 100: 0 - 20 = Not acceptable 20 - 55 = Acceptable 55 - 80 = Enhanced or Good 80 - 100 = Excellent or Best	The same four principles for all the species, determined by the criterion scores (essentially in the same way for the different species): *Good feeding*, *Good housing*, *Good health*, *Appropriate behaviour*.
Overall welfare category	Four categories: Excellent (WQ) or Best (WelFur), Enhanced (WQ) or Good (WelFur) Acceptable Not acceptable	The final category is determined by the four principle scores. The same rules for all the species.

The development and the structure of the scoring systems of the WelFur fox and mink protocols followed the model of WQ (Botreau et al., 2012; with one marked exception: see section 2 below). We describe here the WelFur Finnraccoon scoring system (WelFur 2020) and its development, an emphasis being on highlighting the differences to the fox and mink protocols.

From measurements to criterion scores

A Finnraccoon expert panel was responsible for interpreting how the on-farm welfare measurement data are transformed into the measurement welfare scores (0-100), and how the measurement scores are aggregated into the criterion scores (0-100). The panel consisted of two independent animal welfare scientists, a veterinarian and a scientist both working for the fur industry, and two Finnraccoon farmers. All the panel members were from Finland. The work of the expert panel was facilitated by an external animal welfare scientist who had participated in the development of WQ scoring systems.

The experts were asked to assign welfare scores to various welfare situations on imaginary farms. Due to the differences between the measurements, five different types of tools were required in the scoring, and the imaginary situations were presented to the experts accordingly (Table 2; see also Figure 1 and Tables 3 - 6). Note that the decision tables used in the Finnraccoon scoring system (see Tables 4 - 6) are logically the same as the decision trees used in the fox and mink protocols (c.f. Botreau et al., 2012), i.e. just another form for presenting the same information.

A ‘Delphi-like method’ was used to find consensus among the members of the expert group. The members did the scorings individually, after which the data were compiled to identify the differences in their answers, and finally one shared opinion of the expert group was formed in discussions lead by the facilitator. For the mink and fox protocol the experts did the scoring independently and the consensus opinion was a result of mathematical modelling (Botreau et al., 2012). A weakness of the latter is that it hides the range of the answers. Plenty of true measurement data were available already in this phase, and, thus, the Finnraccoon experts were also very well informed of the real situation on the farms. This helped them to ‘calibrate the level of the scores’.

A marked difference between the WelFur Finnraccoon protocol and the fox and mink protocols is how the period-wise data is aggregated into the criterion scores. In the fox and mink protocols the measurement scores are aggregated into the criterion scores across the periods (Botreau et al., 2012; see also WelFur, 2015a,b). In the Finnraccoon protocol (WelFur 2020) the criterion scores, as well as the principle scores, are calculated separately for each period. If the criterion has only one measurement in a period, its score is the criterion score for that period. If a criterion has more than one measurement, the measurement scores are aggregated to the criterion score by calculating their weighted sum. In addition, a penalty procedure may be applied to reduce the compensation of the lower scores by the higher scores in this aggregation phase. It is applied if, and only if, any of the individual measurement scores to be aggregated has a value lower than 50. The penalty is subtracted from the weighted sum to get the final criterion score. An example of the penalty procedure is presented in Tables 7 and 8. This procedure differs from the WelFur fox and mink protocols where the Choquet integrals are employed for both the weighing the measurements and reducing their compensatory effects while aggregating scores (see also Botreau et al., 2008). We consider the ‘weighted sum with penalty’ method, with the clear steps, more transparent than the Choquet integral method.

Table 2. *The tools in the WelFur Finnraccoon scoring system for transforming the original measurements into welfare scores (0-100). N refers to the number of measurements for which the tool is used.*

Tool type (N)	Tool description, example of measurement and notes
Curve (14)	• Percentage of animals with an impaired welfare state is transformed into the final measurement score using up to third-degree polynomial functions (or curves). • Example: % of too lean Finnraccoons in Periods 1 and 3: the experts were asked to score situations with 0, 1… …100% of too lean animals on the imaginary farms. See Figure 1. • Note: In some cases the final percentages are calculated as a weighted sum of percentages of animals with impaired welfare states of varying severity (e.g. % of Finnraccoons with moderately and severely dirty fur), and then the experts were asked also to weight the severity categories.
Weighted sum (3)	• Percentages of animals in varying welfare situations or percentages of animals showing certain behaviour patterns is transformed into the final measurement score by calculating a weighted sum of the percentages. • Example: % of Finnraccoons with a resting shelter with zero (i.e. no resting shelter), one, two or three walls. See Table 3.
Decision table: farm level only (3)	• Construction of a decision table leading to various possible situations, i.e. different combinations of two or more kinds of categorical data. Each situation has an assigned welfare score, and the score corresponding to the situation prevailing on the whole farm, is considered as the final measurement score. • Example: the killing procedure with altogether up to 4 - 16 situations. See Table 4.
Decision table: individual level and a % rule (4)	• Construction of a decision table leading to various possible situations, i.e. different combinations of two or more kinds of categorical data, with assigned scores. Each situation has an assigned welfare score, and the score for the worst situation observed on at least a pre-determined percentage of the Finnraccoons is considered as the final measurement score for the farm. • Example: protection from wind with altogether 6 situations and a 10% rule. See Table 5.
Decision table: individual level and calculating a mean (1)	• Construction of a decision table leading to various possible situations, i.e. different combinations of two or more kinds of categorical data. Each situation has an assigned welfare score, and the mean of the scores of the individual animals is the final measurement score for the farm • An opportunity to use activity object with five situations. See Table 6.

Figure 1. *An example of a curve converting percentage of too thin animals (x) into the Body condition measurement score (y) (in the Periods 1 and 3). Note that the curve is a 'polynomial spline function' that comprise of more than one polynomial with the polynomial applied changing at certain places, here this 'knot' is at the x-value 10%.*

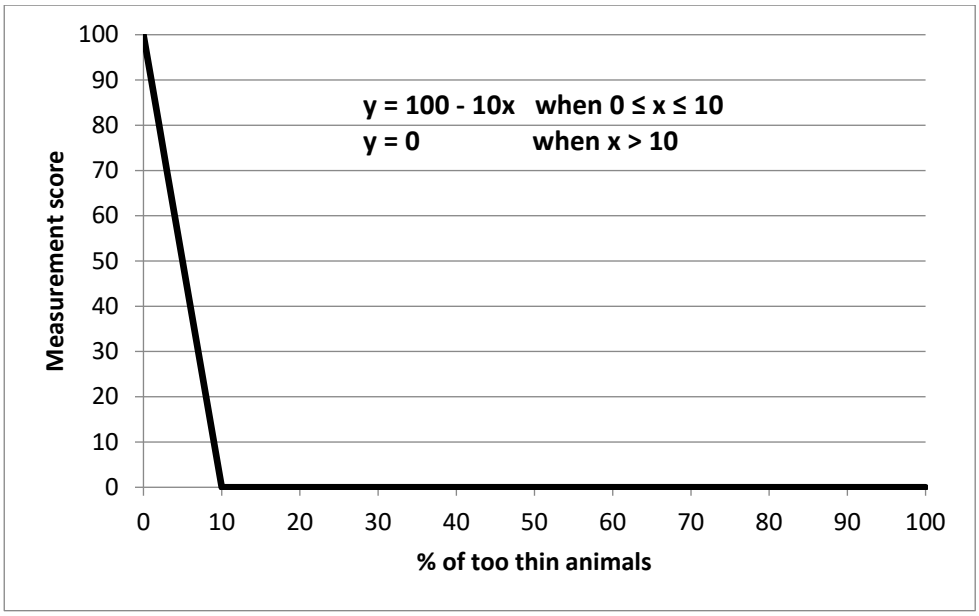

Table 3. *An example of calculating the weighted sums: converting the percentages of animals in various situations with regard to the solid walls in their cages into the Resting shelter measurement score (all periods).*

Percentages of animals in cages with				***Measurement score***
no walls (x_0)	one wall (x_1)	two walls (x_2)	three walls (x_3)	$y = 0.5x_1 + 0.9x_2 + x_3$
100	0	0	0	0
50	50	0	0	25
25	25	25	25	60
0	0	50	50	95
0	0	0	100	100

Table 4. *An example of the decision table method: the score for a farm is read directly from the table. The situation that prevails at the farm level determines the measurement score for the Emergency killing measurement (in the Period 1).*

Situation number	**Killing device**	**Certification of the device**	**Measurement score**
Situation 1	Electrocution	Yes	100
Situation 2	Electrocution	No	60
Situation 3	Other	-	80
Situation 4	No killing device	-	0

Table 5. *An example of the decision table method with the percentage rule. In this example the score for the farm is the worst score (= the one corresponding to the worst situation found on the farm) observed in at least 10% of the animals. For example a farm with 30 animals (25%) in Situation 3, 60 (50%) animals in Situation 5 (50%) and 30 animals (25%) in Situation 5 would get a score 35 for the Protection from wind measurement (in the Period 1).*

Situation number	Environmental protection from wind	Wind shield	Measurement score
Situation 1	Yes	Large	100
Situation 2	Yes	Small	80
Situation 3	Yes	No	50
Situation 4	No	Large	70
Situation 5	No	Small	35
Situation 6	No	No	0

Table 6. *An example of a decision table method: the measurement score is calculated as the average of animals in the various situations. For example, a farm with 60 animals in Situation 4 and 60 animals in Situation 5 would get a score (60 x 40 + 60 x 0)/120 = 20 for the Availability of activity object measurement (in the Period 2).*

Situation number	Type of object	Number of object per animal	Measurement score
Situation 1	At least two different	At least one per animal	100
Situation 2	At least two different	Less than one per animal	90
Situation 3	One type	At least one per animal	80
Situation 4	One type	Less than one per animal	40
Situation 5	No	-	0

Table 7. *An example of aggregating measurements into a criterion score. The Possibility for horizontal movement (y_H) and Possibility for vertical movement (y_V) measurement scores are aggregated to form the Ease of movement criterion score by first calculating their weighted sum, applying the weights (w_H and w_V, respectively) presented in the table below (Step A). Then, the penalty is subtracted from this sum if, and only if, at least one of y_H and y_V is lower than 50 (Steps B and C). See Table 8 for a calculation example.*

Step A: Calculating weighted sum (y_{WS}) of Horizontal movement (y_H) and Vertical movement (y_V)		
Weight: w_H	**Weight: w_V**	**Weighted sum**
0.7	0.3	$y_{WS} = 0.7y_H + 0.3y_V$
Step B: Calculating penalty (y_{Pen})		
A penalty (y_{Pen}) is calculated, if and only if y_H or y_V or both of them have value lower than 50: $y_{Pen} = w_i (50 - 0.402z - 0.0431z^2 + 0.0006228z^3)$, *where z is the lower of the values y_H and y_V and w_i is the weight (see Step A) of this measurement.*		
Step C: Calculating final criterion score C_5		
$C_5 = y_{WS} - y_{Pen}$ If $(y_{WS} - y_{Pen}) < 0$, then $C_5 = 0$.		

Table 8. *An example of aggregating Possibility for horizontal movement (y_H) and Possibility for vertical movement (y_V) measurement scores into the Ease of movement criterion score when one of the measurements (y_H) has a value lower than 50. See also Table 7.*

Score: y_H	**Weight: w_H**	**Score: y_V**	**Weight: w_V**
20	0.7	90	0.3
Step A: Weighted sum = 20 x 0.7 + 90 x 0.3 = 14 + 27 = 41			
Step B: Penalty = 0.7 x (50 - 0.402 x 20 - 0.0431 x 400 + 0.0006228 x 8000) = 0.7 x 29.7024 = 20.8			
Step C: Final score = 41 - 20.8 = 20.2			

From criterion scores to principle scores

The aggregation of the criterion scores to principle scores is done in the Finnraccoon protocol (WelFur 2020: pages 88-92) with the same Choquet integrals as in the mink and fox protocols. The parameters of the Choquet integrals were obtained from the WQ data (Botreau et al 2012). However, unlike for the mink and foxes, the criterion scores and consequently also the principle scores of the Finnraccoon farms are calculated separately for each of the three data collection periods. The benefit of this is that the usability of the assessment results for advisory purposes increases as the period data are less hidden.

Due to the lack of measurements for some of the criteria, there are no criterion scores for all the criteria of the *Appropriate behaviour* principle in all the periods in the Finnraccoon protocol (WelFur 2020). The principle scores cannot be calculated if there are missing criterion scores. Therefore, the missing scores are replaced with other criterion scores within the *Appropriate behaviour* principle or their combinations. There is no criterion score for *Social behaviour* in the Period 1, when there are only adults on the farm, since social conditions are assessed only for the juveniles. Thus, the *Social behaviour* criterion score is replaced in the Period 1 with the *Other behaviour* score. The *Good human animal relationship* and *Positive emotional state* criteria are both assessed in the Period 1 with one test, the *Voluntary approach test*. The test would not be reasonable and is not carried out in the Period 2, when many or most of the females can still live with their small cubs and protect them. Therefore, the *Good human animal relationship* and *Positive emotional state* criteria are replaced in the Period 2 with the average of the *Social behaviour* and *Other behaviour* scores, and the *Other behaviour* score, respectively. In the Period 3, the *Voluntary approach test* is measuring only *Good human animal relationship* and the score for the *Positive emotional state* criterion is replaced in that period with the *Other behaviour* score. The *Other behaviour* criterion includes several measurements concerning the diversity of the housing environment.

From principle scores to final welfare category

Since the principle scores are calculated for each period in the WelFur Finnraccoon protocol, the final welfare category (out of four categories: see Table 1) of a farm can be determined both per periods and across the periods. In both cases the same set of rules as in the fox and mink protocols are used (see WelFur, 2020: pages 94-96). In determining the three period-wise overall categories for the Finnraccoons farms, the period-wise principle scores are used as such. Instead, when determining the overall category across the three periods, the weighted averages of the period-wise principle scores are calculated first, and then these 'combined principle scores' are used for determining the overall welfare category. The winter period has a lower weight (0.2) than the summer (0.4) and autumn (0.4) periods, because of the challenges in the data collection resulting from the inactivity of the animals in the winter.

Concluding remarks

We have described above the score calculation system of the WelFur Finnraccoon on-farm welfare assessment protocol. The lessons learned in the development of the WelFurFR scoring may help in the revision of the fox and mink scoring systems. Apparently, there are no reasons that would speak against the calculation of the criterion and principle scores (and determining also overall welfare categories) per periods also in the fox and mink protocols. The use of weighted sums with a penalty procedure instead of Choquet integrals in aggregating measurement scores into criterion scores requires probably more research. However, if it turns out to be an appropriate method, it could replace the Choquet integrals also when aggregating the criterion scores into principle scores.

Acknowledgements

The authors are grateful to the members of the expert group, their facilitator, and Mr. Jyrki Sura, Head of WelFur Implementation and Management at Fur Europe, for their valuable efforts and fruitful discussions during the scoring system development. The work was funded by Fur Europe.

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

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Abstract
The assessment of the measurement 'Weaning age and procedures' in WelFur-mink is based on scientific knowledge of which much dates back more than 20 years. Recent studies have shown that litter size is a 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 different weaning procedures differs with litter size for dams as well as kits. We therefore hypothesized that a better reflection of the total welfare effect of weaning could be achieved by including the litter size in the assessment on top of the weaning age and procedures that are already in the present WelFur assessment protocol. We tested the effect of litter size in combination with age and procedure of weaning by weaning at least half the kits from litters of six or more kits at six weeks of age and weaning the remaining kits at eight weeks, compared to weaning all kits at eight weeks. We found that this partial weaning at six weeks gave more short-term stress responses after weaning in kits as well as in dams, because the partially weaned group had a response at six weeks while both the partial weaning and the control group reacted similarly after weaning at eight weeks. Partial weaning also had some unexpected positive long-term effects around seven and ten month post-partum, in terms of more explorative and less fearful temperament, and less fur chewing.

Introduction
Weaning age and procedures is the indicator of welfare for the criteria 9. 'Expression of social behaviours' under Principle 4. 'Appropriate behaviour' in the reproduction period of the WelFur-Mink protocol (Møller et al. 2015). The assessment of Weaning age and procedures is based on scientific knowledge of which much dates back 20 years or more. Early weaning at 6 weeks, typically compared to 8-11 weeks is reducing the welfare of the kits in both the short and long term (Hansen et al., 1997; Heller et al., 1988; Malmkvist et al., 2007; Mason 1994; 1996). Kits weaned at 6 weeks of age and housed singly were more in the cage, and croaked significantly more until 15 hours after weaning, than kits placed in groups or in pairs (Hansen et al., 1997).

Based on activity in and out of the nest box, croaking sound from the kits and the level of eosinophil leucocytes in the blood, Houbak and Jeppesen (1988) concluded, that weaning at 6 weeks of age is more stressful for the female and the kits than weaning at 8 weeks of age while weaning at 10 weeks of age is more stressful for the female and possibly also for the kits than weaning at 8 weeks of age. Mason (1994) reported that female kits weaned at 7 weeks of age had more fur chewing on the tail at six months of age than female kits weaned at 11 weeks of age while the difference was not significant for male kits. However, this study did not reveal the welfare consequences of mink under production conditions as the late weaned litters were reduced to four or five kits per litter before the final weaning, in order to prevent crowding, fighting and loos of body weight of the mother. The reduction to four kits in this study indicates that litter size and effects of weaning are related. Recent studies have shown that litter size is a 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 (Malmkvist, 2016). We therefore hypothesized that an assessment focusing not only at the age and procedures at weaning but also on the number of kits in the litter, would more precisely reflect the total welfare effects of

weaning, compared to the present welfare assessment protocol focusing mainly on age and what happens after the separation of the mother and kits.

Materials and Methods

The effects of weaning age and procedure was investigated in 2018 by looking at 160 brown and 139 palomino litters of six to eleven kits of which 153 were weaned at eight weeks of age (Control) and 146 litters was partially weaned at six weeks of age (Partial weaning). At partial weaning, at least half the kits were moved to the neighbouring cage at 6 weeks, depending on litter size in the following way: From a litter of 6, 2 remained with/4 were weaned from the mother. For larger litter the following numbers remained with/were weaned from the mother: 7=3/4; 8=3/5; 9=4/5; 10=5/5; 11=5/6 (Clausen & Larsen, 2015). The effects were observed as the animals position in the cage, activity, behaviour (play, aggression, stereotypy) and vocalisation in the mother and kits observed at six weeks (day 0), day 1 (not stereotypy), 2 and 6 days later in both groups. Long-term effects were measured as stereotypy and temperament in juveniles at around six month of age in November and as fur chewing and injuries inspected at the bodies just after killing for pelting of juveniles in late November. Finally, stereotypy and fur chewing was observed in February while temperament was observed in April in first year as well as in older females selected as breeders.

Results

We found that partial weaning at six weeks gave more short-term stress responses after weaning in kits as well as in dams. This was because the partially weaned group had a response after weaning at six weeks, including significantly more stereotypy in the mothers on the day of weaning, while there were no difference in play or aggression between kits between the two groups. On the day of partial weaning and the day after, the kits that vocalised did so significantly more often, while the percentage of kits that vocalised was not different from the control group. The kits from partial weaning also vocalised more often after weaning at 56 days, than kits in the control group. More females from partial weaning than from the control group vocalised at the day of weaning at 42 days, but not day 1 or later and not after weaning at 56 days.

The long-term effects of partial weaning included more explorative and less fearful juveniles at seven month post-partum ($P<0.05$) (Table 1), and more aggressive and less fearful in the dams selected as next year's breeders. Seven month post-partum it also included less fur chewing in juveniles.

Table 1. *Distribution of temperament in juveniles in October. They were either partially weaned at 6 weeks or weaned normally at 8 weeks (Control).*

	Partial weaning	Control
Exploratory	59.00	49.63
Fearful	22.75	31.48
Aggressive	0.50	0.74
Indecisive	17.75	18.15

Discussion

The results lend support to our hypothesis that weaning procedures focusing not only at the age at weaning but 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 and what happens after the separation of the mother and kits. The extra stress of partial weaning at 42 days, evident in dam and kits acute behaviour (stereotypies, vocalisation), is not surprising given the previous results on full weaning of the entire litter at six weeks pp (Houbak & Jeppesen, 1988; Hansen et al., 1997; Jeppesen et al. 2000). However, the fact

that the dam keeps some kits may shorten the effect to the time span of only one to two days observed. The kits moved at partial weaning were placed in the neighbouring cage. One possibility is that placement of kits in the neighbouring cage rather than out of sight and hearing range, may induce or contribute to these acute effects.

The difference in litter size at weaning at 8 weeks between the 2 to 5 kits remaining in the partial weaning group compared to the 6 to 11 kits in the control group may help to explain why there were no difference between the two groups to the weaning at eight weeks. This may be because the burden of having 2 – 5 kits at 8 weeks is limited, increasing the burden of weaning, while having 6 – 11 kits is a heavy burden for which weaning at 8 weeks is not only a burden but also a relief to the female.

Compared to the negative effects of weaning all kits in a litter at 6 weeks it appears that partial weaning at 6 weeks does not have such negative long-term effects. In contrast, we found some rather unexpected positive effects, in terms of more exploratory and less fearful juveniles, and less fur chewing, compared to weaning all kits in a litter at eight weeks of age. As the weaning age in itself should have negative long-term effects, these positive effects are likely to stem from a positive effect of the litter size being reduced by the partial weaning. These long-term effects on temperament and behaviour are interesting and surprising. The repeatability therefore needs to be investigated as well as potential mediation or causing factors behind the effects.

The knowledge gained from these studies can be included in a future version 2 of the WelFur-Mink protocol but before that, the particular ‘Partial weaning’ procedure used on some Danish farms might be assessed more correctly.

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

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Abstract
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 pilot and first two actual measurement years are presented. At the beginning of the 2018 Fifur implemented the BMI into the Finnish fur certification system and the first certification auditions were performed during autumn 2018 and 2019. The calculations yielded average body weights for production animals between 14.2 and 16.2 kg. The results from the breeding animals were found to be lower than those of the production animals with values between 7 and 16.6 kg. On the other hand, the average body length did not vary as much between production and breeding animals. Consequently, the average BMI of production animals was 20.7-22.1 while for breeding animals it was 14.7-25.8. 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.

Keywords: weight, body length, fatness

Background
The fatness of Finnish blue foxes has increased significantly during the last two decades. From an animal welfare point of view, fatness has several negative consequences due to phenotypic and genetic correlations with health and fertility traits (Kempe et al. 2013; 2015). Therefore, in 2017 the Finnish Fur Breeders Association decided to develop an objective and effective, but at the same time simple and cheap, method to evaluate the level of fatness of blue foxes. As a result, the body mass index (BMI) for the blue fox was developed (Peura et al. 2017, Viksten 2017) and tested in autumn 2017 (Peura 2018). BMI was implemented into the Finnish blue fox certification system in autumn 2018. This article presents the results of the pilot test and the first two years.

Methods and materials
50 farms among certified Finnish blue fox farms were selected randomly in 2018 and 2019. Moreover, 20 breeding and 20 production animals were selected randomly within each selected farm. In 2018 the measurements were made during weeks 43-44 and in 2019 weeks 45-46. The theoretical background of weigh-for-length index (later body mass index, BMI) is described in detail in Peura et al. (2017) and Viksten (2018). BMI was calculated and standardized using the following equations:

$$\boldsymbol{BMI_{production\ animals}} = 25 + 5 * \left(\frac{BMI_{pre} - mean}{std}\right)$$

$$\boldsymbol{BMI_{breeding\ animals}} = 20 + 5 * \left(\frac{BMI_{pre} - mean}{std}\right)$$

where $BMI_{pre} = \frac{W}{L^P} * constant$, *W = animal weight (kg), L = body length (cm)* and parameter values as shown in Table 2.

Table 2. *Parameters used in equations*

	Males		Females	
	Breeding	Production	Breeding	Production
Constant	155,69	155,69	52,41	52,41
P	1,79	1,79	1,54	1,54
mean	0,925	1,285	0,734	1,289
std	0,192	0,197	0,151	0,189

Results

Figures 1-3 present the average body weight, body length and BMI for production and breeding animals. For production animals, the results from pilot year 2017 and actual measurement years 2018 and 2019 are presented. The results from breeding animals are presented only for 2019.

Figure 1. *Average body weight (kg) (± std) of production (left) and breeding (right) animals.*

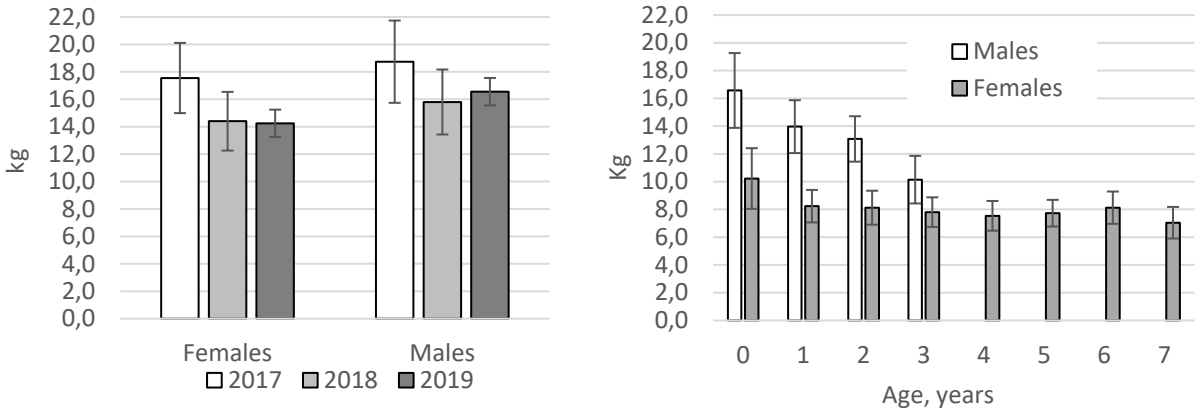


Figure 2. *Average body length (cm) (± std) of production (left) and breeding (right) animals.*

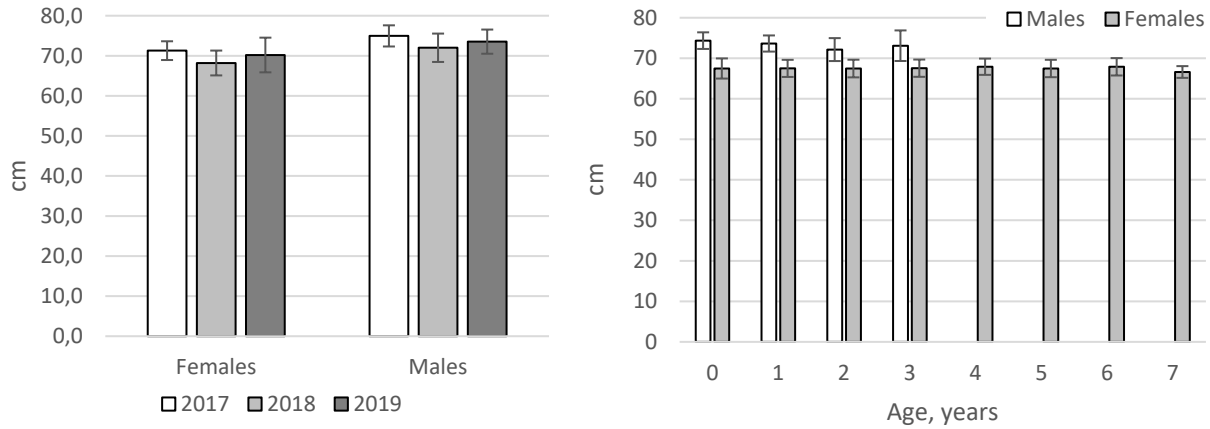
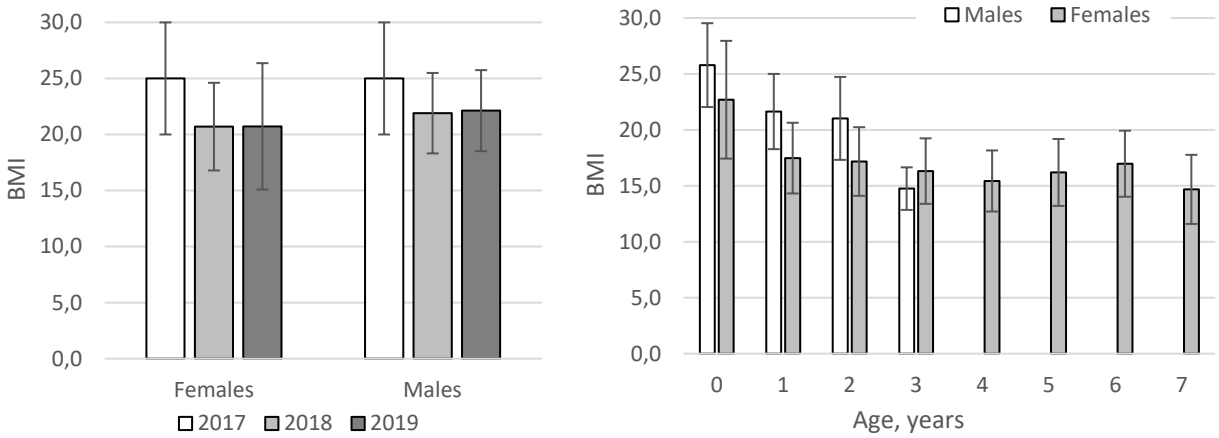


Figure 3. *Average BMI (± std) of production (left) and breeding (right) animals.*



Discussion

The BMI seems to be an efficient tool to evaluate fatness of blue foxes. Even though the measurements between years 2017, 2018 and 2019 are not completely comparable due to mild changes in measurement weeks, the results indicate, that the average fatness of blue foxes has not increased during the first measurement years. For females, fatness has even moderately decreased. In the Finnish blue fox certification system, the upper limit of BMI for production animals was 35 in 2018 and 2019 but for the autumn 2020 measurements the upper limit has been lowered to 32. The upper BMI limit of breeding animals is 30 and in autumn a new low limit will also be tested, which is set to be 14 and 12 for young (born in measurement year) and old breeding animals, respectively. Fifur has currently several ongoing projects, where optimal BMI for breeding animals in different production stages is studied. For breeding animals BMI is mainly a management tool to help farmers optimize their production since the fatness is not a big problem for the breeding animals.

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Effects of cage size on the welfare of farmed American mink (*Neovison vison*)

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Abstract
Canadian cage (C) sizes recently increased to improve mink welfare, but remain smaller than European cages (E). We assessed welfare in C- or E-raised Black females, and Pastel, melatonin-implanted pair-housed males. Female pairs (64) were split after 3 months; remaining 64 females spent 3 more months in E or C. Subsequently we collected data on stereotypic behaviour (SB), fearfulness, and faecal glucocorticoid metabolites (FCM). For half, *post mortem,* we assessed tail chewing and physiological/anatomical stress signs, while remaining females now gained access to both C and E cages to allow preference assessment. For male pairs (64) we recorded thermoregulatory behaviour (e.g. proximity to water line, drinking, lying spread out, avoiding touching) and temperature during 7 hot (>27ºC) and 7 cooler summer days (<27ºC). After 5 weeks, we removed alternate pairs, and gave remaining pairs access to both C and E cages again to allow them to show preference. C-raised females had higher levels of FCM, and tended to show signs of fear when handled. C-raised males were in contact with cagemates more, and showed fewer spread out postures, even on hot days. When offered a cage choice, females showed no preference. In contrast, males preferred to spend their time together in the type of cage they were not reared in, but this was most marked for C-raised males who, on hot days, preferentially used E cages for resting together in spread out postures. Results suggest that mink welfare in E is better.

Keywords: floor area, fur farmed animals, preference, thermoregulation, stress

Background
Canadian cage size recommendations for farmed mink have recently increased minimum floor areas as assumed to improve welfare (NFACC/CMBA 2013), yet these remain smaller than those under European regulations. In addition, while in Europe floor areas remain constant across the mink's life cycle, in Canada floor area varies with age, sex, and reproductive status. This is relevant because, for adult, single-housed females, Canadian floor areas can be 57% smaller than European cages. In addition, smaller floor areas might restrict movement and thus impair thermoregulation (Wustenberg and Wustenberg 1988): important to investigate generally and globally due to regional temperature differences; and particularly if individuals are implanted with melatonin to induce faster winter coat growth during the summer (Rose et al. 1984), as is common practice in Canada. We tested whether Canadian cages (C) underperformed in terms of welfare compared to European cages (E) in two subpopulations of mink: single-housed adult females (kept on-farm the longest, thus most at risk of experiencing chronic housing effects) and pastel, melatonin-implanted pair-housed males during summer (at higher risk of experiencing heat stress in smaller cages due to their size and earlier growth of winter fur).

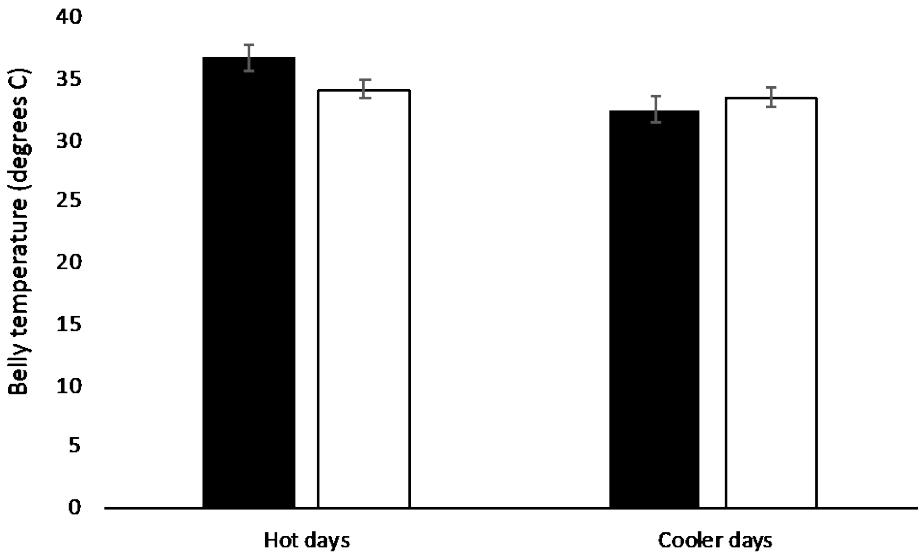
Methods and Materials

In two experiments, we raised 64 pairs of non-sibling Black females and 64 of Pastel males from weaning in either E or C. Each cage housed two mink and was provided with an identical nestbox, shelf and two enrichment items. Female pairs were split after 3 months and the remaining 64 females spent 3 more months individually housed in their E or C. At the end of this period we collected data on stereotypic behaviour (SB), fearful responses, and samples for faecal glucocorticoid metabolite (FCM) analyses. Half of our females were then humanely killed. *Post-mortem*, we estimated degree of tail chewing, and assessed housing effects on stress physiology (weight of adrenal glands), immune function (weight of thymus and spleen) and developmental stress (mandibular fluctuating asymmetry). The remaining females were given free access to the cage they had not been raised in and their preferences for either cage were assessed. For males, we recorded thermoregulatory behaviour (e.g., proximity to the water line, drinking, and the extent to which cagemates were touching when co-resting on the cage floor). When co-resting on the cage floor, temperature of heat dissipating areas (e.g. paws, belly) was also recorded via infrared thermography. Measurements were taken during 7 hot (>27ºC; mean±SD: 29.3±1.1ºC) and 7 cooler days (<27ºC; average±SD: 24.6±1.2 ºC). After 5 weeks, we removed every other pair. Remaining pairs were given free access to the cage they had not been raised in, and their preferences for either cage were assessed.

Results

There were no cage effects on female SB or tail chewing, but when C-raised females were handled, they tended to scream more ($p=0.07$) and had higher levels of FCM ($p<0.05$). When a choice of cage was offered, females allocated their overall time equally between E and C. Males spent more time by the water line ($p<0.001$), touched each other less ($p<0.001$), and adopted spread out postures more ($p<0.05$) on hotter days compared to cooler days. Compared to E-housed males, however, C-housed males were more likely to have body contact with a cagemate ($p<0.001$), and less likely than E males to adopt spread out postures or rest with their belly exposed ($p<0.001$ and $p<0.01$, respectively) when hot. Belly temperatures of C-raised males were also 2.5°C higher than E-raised males when hot, although this result was not statistically significant (Fig. 1). Rearing cage and ambient temperature did affect where and how paired males chose to rest together: while E mink showed no preference, C males preferred to co-rest displaying spread out postures in E when hot.

Figure 1. *Belly temperatures across 7 hot days and 7 cooler days. Black bars = Canadian-housed males; empty bars = European-housed males.*



Discussion

Overall, our results suggest that single-housed females in E cages might experience better welfare as they seem less fearful and physiologically stressed there; and that E cages also allowed paired melatonin-implanted males to express thermoregulatory behaviours more successfully. This is the first evidence that floor area *per se* affects mink welfare (cf. Hansen et al. 2007). These findings have important implications for cage size regulations.

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

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Summary
We studied the effects of concealment screens (S) and extra enrichments (E) on the ease of handling and temperament in 40 adult and 40 juvenile female blue foxes. The animals were allocated into four treatment groups BN, EN, BS and ES (basic E only=B; basic+extra E=E; no S=N; S=S). The foxes' behaviour was measured just before (August) and 3-4 months after (December) the onset of the experiment with three behavioural tests: the catch test (CT), the subjective evaluation of HAR (SEH) and the stick test (ST). In August, the animals of all groups responded similarly in the behavioural tests. In December, the juveniles in the S groups were more difficult to catch than the juveniles in the N and the adults in the S and N groups. The foxes with the screens approached the assessor in the SEH and explored the stick in the ST less frequently than the foxes without the screens. For the SEH the effect was confounded by the E and for the ST the effect was confounded by the age and E. The concealment screens lengthened the catching time of the blue foxes and had negative effects on the human-animal relationship.

Keywords: fur farming, animal welfare, catching, temperament, *Vulpes lagopus*

Introduction

The environment we provide for our production animals is of great importance from their welfare point of view. In the Finnish farm conditions, the blue foxes (*Vulpes lagopus*) are typically housed in wire mesh cages furnished with platforms and wooden blocks and/or bones as gnawing objects (as recommended by the Council of Europe 1999). The blue foxes make use of any movable objects (Korhonen et al. 2002). They use platforms for observation, resting with an unrestricted view (Mononen et al. 2001), withdrawing and/or escaping (Korhonen et al. 1996). It has been suggested that that the farmed foxes should have also an opportunity to hide (Council of Europe 1999). However, a hiding place may impair the development of an appropriate human-animal relationship (HAR) (Harri et al. 1998, Pedersen 1991) and complicate routine daily care of the animals, such as health checks. These effects might be mitigated by enrichments that promote the sense of predictability and controllability of the environment, and consequently the coping ability of the foxes (Keeling and Jensen 2009) and good human-animal relationship. We investigated the effects of concealment screens and extra enrichment objects on the ease of catching and temperament in female blue foxes.

Material and Methods

Treatment groups

The study was conducted at Kannus Research Farm Luova Ltd, Finland, in August-December 2019. The experimental animals were 40 adult (> 1 year old) and 40 juvenile (<1 year old) female blue foxes housed in wire-mesh cages (width: 107cm; length: 114 cm; height: 72 cm) in a shed with two rows of cages. The adults were housed singly in the front part of the shed. The juvenile females were in the rear part of the shed, and

they were pair-housed with juvenile males until mid-November. All the cages were furnished with a platform and a wooden block. In late August, the 40 juvenile (J) and 40 adult (A) female blue foxes were allocated into the four treatment groups, 10 juveniles and 10 adults in each group (Table 1). B groups had basic furnishing (wooden block + platform) in the cages. In addition to the basic furnishing, the E foxes had two extra enrichments that were changed every second week and they also received an edible 'super enrichment' on every Monday. The N groups had no concealment screens and the S groups had concealment screens installed under the platform (mounted to the outermost wall of the cages) so that there was a tunnel-like space behind the screen under the platform. The data were collected as a part of a study focusing on fur chewing, and therefore half of the adults had fur chewing history and half of the juveniles had mothers withfur chewing history. The treatments and fur chewing history were balanced between the two cage rows.

Table 1. *The numbers of animals in the treatment groups. The numbers illustrate the number of juvenile (J) and adult (A) foxes at the behavioural testing in December, and only data from these animals were used also for August. Five foxes died in the course of the study.*

	Concealment screen	
Enrichment	**No screen (N)**	**Screen (S)**
Basic enrichment (B)	BN: 10 J + 10 A	BS: 10 J + 9 A
Extra enrichment (E)	EN: 8 J + 10 A	ES: 10 J + 8 A

Behavioural tests

In August (i.e. one week before starting the experimental treatments) and December, the foxes' behaviour was measured with three behavioural tests: the stick test (ST), the subjective evaluation of HAR (SEH) and the catch test (CT). The ST is supposed to measure explorative behaviour, i.e. an aspect of positive emotional state (PES) (WelFur 2015), whereas the SEH and CT can be assumed to measure HAR. In addition, CT measures also the ease of handling in a practical sense. The ST and SEH were carried out one after the other, the ST first. In the ST the assessor approached the cage of a fox calmly and inserted 10-20 cm of a 150 cm long stick into the cage through the front wall of the wire mesh cage at a height of 30-40 cm from the cage floor. The assessor avoided eye contact with the fox, kept hold from his end of the stick and stood still at a distance of approximately 1 m from the cage of the fox for 10 s. The fox's reaction towards the stick was scored 'explorative' (0) when the fox approached a distance of 10 cm or closer, and touched, sniffed or nibbled the stick in an explorative way. The fox was scored 'non-explorative' (1), when it did not respond to the stick or kept a distance longer than 10 cm to the stick (see WelFur 2015). After finishing the ST the assessor draw the stick out of the cages moved closer to the same fox observing its location, movements, gestures, facial expressions and vocalizations and scored SEH on a six-point scale from 0 to 5 (Table 2).

Table 2. *Description of the subjective evaluation of human-animal relationship (SEH).*

SEH	
Scale	*Description*
0	**Very curious and very confident:** The fox stayed in the front part of the cage or approached the assessor willingly andsought contact with him. The fox did not withdraw when the assessor extended his hand close to the cage netting, first the front wall and then the roof. The fox sniffed the hand kept on the roof by leaning against the front wall with its front legs.
1	**Very curious and quite confident:** As 0 above, but the fox was more hesitant to sniff the hand and occasionally withdrew further away.
2	**Curious but slightly cautious:** As 1 above, but the fox did not sniff the hand, and withdrew slightly from the assessor as he put his hand close to the cage netting.
3	**Cautious or passive:** The fox moved around in the rear part of the cage or on the platform and could show some attempts to explore the assessor. When the assessor extended his hand towards the cage, the fox was suspicious and withdrew slowly further away. This category also includes the foxes that did not show any kind of approaching or withdrawing reaction; i.e. the fox was inactive (lying or sitting) on the platform or somewhere else in the cage.
4	**Fearful:** The fox avoided human contact. It moved mainly in the rear part of the cage or stayed on the platform. When the assessor extended his hand towards the cage, the fox withdrew even further away. The body language expressed signs of suspiciousness: the body was lowered and/or ears turned back.
5	**Very fearful:** As 4 above, but the fox showed severer or more active fear reactions. No foxes belonged to this category in this study.

A three-point scale was used in CT to score how easily a fox was caught with the traditional neck tongs The CT score was assessed by the same assessor who conducted ST and SEH, but the catching itself was carried out by an experienced animal caretaker. The animal was scored as 0 (‘easy to catch’) when the animal was easy to catch and the catching took less than 10 seconds. If the catching took 10-30 seconds, the animal was scored as 1 (‘some difficulties to catch’) and if it took more than 30 seconds it was scored as 2 (‘major difficulties to catch’).

Statistical analyses

The effects of the treatment and age (independent variable) on the behavioural test performance (dependent variable) were analysed with binary logistic regression models (forward step) by using the IBM SPSS Statistics software for Windows version 25 (SPSS, 2019). The SEH and the CT were re-scored into dichotomous variables. For the SEH the scales from 0 to 2 (= ‘approaching’) and the scales from 3 to 5 (= ‘avoiding’) were merged. For the CT the categories ’some’ and ’major’ difficulties to catch were combined resulting in the classes ’easy to catch’ and ’difficult to catch’.

Results

In August, all the foxes scored 0 in the CT, i.e. were easy to catch. In December, the juveniles in the S groups were more difficult to catch than the juveniles in the N groups and adults in both the S and N groups (S*Age: $P = 0.001$, Figure 1). The concealment screens affected also the foxes’ behaviour in the SEH in December: the foxes with the screens approached the assessor less frequently than the foxes without the screens ($P = 0.015$, Figure 2). The effect of the concealment screen was confounded by the enrichment treatment: the BS foxes approached the assessor the least and the BN foxes the most frequently, the two other groups being

intermediate (S x E, P = 0.044) (Figure 2). In August, the behaviour of the foxes in the SEH did not differ between the experimental groups (P > 0.05 for all main effects and interactions), where 22 – 45% from the groups approached the assessor (BN: 45 %, BS: 31,6%, EN: 22,2%, ES: 22,2%).

Figure 1. *The percentages of foxes that were easy to catch in the CT in December. S = concealment screen, N = no concealment screen, B = basic enrichment, E = extra enrichment, J = juveniles, A = adults.*

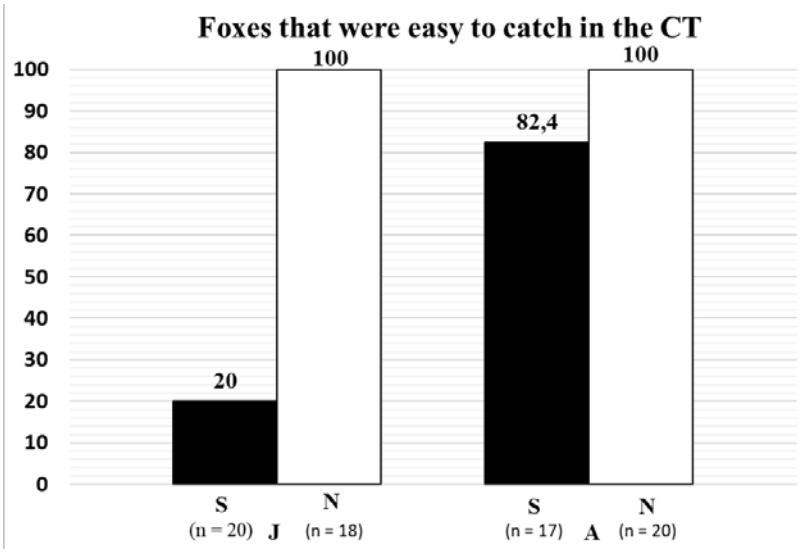
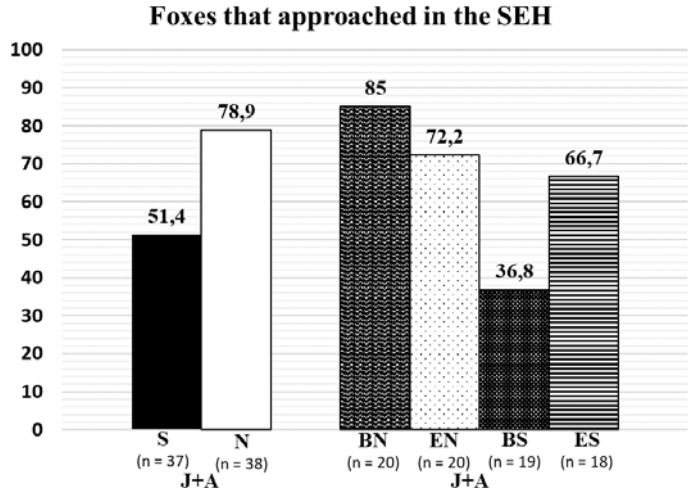
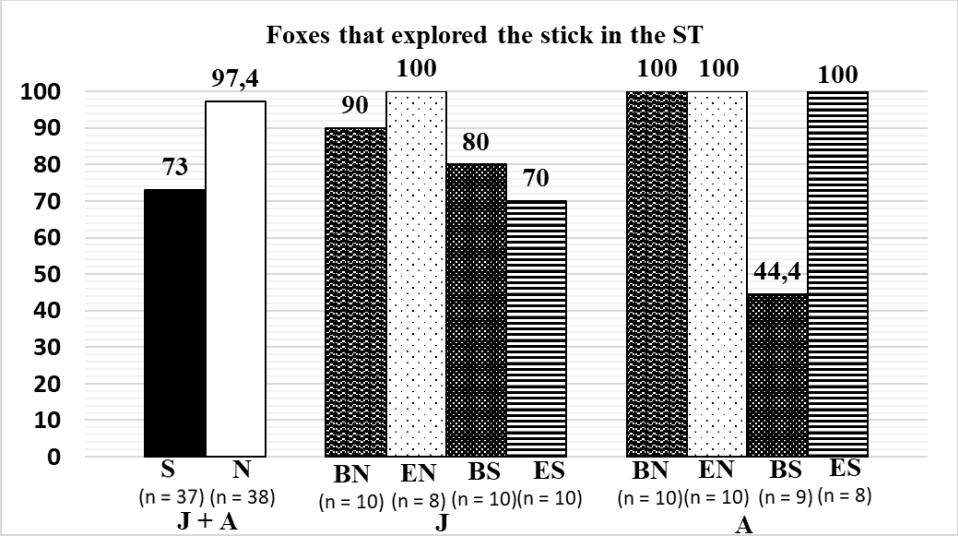


Figure 2. *The percentage of foxes approaching the assessor in the SEH (on the right) in December. Abbreviations as in Figure 1.*



In December, the foxes with the concealment screens explored the stick in the ST less frequently than the foxes without the screens (P = 0.011, Figure 3). This effect was confounded by the age and enrichments (S x E x age: P = 0.031): The foxes with concealment screen and basic enrichments was the least explorative adult group, whereas all the juvenile groups responded in a rather similar way. In August, the responses of the foxes in the ST did not differ between the experimental groups (P > 0.05 for all main effects and interactions): from juveniles 20-50 % touched the stick (BN: 40%, BS: 20%, EN: 12,5% ES: 50%) and from adults 78 – 90% touched the stick (BN: 90 %, BS: 77,8%, EN: 80%, ES: 87,5%).

Figure 3. *The percentage of blue foxes exploring the stick in the stick test in December. Abbreviations as in Figure 1.*



Discussion

It can be concluded that the concealment screens had a negative effect on the temperament of the foxes and human-animal relationship 3-4 months after the screens were added to the foxes' cages. The catching of the juveniles with the screens was very challenging and the foxes with screens were not as explorative or did not approach human as often as the foxes without the screens. Delivering the enrichments to the foxes meant regular positive human contact, and this may partly explain the result that a larger percentage of adult foxes with than without the extra enrichments explored the stick in the ST, but the enrichments did not mitigate the negative effects of the concealment screens in juveniles. To sum up, the structural solution used in the present study for providing the foxes with a place for hiding and/or withdrawing is unpractical and leads very probably to unnecessary stress in the proximity of human, hindering their daily care. Thus, this kind of structural solution cannot be recommended to commercial fox farms.

Acknowledgements

We thank the farm staff of Luova for their help in carrying out the experiment. The study was financed by the Finnish Ministry of Agriculture and Forestry and Finnish Fur Breeders' Association.

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

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Background

The WelFur on-farm welfare assessment systems have been implemented on all European mink and fox farms since 2017. Development of the protocols that were published in 2015 (WelFur 2015a, b) had started already in 2009 (Mononen et al. 2012). Initial work for the protocol for Finnraccoon (*Nyctereutes procyonoides*) was started at the same time with the other protocols. The work has progressed more slowly, but is now about to be completed.

Here we describe the measurements of the on-farm welfare assessment protocol for Finnraccoons, as well as how they were selected.

Materials and methods

First, similar to WelFurFox and WelFurMink protocols, the potential welfare measures were identified based on a literature review. A preliminary selection of measurements was tested on farms in a very early stage of the development (Koistinen et al. 2013), and the results were used for the further development work. Thereafter, implementation of the early version of the protocol on farms enabled an iterative process in the refinement of the protocol. This ensured high feasibility of the whole protocol.

Measurements of the protocol

The protocol following, the same structure of four welfare principles and 12 criteria as the WelFurFox and WelFurMink protocols and Welfare Quality project®, includes 25 measurements (Table 1). Not all measures are taken in all of the three Periods within the production cycle and not in all types of animals.

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 (Table 1). *Absence of prolonged thirst* is covered by an input based measure of *Continuous water availability*, including sub-measurements of the *Type of the watering system* and *Availability of potable water*.

Comfort around resting is measured by *Opportunity for allohuddling* and *Resting shelter*, since studies show a strong preference for allohuddling and use of shelters while resting (Table 1). *Protection from exceptional hot weather*, *Protection from wind* and *Cleanliness of the fur* describe *Thermal* comfort, depending on the Period. *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* (Table 1). *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 at the end of P3* describe *Absence of pain induced by management procedures*.

Expression of social behaviour is described by *Social housing of juveniles* (Table 1). *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 of these measurements are abnormal behaviours, whereas the other three are input based measurements and measure opportunities for performing species specific behaviours.

Table 1: *The welfare measurements describing the 12 welfare criteria in the on-farm welfare assessment protocol for Finnraccoons. Period refers to the data collection windows within the production cycle, ie 1 = winter, 2 = summer and 3 = autumn, when the measurement is taken.*

Principle	Welfare criterion	Measurement	Periods
1. Good feeding	1. Absense of prolonged hunger	Body condition	1, 2, 3
		Availability of nutritional fibre	1, 2, 3
	2. Absence of prolonged thirst	Continuous water availability	1, 2, 3
2. Good housing	3. Comfort around resting	Opportunity for allohuddling	2, 3
		Resting shelter	1, 2, 3
	4. Thermal comfort	Cleanliness of the fur	1, 3
		Protection from exceptional hot weather	2
		Protection from wind	1, 3
	5. Ease of movement	Possibility for horizontal movement	1, 2, 3
		Possibility for vertical movement	1, 2, 3
3. Good health	6. Absence of injuries	Difficulties in moving	1, 2, 3
		Skin lesions and other injuries to the body	1, 2, 3
	7. Absence of diseases	Bent feet	2, 3
		Diarrhoea	1, 2, 3
		Other disease	1, 2, 3
		Mortality	1, 2, 3
	8. Absence of pain induced by management procedures	Emergency killing	1, 2
		Killing at farm at the end of P 3	3
4. Appropriate behaviour	9. Expression of social behaviours	Social housing of juveniles	2, 3
	10. Expression of other behaviours	Stereotypic behaviour	1, 2, 3
		Fur chewing	1, 3
		Availability of straw	1, 2, 3
		Opportunity to use activity object	1, 2, 3
		Quality of the available area	1, 2, 3
	11. Good human-animal relationship	Voluntary approach test	1, 3
	12. Positive emotional state		

The categorization of the measurements, and data collection and sampling methods are adapted to the activity level of the animals. Thus a less detailed inspection of the animal is done in winter due to the natural lethargy of the Finnraccoons.

Concluding remarks

The challenges encountered during the implementation of the WelFurFox and WelFurMink protocols were avoided by modifying the development process in the Finnraccoon. The protocol for the Finnraccoon describes welfare of this species comprehensively. According to the assessors, data collection on farm is feasible. All the Finnraccoon farms in Europe have been assessed during the production cycle 2019-2020.

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

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Abstract
Chewing one's own fur is a form of self-injurious behaviour that can be regarded as an indicator of impaired welfare. We studied the association between fur chewing and coping style in blue fox vixens (n=1393). The fur chewing status 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 a 'proactive coping style' (bold, explorative, active) and animals with the CSI sums 2 and 3 a 'reactive coping style' (shy, unexplorative, inactive). There was a higher percentage of fur-chewers among the proactive (33.9%, 171/504) than the reactive (26.5%, 236/889) blue foxes ($P<0.001$, logistic regression). Our results demonstrate that proactive blue foxes, may have an increased tendency for fur chewing.

Keywords: animal welfare, animal behaviour, fur farming, *Vulpes lagopus*

Background
Self-injurious behaviour (SIB) is observed in numerous animal species kept in captivity (Devine, 2012). In the two major species kept for their fur, blue foxes (*Vulpes lagopus*; Ahola et al., 2014) and mink (*Neovison vison*; Malmkvist and Hansen, 2001), SIB is expressed as fur chewing. The prevalence of fur-chewing in farmed blue foxes peaks in winter and is observed then in 10% of animals (Ahola et al., 2014), but there are few studies on its causation or aetiology. In general, SIB in animals results from deprivation and/or stress, but also genes can affect its development (Devine, 2012, Malmkvist and Hansen, 2001). Muehlmann and Lewis (2012) pointed out that SIB shares many features with abnormal repetitive behaviours; such as stereotyped behaviour, tics and compulsions. Ijichi et al. (2013), in turn, suggested that personality factors relating to proactive coping style may be linked to stereotyped behaviour. Thus, one could hypothesise that also SIB is associated with proactive coping style that is reflected by bold, explorative and active behaviour (Finkemeier et al., 20218). We tested this hypothesis by comparing coping styles in blue foxes with and without signs of fur-chewing.

Material and methods
The data were collected on a private Finnish fox farm in February-March in 2017 (n = 660), 2018 (n = 156) and 2019 (n = 577). The experimental animals were blue fox breeding females. Each fox was included in the data only once, i.e. in the year when it appeared first time on the farm population. The foxes were classified to four age categories: 1 year (n = 498), 2 years (n = 247), 3 years (n = 307) and 4 years or older (n = 341). The fur chewing status of each fox was measured with the WelFur scale: no (0) or clear (1) signs of fur chewing (WelFur 2015). The coping style of the foxes was assessed with a 'coping style index' (CSI) based on three temperament tests (Table 1). The sum (ranging from 0-3) of the three test results was calculated, and finally a

dichotomous CSI was calculated. Animals with the CSI sums 0 and 1 were interpreted to have a 'proactive coping style' (bold, explorative, active) and animals with the CSI sums 2 and 3 a 'reactive coping style' (shy, unexplorative, inactive; Finkemeier et al. 2018).

Table 1. *Description of the three behavioural tests.*

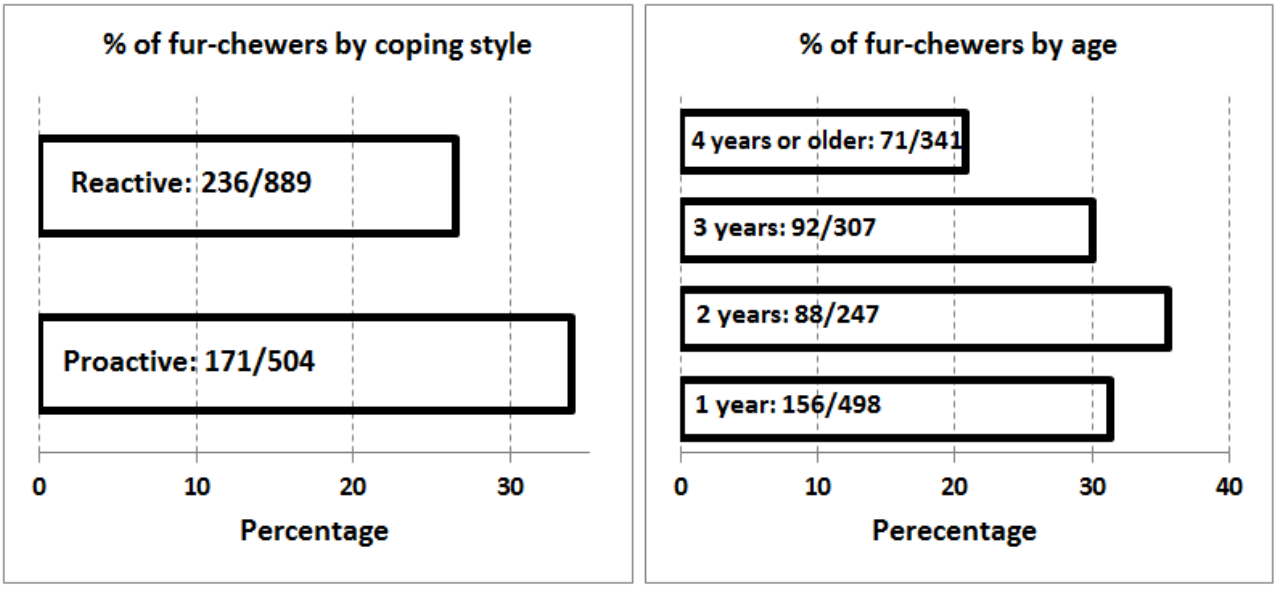
Test	Duration	Rationale and reference
Stick Test (ST)	10 s	Whether a fox explores (0) or not (1) a stick inserted into its cage by the assessor (WelFur, 2015).
Subjective Evaluation of Human Animal Relationship Test (SEH)	10-180 s	Whether a fox approaches the assessor (0) or not (1) as he/she approaches very close to its cage (modified from Mononen et al., 2017).
Feeding Test (FT)	30 s	Whether a fox eats (0) or not (1) in the presence of the assessor (WelFur, 2015)

The results were analysed with a binary logistic regression model where FCS was the dependent variable and CSI and AGE the independent variables. CSI x AGE was non-significant (P = 0.31), and it was dropped from the final model.

Results

The percentage of fur-chewers was higher among the blue foxes with a proactive coping style (33.9%) than among the foxes with a reactive coping style (26.5%) (P < 0.001, odds ratio = 1.508, 95% Confidence Interval 1.186-1.918; Figure 1: left). 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 < 0.001; Figure 1: right).

Figure 1. *Left: The percentages of fur-chewers among the blue foxes with proactive and reactive coping styles. Right: The percentages of fur-chewers among the blue foxes in the four age categories.*



Discussion

Fur chewing can be considered to reflect impaired welfare. However, our preliminary results here demonstrate that it is slightly more frequent among the animals which had a positive response, in terms of animal welfare, in at least two of the three behavioural tests (i.e. CSI sum was 0 or 1): ate in the FT, explored the stick in the ST and/or approached the assessor in the SEH (see WelFur, 2015 and Mononen et al., 2017 for the welfare interpretations). This finding may seem paradoxical but supports our hypotheses that fur chewing is probably associated with a proactive coping style in blue foxes (see Ijichi et al., 2013).

The main effect of the age on the fur chewing behaviour may be either a true finding, or an artefact resulting from large amounts of new breeding animals bought to the experimental farm in the course of the study years.

We conclude that further studies are needed to elucidate the associations between fur chewing coping style, and foxes' age, as well as the connection of coping style to welfare of blue foxes.

Acknowledgements

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Poster session

Evaluation of human impact on the behavior of the farmed chinchilla

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The aim of the study was to establish whether human contact (handling) will influence the behavior of the chinchilla. The investigations were performed on juvenile chinchillas twice: when the animals were 30 and 240 days old, respectively. The differentiating factor was **handling** process. The behavior of the animals was evaluated with the use of **an elevated plus-maze test**. It was find, 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.

Key words: chinchillas behavior, handling, elevated plus-maze test

The farmed chinchilla (*Chinchilla langiera*) is a species which is primarily bred for its fur. In order to utilize it properly, we needed to determine its needs. In effect, its dietary norms have been established, the specificity of its reproduction has been determined, and a caging system has been implemented. These solutions formed the basis for the further development of the breeding process (Barabasz, 2008).

However, the issue of chinchilla behavior is still poorly understood, although it appears to have a significant impact on breeding results. Some questions on the behavioral issues of the species still require answers. Such knowledge will allow us to ensure that the animals are provided with the highest degree of welfare in the conditions of farm breeding, as well as point to the possibilities of their improvement.

One such issue is the level of confidence of chinchillas in humans. Increasing the animals' confidence may have an influence on their welfare and contribute to raising their breeding results (Broom, 1988, sustainablefur.com).
The chinchillas confidence level can be improved by more frequent human contact (handling). This term *handling* refers to direct contact of the animal with the human hand. Such contact lowers the level of fear, as well as mitigates the influence of other stressors (Pisula, 1999).

The evaluation of the animals' behavior is performed with the proper behavioral tests. There are no ready-made behavioral tests which would allow for the evaluation of chinchillas specifically. However, there exist tests for other species of rodents (primarily mice and rats). In effect, the study made an attempt to evaluate the usefulness of the elevated plus-maze test (Pellow et al., 1985) as an indicator of the chinchillas' level of confidence.

The aim of the study was to establish whether human contact (handling) will influence the behavior of the chinchilla estimated by using an elevated plus-maze test.

Materials and methods

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 the **handling** process. Animals from the experimental group (handled) were taken out of their cages several times per week, touched, and caressed by ca. 5 min form the age of 30 days. In turn, animals from the control group were kept in a manner typical for farm production. In effect, their direct contact with humans was minimal.

The behavior of the animals was evaluated with the use of an **elevated plus-maze test**. This is a construction made of two 2-meter bars, which intersect in the middle at 90 degree angles. Two of the bars are covered by walls at their sides, while two are left uncovered. The entire construction is placed at a height of 1 meter above the floor. The bars are 15 cm wide. Similar tests are used in behavioral tests of laboratory animals, mainly mice and rats (Boguszewski, 2004; Tyl-Bielecka, 2008). For the purpose of this study, the maze was specifically constructed and adjusted (size-wise) to accommodate chinchillas.

The animals were placed in the center of the observation field of the maze (the intersection of the covered and uncovered bars) and observed for 5 minutes.

The specific behaviors of the animals in the maze were awarded points depending on the place to which the animals have moved.

The points were awarded as follows:

- the middle of the maze, or the place in which the animal was placed (no movement) – 0 points;
- the bars covered with walls – 1 point;
- the uncovered bars – 2 points;
- leaning out from the covered and uncovered bars – 3 points;
- jumping from the bars (escaping the test) – 4 points.

Points were awarded for physical activity. Those animals which leaned out and jumped from the maze were awarded the most points. All the points awarded during the observation were added in a spreadsheet.

In order to account for the influence of the age of the chinchillas on the results of the test, two series of the study were performed:

Series I – 30-day-old animals (+/- 2 days) – 12 males and 9 females
Series II – 240-day-old animals (+/- 2 days) – 11 males and 8 females

Depending on whether the examined variable is normally distributed or not, various methods of analysis were used: variables for which it was not possible to exclude compliance with normal distribution were analyzed with the Student's t-test for paired samples. Remaining variables - Wilcoxon signed-rank test.

Results and discussion

The results of the evaluation of the efficiency of handling were presented in Table 1.

Table 1 - *The efficiency of handling (average sum of points in the group)*

Age (days)	Sex	Group		Average for both groups
		Handled	Control	
30	Males	15.3a	50.0a	34.6
	Females	39.0	64.0	51.6
	Average for both sexes	28.3A	60.0A	44.2
240	Males	25.8	41.2	34.3
	Females	20.0	43.0	32.7
	Average for both sexes	22.6	40.4	32.1

A – significance of differences on level 0,01
a – significance of differences on level 0,05

The obtained results show that in most cases, the 30-day animals were more active than 240-day-old animals (Table 1).

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. Among dogs, we can observe higher physical activity in the early stage of development, which is lowered by the time the animals attain the age of sexual maturity (Kaleta and Fiszdon, 2002; Serpell, 1995). In the case of rats evaluated with the use of a plus-maze test, mature animals were more active than when they were younger, with females displaying more activity in comparison to males (Tyl-Bielecka, 2008). Differences in the physical activity of 30-day-old and 240-day-old chinchillas may, in effect, be the result of subsequent stages of development.

The only exception in this regard was a group of 30-day-old handled males, who displayed exceptionally low physical activity. In comparison with 30-day-old females from the control group, their activity was lover with a significance level of $p<0.05$. When all of the handled animals were compared to the control animals of the same age, the difference turned out to be even more significant ($p<0.01$).

The reason behind the lower physical activity of the animals from the handled group in comparison to the control group, both in the case of 30-day-old and 240-day-old animals, may be a higher level of confidence in humans (no fear of humans). Because the animals were accustomed to human contact thanks to being handled daily, they did not feel threatened during the observation process. They did not try to run or jump from the maze, as was the case with animals from the control group.

In conclusion, it can be stated that:

- The handling process has resulted in the change of physical activity among the animals: animals from the experimental group seemed to be more calm and more trustful with regard to humans;
- The proposed plus-maze test proved to be useful in the evaluation of the physical activity of chinchillas;
- A larger degree of physical activity among younger animals may be the result of their stage of development; however, the confirmation of this thesis would require further studies.
- The observed differences in physical activity among 30-day-old males and females are an interesting new insight, which was not noted in literature to date and which requires confirmation in the course of further studies.

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Fox Farmers in Focus

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Summary
The aim of the project "Fox fur farmers in focus" is to improve both profitability and the welfare of people and animals on fox fur farms by developing work methods. The project aims to find out restrictive factors of work productivity and of farmers welfare on fox fur farms as well as to discover targets in work processes that need to be developed. To compile information on fox fur farming, interviews of advisors, questionnaires for fox fur farmers as well as work studies that are required to be carried out. Essential topics to be developed are time-consuming tasks and tasks that have problems in workflow. Also, tasks in which the physical workload is high due to lifting of mature animals, repetitive movements and poor working postures need to be developed. So far only preliminary results are available because the project is still on going.
Keywords: work methods, welfare, profitability

Introduction
Fur farming has a significant economic weight in many municipalities in western Finland. The most essential production costs are feed and labor costs. Feed cost is about 50 % of total production cost whereas salaries and fixed labor costs constitute 13–15 % of total production cost in fox fur production (STKL 2018). Due to the SARS CoV 2 pandemic the fur trade has temporarily weakened, circumstances in the market have changed significantly and the livelihood of fur farmers has become uncertain. Because neither production costs nor the amount of work have decreased on a fur farm, quick measures are needed to improve both profitability and human welfare on fur farms.

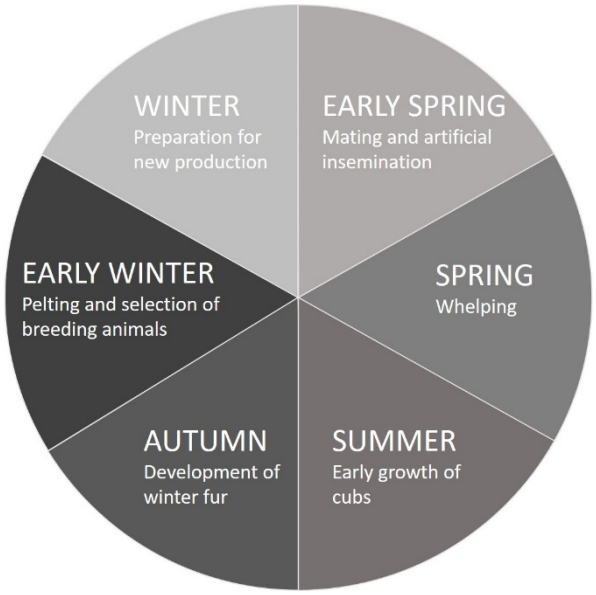
Concerns about the economy and own welfare are common stress factors among fur farmers currently. The aim of the project "Fox fur farmers in focus" is to improve both profitability and the welfare of people and animals on fox farms by developing work methods.

The project aims to find out restrictive factors of work productivity and of farmer's 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 fur farms and to produce practical operation models for developing work.

Material and methods
Fur farms have a certain (regular, particular) annual cycle: some tasks are repeated year round as a everyday routine, whereas some are carried out only at a certain time of the year (Hernesniemi 2019). In the project the whole annual cycle is examined (figure 1). To compile information on fox fur farming, interviews of advisors, questionnaires for fox farmers as well as work studies are required to be carried out. By interviewing the advisors, the general view of fox farming during the annual cycle was examined as well as the most essential problems and good practices they have met on the farms. Questionnaires for fox farmers aim to chart work

methods, labour force, resources and management practices on fox fur farms. Also, fox fur farmers' experiences of work fluency and work load are inquired. According to the results of interviews and questionnaires work studies – 8–12 research days – will be focused on the most critical phases and challenges of the annual cycle on fox fur farms. Work studies include both methods studies (ie workflow) and time studies (tasks and their labor time). Also work-load, work safety and animal welfare are in the focus.

Figure 1. *The annual cycle of fur farms (STKL 2016).*



Identified needs for developing will be discussed in workshops to concretize the topics and to find out practical solutions. Small-scale workshops will be arranged locally in order to get fox farmers involved in meetings.

The solutions will be reported as information cards and videos as well as introduced in the result seminar. Electronic information channels available for fox fur farmers will be used to deliver the information of cost-effective and animal friendly operation models.

Results

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 (tags). During winters freezing can cause problems and extra work in order to give the animals water to drink. 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).

Discussion

The project is carried out in the strong fur farming provence of Pohjanmaa in Finland by the Finnish Fur Breeders' Association (FIFUR) and TTS Work Efficiency Institute in 2021–2022. So far the advisors have been interviewed and the inquiry of fox farmers is currently on going. Work studies will begin in autumn if the epidemic situation makes it possible. Workshops, informing and reporting will be dominate the project in 2022. Information cards and videos will also be available for other fur farmers, industries connected to fox fur farming and other stakeholders.

Acknowledgements
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Running wheel activity in mink with different forms of abnormal behaviour

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Abstract
Stereotypies can take many different forms 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 welfare 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 (SB). We screened in 2019 c. 1100 Palomino and Brown mink dams, individually housed at the AU-farm, into six groups based on their behavioural phenotype: CONTROL (n=18) free from abnormal behaviour, FURCHEW (n=14) fur-chewing, ORALSB (N=11) with licking SB, STATSB (n=15) with stationary SB, PACERS (n=16) with pacing, and MIXED (n=14) with several forms of abnormal behaviour. These 88 mink were relocated to cages with running wheel access for 10 days. We analysed 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. per mink 960 rpd on the first day, 1025 rpd on day 10). There was a considerable variation in running wheel activity between mink. The major finding was that running wheel activity differed between groups with PACERS, STATSB and MIXED 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.

Keywords: Enrichment, fur-chewing, motivation, Neovison vison, stereotypic behaviour, welfare.

Introduction

Understanding animal motivation, stress responses and abnormal behaviour is important for pursuing good welfare in husbandry. In farm mink, fur chewing and stereotypies are the main types of abnormal behaviour (e.g. Malmkvist et al., 2012, and many others). Previously, Hansen and Damgaard (2009) reported that running wheel activity substitutes pacing: Mink with access to a running wheel used the running wheel for the same amount of time as mink without access to a running wheel performed stereotypies, and the daily rhythms of the two types of activity were identical. The experimental mink in the study originated from a selection line of mink with a high level of stereotypic behaviour, however, with no further specification of type and intensity of individual stereotypic behaviour (Hansen and Damgaard, 2009). In practice, stereotypies can take many different forms in mink– such as repeated pacing, licking, and head-twirls – but many studies, and the WelFur protocol, pool these together (FurEurope, 2015). It is currently unknown if these different forms of stereotypic behaviour (SB) share a similar motivational background and have equal welfare significance for the mink. Based upon the diversity in the expression of stereotypic behaviours, we hypothesised that the different forms have a different causation or, in case of common causation, indicate different degrees of severity of thwarted motivation. Still, some subtypes of abnormal behaviour may share a common background – such as a general frustration due to e.g. barren housing or hunger - with differences in individual performance just being coincidental. 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

hypothesised that use of a running wheel would be higher in pacing mink and that running wheel access would substitute stereotypic pacing, with little effect on the other types of stereotypies such as stationary and licking SB in mink.

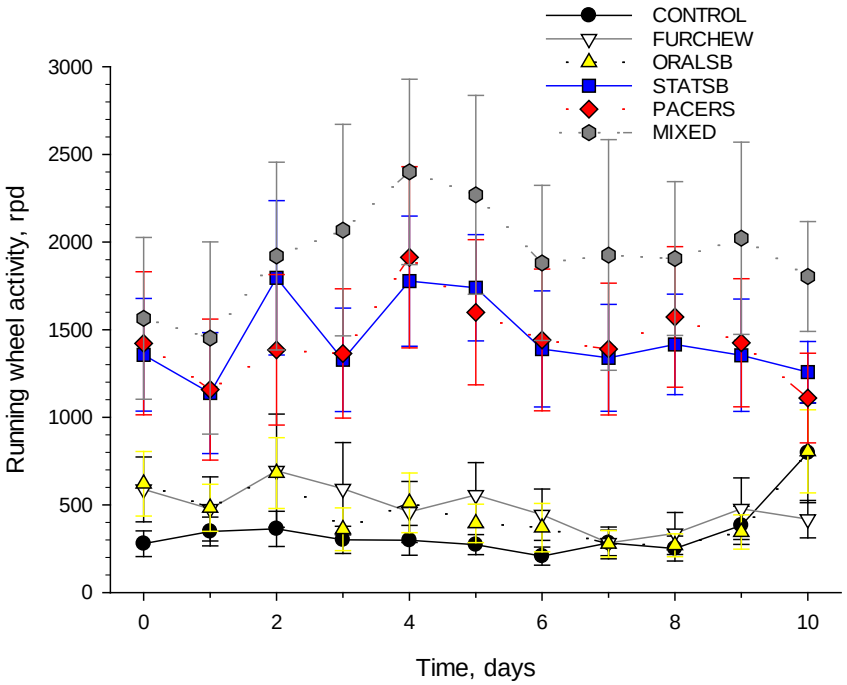
Methods and materials

We screened in 2019 c. 1100 Palomino and Brown adult mink females, individually housed at the farm at Aarhus University, into six groups based on their behavioural phenotype: CONTROL (n=18) free from abnormal behaviour, FURCHEW (n=14) fur-chewing, ORALSB (N=11) with licking SB, STATSB (n=15) with stationary SB, PACERS (n=16) with pacing, and MIXED (n=14) with several forms of abnormal behaviour. In total, 88 mink were transferred to cages with running wheel access for 10 days. We analysed automatically recorded running wheel activity as rounds per days (rpd, i.e. per 24h) using repeated measures mixed ANOVA. Additionally, location in the cage, normal and abnormal behaviour were measured days -1 (before), 5, 10 (during), and 15, 20 (after) the housing in a cage with running wheel access (day of transfer: day 0). Observations were conducted 8-11h as 120 sec continuous observations twice per day per mink by trained observers, blinded in relation to the experimental group. When not in the running wheel cage, the mink were housed in standard Danish commercial cages. The experimental period was in November-December.

Results

There was a considerable variation in running wheel activity between mink. 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 (in average 1474, 1404 and 1753 rpd per mink, 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 (Figure 1).

Figure 1. *Running wheel activity as mean ±se rounds per day (rpd) during 10 days in cage with running wheel access.*



Discussion

Different forms of abnormal behaviour influenced the running wheel activity in mink. In line with previous findings (Hansen and Damgaard, 2009), we confirmed the expectation of a high level of running wheel activity in pacers. However, our study provided several novel findings. The group of mixed SB (mink with more than one type of SB, including fur-chewing) used the running wheel to the same extent as the pacers. Furthermore, in contrast to our *a priori* predictions, stationary SB mink also used the running wheel extensively. Previously, stationary and licking SB may have been classified as the same type of stereotypy.

Oral SB is prevalent in other species (Mason and Mendl, 1997), but we found no previous records on mink. From the present results, it is clear that licking SB, fur-chewers and control mink use the running wheel much less than the other groups. This indicates a different causation of these SB, and the interesting question is whether these forms have different welfare significance for the mink. Whether running wheel access reduced the actual occurrence of the different forms of stereotypies will be presented at the IFASA conference.

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Session III: Breeding, genetics and reproduction

Breeding, Genetics & Reproduction

Oral presentations

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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Abstract
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 (Robinson, 1975). 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 mink with four distinct 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 of 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+1 G>A) and *MITF-M* (c.33+1 G>A) genes in silverblue and Hedlund white mink 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 mink we identified 2 frame shift mutations in *RAB38* gene (c.20-21dup, c.574-589del).

Background
Silverblue (*p/p*) shade of the coat was described in 1931, it is the first mink fur-colour mutation. It inherited as a Mendelian autosomal recessive trait and to date is the most common mutations in the mink fur industry.

Furthermore, silverblue is used in combination with other mutations to generate popular fur colours, such as violet (*a/a m/m p/p*), sapphire (*a/a p/p*), and pearl (*k/k p/p* or *a/a k/k p/p*) (Cirera et al., 2013).

Hedlund white is another widely used mutation, that lead to generation of albino-like white coat. This phenotype is the result of a recessive mutation (*h*) with pleiotropic and codominance effects: homozygous (*h/h*) mink are completely white with dark or blue eyes and deaf, while heterozygous animals (*h/+*) are piebald with no hearing defects (Markakis et al., 2014).

Moyle is a light brown mink coat colour, it a member of wide range of brown phenotypes that are highly similar to each other, which significantly complicates selection and breeding. Moyle phenotype is inherited as a Mendelian autosomal recessive trait, and three alleles were postulated: moyle (*m*), cameo (m^c), and wild type (+). The cameo allele produces a darker shade of brown than the moyle allele and seems dominant to it. Both alleles are recessive to wild type, which is a standard dark brown colour ($+ > m^c > m$) (Robinson, 1975).

To identify mutations underlying silverblue, Hedlund white and moyle phenotypes we performed sequencing and comparing of whole genomes of five distinct fur colours: silverblue (*p/p*), Hedlund white (*h/h*), moyle (*m/m*), violet (*a/a m/m p/p*) and standard dark brown.

Methods and materials

For this study we collect biological material from farm-bred American mink maintained in Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (7 silverblue (*p/p*), 12 Hedlund white (*h/h*), 2 pearl (*k/k p/p*), 1 violet (*a/a k/k p/p*), 2 shadow silverblue (S^H/+ *p/p*), 2 royal pastel (*b/b*), 2 American palomino (*k/k*), 3 moyle (*m/m*), 1 lavender (*a/a m/m*)), 4 violet (*a/a m/m p/p*), and 17 standard dark brown. Additional samples (10 Silverblue and 4 Hedlund white) were collected from animals maintained in «Mermeriny» fur farm, Tver region, Russia.

Whole genome sequencing of 3 silverblue, 3 Hedlund white, 1 moyle, 1 violet and 3 standard dark brown animals was performed on a HiSeq 2000/2500 and NovaSeq 6000 sequencer (Illumina) at the Vavilov Institute of General Genetics RAS (Moscow, Russia) and Genetico Company (Moscow, Russia).

Resulting reads were mapped to the mink (*Neovison vison*) genome with BWA-MEM algorithm. Duplicate reads were detected and removed from analysis with Picard MarkDuplicates algorithm. Genetic variants in sequenced mink genomes were predicted using GATK HaplotypeCaller software.

To detect the genetic factor underlying the silverblue phenotype, we selected common homozygous variants, with depth of coverage more than 2, in *p/p* mink that are not homozygous in standard dark brown wild-type. The same analysis was performed for Hedlund white and moyle/vilolet genomes. Annotation and effect prediction of selected variants were performed in SnpEff and VEP software, using the mink genome annotation.

Selected candidate mutations were confirmed using Sanger sequencing. Effects of *MITF* and *MLPH* mutations on splicing were validated using reverse transcription polymerase chain reaction (RT-PCR) and Sanger sequencing. Allele-specific RT-PCR and Sanger sequencing was also performed to confirm the chromosome location (*cis* or *trans*) of *RAB38* mutations in double heterozygotes animals.

Results

We performed whole genome sequencing with x5-40 average coverage of American minks with five distinct fur colours.

Among common homozygous variations in three Silverblue mink that were not homozygous state in wild-type minks, we identified single nucleotide variation (MLPH c.901+1 G>A [$MLPH^{p}$]) in splice donor site of melanophilin (*MLPH*) gene that potentially resulted in loss of function. RT-PCR and Sanger sequencing of the *MLPH* cDNA region encompassing exons 6–9 revealed that $MLPH^{p}$ mutation lead to complete loss of exon 7 (196 bp). $MLPH^{p}$ mutation was found to be homozygous in all tested silverblue minks as well as in mink with *p* allele possessing colours (pearl, violet and shadow silverblue). Moreover, wild-type mink, as well as mink with other colour coats, are not homozygous for this mutation.

Among common homozygous variations in three Hedlund white mink that were not homozygous state in wild-type and silverblue mink, we identified single nucleotide variation (MITF-M c.33+1 G>A [$MITF^{h}$]) in splice donor site of melanocyte-specific isoform of *MITF* gene. $MITF^{h}$ mutation was shown to be homozygous in all tested Hedlund white mink from the two unrelated test populations, but not in mink with other coat colour phenotypes. Using RT-PCR we confirmed absence of *MITF-M* transcript specific region (encompassing splicing site exons 1M and 2) in cortex of minks, homozygous for $MITF^{h}$ mutation, by comparison with mink without this mutation.

Among homozygous variations in moyle/violen minks that were not homozygous state in wild-type and silverblue mink we identified a homozygous 16-bp deletion (RAB38 c.574-589del [$RAB38^{3del}$]), in the moyle sample, at the third exon of *RAB38* gene. Also, we found a homozygous 2-bp duplication (RAB38 c.20-21dup [$RAB38^{1dup}$]), in the violet sample, at the first exon of the *RAB38* gene. Both mutations potentially resulted in the loss of function of RAB38 protein. All tested moyle mink, as well as well as in mink with *m* allele possessing colours (lavender and violet) were homozygous for $RAB38^{1dup}$ or $RAB38^{1dup}$ mutations or were heterozygous for both. Moreover, wild-type mink, as well as mink with other colour coats, are not homozygous od double heterozygous for this mutation. Allele-specific RT-PCR and Sanger sequencing confirmed that the $RAB38^{3del}$ and $RAB38^{1dup}$ mutations were located on different chromosomes in double heterozygote animals.

Discussion

The *MLPH* gene encodes melanophilin, a Rab effector protein involved in melanosome transport. It acts as a linker between melanosome-bound RAB27A and the motor protein MYO5A in melanosome trailing. A tripartite complex (RAB27A-MLPH-MYO5A) is among the most important elements of mature melanosome intracellular trafficking. In silverblue mink, complete loss of *MLPH* exon 7 leads to a frame shift and a premature stop-codon at amino-acid position 308 that results truncation of MYO5A-binding domain.

Dysfunction in MYO5A recruitment disturbs the transport of mature melanosomes to actin-rich dendritic tips of melanocytes, where, melanosomes are passed to the nearest keratinocytes. Disturbs of this process eventually diluting coat colour.

MITF gene, especially its melanocyte-specific isoform MITF-M, is well known to be critical for the development of neural-crest-derived melanocytes. *MITF* gene mutations cause abnormal depigmentation of hair and skin, sometimes associated with total or partial deafness. A strong association was previously indicated between the locus containing *MITF* and the Hedlund phenotype in mink. However, the earlier study

did not reveal any mutations in coding and intron flanking sequences of the MITF-M in Hedlund white mink (Markakis et al., 2014).

The *RAB38* gene encodes the member of the Rab small G protein family, which is involved in intracellular vesicle trafficking and melanosome biogenesis. RAB38 participates in the transport of newly synthesized tyrosinase and Tyrp1, which are key enzymes in melanin production from the trans-Golgi network endosomes to maturing melanosomes. The $RAB38^{3del}$ mutation may result in a frame shift at the 192 protein position and lead to the loss of a stop-codon at the 212 position. A novel potential stop codon occurs only at the 277 protein position, which results in a 30% enlargement of protein size and a C-terminal end that is completely different from wild-type. C-terminal domains of Rab-proteins seems to be involved in their interaction with Rab escort protein. The $RAB38^{1dup}$ mutation may result in a frame shift at the 8 protein position and lead to a premature stop-codon at the 15 position.

Taking all data together we suggest that mutations $MLPH^{p}$, $MITF^{h}$ and $RAB38^{1dup}$ / $RAB38^{3del}$ 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.

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The Contribution of Molecular Biology to Improving Mink Fertility

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Summary

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.

Introduction

The American mink *(Neovison vison)* has been raised as a semi-domestic species since the 1860's. The original stocks came from wild caught animals in Québec, augmented in the 20th century by live-trapped animals from the Yukon and Alaska. The commercial cultivation of mink grew and developed over the 20th century to become a worldwide industry, with farms in North and South America, Europe and Asia. From an economic view, the industry has proven to be markedly cyclical, with recurring booms and busts during its history. There are multiple causes for these fluctuations, perhaps the most significant being the short generation time of the species that allows for rapid escalation in number of animals raised. This occurs when auction prices increase and more animals are kept in production and is usually followed by a glut of pelts on the market with consequent decline in the pelt price. Other factors have contributed to the instability of the market, including disease, the anti-fur movement and consequent public sentiment. The sequelae to the 2019-2020 corona virus pandemic have had extensive consequences in the industry and many more are expected.

Economic success and even survival in the precarious mink industry depends on optimizing all aspects of raising these animals. It is indisputable that maximizing reproductive success is a key element in the sustainability of the enterprise. While research over the last decades has revealed many of the mechanistic and endocrine aspects of the reproductive process, it remains relatively inefficient. The chronic issues include male infertility occurring in a frequency of from 15-20 %, failure to breed in some 10 % of females, embryonic mortality that can exceed 50 %, and neonatal losses of 25 % or more. Disease and xenobiotic influences also reduce reproductive success. Research into these issues employing the classic tools of biology have better defined, and, in some cases, ameliorated these problems. The rapidly advancing technology of molecular biology has provided the tools to better understand, and ultimately remove the constraints on optimal fertility in mink. In the following paragraphs, the role of modern methods in unravelling the issues that contribute to reduced fertility are discussed.

Genomic Studies

The human genome was first sequenced nearly 20 years ago, at great expense. New methods, in particular, next generation sequencing, have dramatically reduced the costs of determining the genetic sequences of an individual or a species. The determination of genome sequences in the dog, cat and ferret provided carnivore examples, but it was not until 2017 that the first draft of the complete mink genome was reported (Cai et al., 2017). It revealed a number of similarities in the mink genome to the genes of other carnivores, particularly to the ferret, and a number of anomalous sequences, the function of which his yet to be discovered.

Comparison of sequences among individual mink has shown that there is also intraspecies genetic variation in the form of single nucleotide polymorphisms (SNPs) (Vitti et al., 2013). The sequences from the mink genome have served as a powerful tool to determine the genetic variations in farm populations, as employed by Miar and co-authors at Dalhousie University (Karimi et al., 2021b). Genomic technology is currently being used to investigate problems significant to mink health and production, including the genes that respond to the Aleutian disease virus (Karimi et al., 2021a). Information derived from these of studies will be of great value in understanding the basis for tolerance to this disease.

Those who raise mink have long known the importance of genetics to reproduction and have used practical information such as litter size and kit survival rates to select breeders for subsequent production years. Litter size is a complex trait that includes factors such as ovulation and fertilization rates and embryonic mortality. Early work by Einersson (Einarsson, 1987) demonstrated that the heritability of litter size is 0.16, indicating that a genetic gain of 0.1 kits per year can be obtained by selection of females for the number of kits. It has been shown that there are significant effects of color phenotypes on litter size, indicating differences in populations that have been selected for other traits (Thirstrup et al., 2014). Genetic traits from the sire may also contribute to variation in litter size. Recently, classic genetic methods have been employed to explore the variation in reproductive success in mink, and have shown that selection for higher litter weights at birth can improve both survival rate and number of offspring (Karimi et al., 2018). Given these evident variations between phenotypes and populations, it is expected that the use of SNPs and other measures of genomic variation will have great potential for improvement of fertility in the mink. To date, this potential has not been exploited.

Fertility in Males

The male mink is an example of a seasonal breeder. Growth of testis in immature animals and testici;ar recrudescence in adults commences in short days, and can be brought about by treatment with melatonin (DiGregorio et al., 1994). As noted above, there is an elevated frequency of infertility in male mink, due to multiple factors. These include developmental issues such as testicular hypoplasia, cryptorchidism, aplasia of the epididymis and autoimmune orchitis (Sundqvist et al., 1989). With the exception of orchitis, these conditions have been only superficially explored, and none at the level of either genomic or global transcriptomic analysis. Candidate gene studies have been reported for autoimmune orchitis, a condition that affects some 30 % of black mink (Pelletier et al., 2011). These include the Gap junction, cholesterol transport and apoptosis genes, as identified in a series of studies by Pelletier and associates (Pelletier et al., 2000). Their findings show that the blood-testis barrier is open during the season to testicular regression. Variant expression of connexins, the genes associated with maintenance of the blood-testis barrier, is an important factor in the development of orchitis (Pelletier et al., 2011, Pelletier et al., 2015), presumably because seminiferous tubular proteins are exposed to the vascular system. Genomic and transcriptomic studies have yet to be reported, and these analyses have significant potential to better understand and select against the factors that cause male mink infertility.

Uterine regulation of diapause and gestation

The number of live born kits varies greatly among female mink under husbandry conditions, with litters from one to 15 or more having been recorded. As noted above, the estimated heritability of litter size is low to moderate (Karimi et al., 2018). Selection for color phase results in variation in litter size, again demonstrating that there is a have shown that there is a genetic component to this trait, but little is known about the genetic factors that contribute to this variation. The length of embryonic diapause, as reflected in gestational length correlates negatively with litter size (Karimi et al., 2018) suggesting that the duration of the period when the embryo is free in the uterus is critical to reproductive success. Thus, understanding of the uterine factors contributing to the length of diapause and to consequent reactivation of the blastocyst from developmental arrest bear significance to fertility.

There have been number of candidate gene studies, mostly to explore factors known to be associated with implantation in laboratory animals. The first gene germline deletion that showed a failure of implantation phenotype in the mouse was the cytokine, LIF (Stewart et al., 1992). Transcriptomic and spatial analysis of the mink uterus during diapause and activation demonstrated expression of LIF coincident with implantation (Song et al., 1998a). Knockout of the rate-limiting enzyme of prostaglandin synthesis, PTGS2, also resulted in a failure of embryo implantation in the mouse (Lim et al., 1997). Analysis of the uterus during mink gestation demonstrated that PTGS2 is not detectable in the uterus during diapause, but that its protein form was strongly expressed within 48 h after the initiation of embryo activation (Song et al., 1998b). Another gene of interest, MSX1 was found to be expressed during diapause, but disappeared in the uterus after activation of the embryo (Cha et al., 2013, Cha et al., 2020). Remarkably, this expression pattern is conserved among three species that display diapause, the mouse, the mink and the tammar wallaby (Cha et al., 2020, Cha et al., 2013).

While investigation of candidate genes is informative, this approach fails to provide a global understanding of uterine changes during the critical transitions of the embryo from diapause to activation to implantation. The first global analysis in the mink employed suppressive subtractive hybridization (Lefevre and Murphy, 2009). While this method has a practical limitation in number of genes recognized, it nonetheless revealed striking differences in the uterus between the diapause and activated state in transcription factors, metabolism, cell cycle and cell structure genes (Figure 1). In further analysis, it was shown that a cluster of genes related to polyamine synthesis were upregulated associated with embryo activation (Lefevre et al., 2011a). By subsequent exploration of polyamine metabolism in the mink uterus, it was shown that one of these, the polyamine, putrescene, is an essential signal for activation of the blastocyst from diapause, both *in vivo* (Lefevre et al., 2011a, Lefevre et al., 2011b) and *in vitro* (Fenelon et al., 2016). Another factor emerging from this analysis was the presence of the prolactin receptor in the mink endometrium (Lefevre and Murphy, 2009). Prolactin is well known as the hypophyseal factor that terminates diapause in mink by actions on the corpus luteum (Murphy et al., 1981). Further investigation demonstrated a direct effect of prolactin on promoting polyamine synthesis in the uterus (Fenelon et al., 2016). Thus, studies that began with global analysis, ultimately resulted in linking the photoperiodic prolactin response that induces implantation in the mink with the cascade of ovarian and uterine mechanisms that terminate diapause and bring about embryo reactivation. These findings clearly demonstrate the value of global transcriptomic analysis on exploring and understanding the physiological aspects of mink fertility.

Transcriptomes in the uterus between diapause and day 5 following activation were compared by a more modern method, next generation RNA sequencing, with the goal of providing to a more global appreciation of the uterine events associated with embryo activation and implantation (Cao et al., 2019b). In the uterus, this analysis identified 1684 differentially expressed genes associated with reactivation of the embryo from

diapause, comprising 963 upregulated and 564 downregulated genes. Genes associated with prolactin signalling, extracellular matrix remodelling and intracellular signalling pathways emerged as potential regulation of the activation process. In terms of prolactin signalling, the results support the view of the direct effects of this hormone, via the JAK/STAT pathway, on induction of polyamine synthesis, epidermal growth factor signalling, LIF and angiogenesis. It was of interest that activation was associated with expression of genes in PI3-Akt intracellular signalling, upstream of the mTOR pathway. This finding is consistent with a recent study of mouse diapause where these two pathways have been implicated (Murphy, 2020).

Postimplantation embryonic and fetal loss account for as much 40 % of neonatal mortality in mink. There are a multiple factors that may interfere with successful gestation after the embryo has been reactivated and implantation ensues. Thus, the molecular events of postimplantation pregnancy are therefore of interest to understanding and ameliorating infertility. A recent investigation by Cao et al.(Cao et al., 2019a) focused on the comparison of uterine transcriptomes between implantation sites and interimplantation regions of the mink uterus during early postimplantation. It resulted in identification of 582 differentially expressed genes. Of these some 245 were upregulated and in the implantation sites relative to the spaces between them, and 153 transcripts were more abundant in the interimplantation regions relative to the sites of embryo attachment. Not surprisingly, LIF, known to be essential for implantation (Song et al., 1998a) was greater at attachment sites. Other overexpressed genes included proteolytic enzymes and intracellular signalling molecules. Pathway analysis revealed major changes in extracellular matrix molecules and those associated with remodelling of the cytoskeleton. These studies represent a major advance, but further investigation is required to establish the significance to embryo mortality of differentially expressed genes present in the uterus during the early implantation phase of gestation.

Transcriptomic information of the uterus in public databases of the mink and other mammalian species during diapause and reactivation was recently evaluated in an *in silico* study (Ajit et al., 2021). This comparison demonstrated that a number of genes associated with diapause and the reinitiation of development is conserved across species that display the diapause trait. These include, as noted above, the MSX homeobox genes during diapause, as well as genes in the arginine metabolism pathway, the PI3K/Akt pathway, and the extracellular matrix signalling pathway during reactivation. While this approach was based not on experimentation, but on bioinformatic analysis, it was informative in terms of providing potential hypotheses that can be tested in in vivo and in vitro.

Unravelling the mysteries of the mink embryo

A defining feature of mink reproduction is the occurrence of obligate diapause, a condition in which the development of the embryo is arrested at the blastocyst stage (Murphy and Fenelon, 2020). This prolonged arrest of development has been implicated in the elevated levels of embryonic loss during gestation (Schneider and Hunter, 1996). It is not clear whether this condition results from the deficiency of uterine factors, or from the active suppression of development by the uterus (Fenelon et al., 2014). The termination of the arrest in embryo development is associated with expansion of the blastocyst, an increase in protein synthesis and cell proliferation (Desmarais et al., 2004). Candidate gene approaches to understanding activation are sparse, but it has been shown that FGF4 (Desmarais et al., 2004) and the nuclear receptor PPARγ can induce proliferation of mink trophoblast cell lines *in vitro* (Desmarais et al., 2007).

There have also been a few studies comparing the transcriptome of the mink embryo during diapause and during the reactivation of development. In the first of these, employing subtractive hybridization revealed 216 transcripts that were differentially expressed in diapause vs. activated blastocysts (Fenelon et al., 2016). Some

66 % were homologues of known sequences, determined by comparison of other carnivores (Figure 2). Pathway analysis indicated that 40 % of the differentially expressed genes were involved in signal transduction, while there was a significant component (11%) related to the cell cycle (Lefevre and Murphy, 2009). Verification of relative abundance by qPCR indicated that transcripts for *Adipor1, Ilk, Trip, Inc200, Atrx and Tra1* were upregulated as the blastocyst transitioned from diapause to reactivation. Another cluster of transcripts associated with polyamine synthesis, including *Odc, Azin, and Sat1* were likewise in greater abundance in the actively developing embryo. As seen below, polyamine related genes are involved in uterine regulation of diapause. An *in silico* study of differentially expressed genes during reactivation of the mouse blastocyst from diapause demonstrated that the similar to the mink, expression of cell cycle genes was repressed during diapause (Ajit et al., 2021).

The RNAseq study of Cao et al. (Cao et al., 2019b) on the uterus yielded potential clues the escape from diapause in the mink embryo, given activation of the PI3/Akt pathway. Further investigation demonstrated that pharmacological blockade of this pathway in mink embryos *in vitro* resulted in reduced embryo survival and reduced embryo expansion (Cao et al., 2019b). As noted above, these results recapitulate previous studies of the mouse embryo in diapause (Murphy, 2020).

The *in silico* approach of Agit et al. (Ajit et al., 2021) to determine transcription factors of interest in diapause and reactivation was also applied to embryos. It showed that the conserved transcription factors *Foxo3, Tead2 and Hic1* are associated with diapause. Of these, both *Foxo1* and *Hic1* are associated with cell cycle repression, and may be significant factors in maintaining developmental arrest. The transcription factors *Klf5, Egr1, Tfpa2b, Egr4* and *Hoxa11* are expressed during the transition from diapause to activation. The many interesting hypotheses emerging from this study await *in vivo* and *in vitro* experimentation.

Conclusions

The goal of this presentation was to illustrate the significance of the techniques of modern molecular biology, genomics and transcriptomics to the understanding and improvement of mink reproduction. Genomics is the frontier for study of production issues as diverse as coat color genetics, pelt quality and feed efficiency. Aleutian disease is most likely the most significant constraint on mink production, and, as some populations have been shown to be resistant to the disease (Karimi et al., 2021a), thereby providing the potential for selecting for this resistance. Genomics also has massive strength to study reproductive issues, particularly the little-known causes of problems such as male infertility, failure to mate in females, and pre- and postimplantation embryonic mortality. From the information presented above it is clear that transcriptomic analysis has been responsible for major breakthroughs in determining the physiological elements in the regulation of diapause and implantation. Indeed, the recognition of the critical role of polyamines as uterine signals to induce reactivation of the embryo began with exploration of the transcriptome (Lefevre et al., 2011a). Comparative analyse across species can also provide new insight into the mechanisms of reproductive function.

Other global evaluation methods including proteomics, epigenomics, lipidomics and metabolomics have become available that can and will be employed in the future to address mink fertility. It is therefore expected that these systems biology approaches will improve not only our understanding of mink reproductive physiology, but also will contribute to future strategies to maximize production.

Figure 1. **A.** *Distribution by function of differentially expressed genes in the mink uterus during diapause versus day 5 after activation of the embryo.* ***B.*** *Immunohistochemistry demonstrating expression in the uterus of diapause of ornithine decarboxylase 1 (brown pigment), one of the differentially expressed genes in the polyamine pathway.* ***C.*** *Expression of ornithine decarboxylase 1 in the uterus day 5 after activation of the embryo from diapause.*

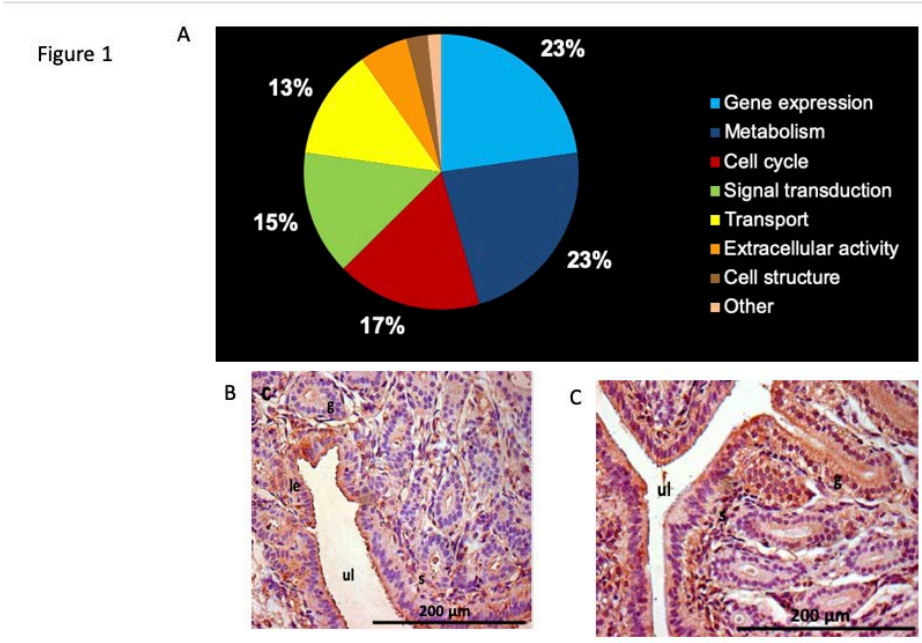
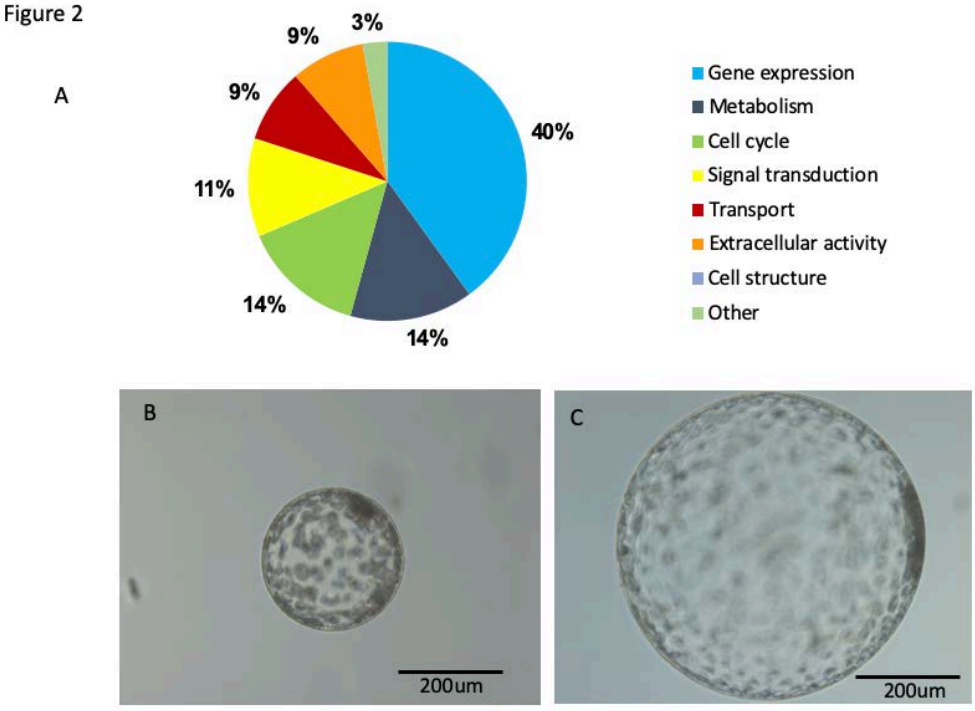


Figure 2. **A**. *Distribution by function of differentially expressed genes in the mink blastocyst during diapause versus day 5 after activation of the embryo.* ***B.*** *The mink embryo collected in the state of diapause.* ***C.*** *The mink embryo at day 5 showing the rapid expansion that after activation.*



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

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Summary
The main aim of this research was to investigate the early selection, genetic associations and suitability of two leg conformation traits, carpal hyperlaxity (LAXITY) and valgus deformity (VALGUS), for introduction into the breeding scheme. Results showed that LAXITY1 occurred at an early age (5-14 weeks) in 44% of blue fox puppies. LAXITY1 had moderate heritability (0.20±0.06) and positive genetic correlation (0.40±0.28) with LAXITY2, which was assessed at the end of growth. 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±0.04 in VALGUS2. Genetic correlation between the two VALGUS traits was positive, even though the standard error was high (0.29±0.68). Obtained genetic parameters indicated that early selection has potential to improve carpal hyperlaxity of blue foxes. However, ideal evaluation and selection time point of valgus deformity is at later growth phase. Selection against VALGUS and LAXITY is highly recommended in order to maintain blue fox's ability to move about. 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.

Key words: Alopex lagopus, blue fox, correlation, genetics, heritability, leg conformation

Introduction
Carpal deformities are a common problem among Finnish blue foxes (Kempe 2018). The condition involves excessive hyperflexion at the carpus, sometimes with valgus deformity. Flexural leg deformity may occur already at an early age (1.7-3 months), but is most common at the end of growth period on Oct/Nov (Korhonen et al. 2005, Kempe 2018). Recent WelFur results show no improvement in leg conformation (n=4688 on 81 farms), reporting a high frequency of mild or clear laxity of carpus in production animals: 56.5% and 30%, respectively (Ahola et al. 2014). Carpal laxity is moderately heritable trait in blue foxes (Kempe 2018). Other predisposing factors are fast growth rate, high body weight and fatness.

It seems that flexural leg deformity is not the only conformation problem in the Finnish blue fox population. Preliminary studies on orthopaedic abnormalities in blue foxes indicate that juvenile individuals may suffer from incipient and mild skeletal problems (Korhonen et al. 2005, Mustonen et al. 2017, Svenns 2018). The prevalence, severity and genetics of these problems have not been studied. According to Svenns (2018) abnormal conformation and rotation of a blue fox's legs (angular limb deformities, radius curvus) occur when the growth plates close prematurely and radius and ulna are not grown in a synchronized manner. Also nutritional imbalances, trauma or inflammatory process can damage cartilage cells, which are very sensitive to pressure changes, and cause early closure of growth palates (Knapp et al. 2016). This cause the radius to deform in the cranial-caudal plane (radius curvus), external rotation and ultimately to a valgus deformity of the carpus (Knapp et al. 2016).

The objectives of this research were to develop practical evaluation method for on-farm measurement of the carpal laxity and valgus deformity, to determine statistical models for the estimation of (co)variance components and genetic variation in the leg conformation traits, to understand the co-responses among the studied traits and to determine the suitability and feasibility of the new traits for the blue fox breeding programme.

Material and methods

The experiment was carried out on five commercial fur farms in Finland. Data were collected during growth period in 2018 from altogether 3290 blue foxes. The pedigree was obtained from Saga Furs Oyj. The pedigree structure in the data was monitored using RelaX2 (Strandén and Vuori, 2006). Original pedigree was purned to have only informative animals (n=11 423) in variance component estimation.

The offspring of preselected dams were born between May 18 and June 26 in 2018. Blue foxes were classified into four classes by their time of birth: 104-129, 130-144, 145-160 or 161-180 days from the beginning of the year (Table 2). Litters of more than four pups were selected for the experiment. Pups from the same litter were divided into full-sib pairs as follows: male-male, male-female, or female-female pairs. Uneven animals (70) were housed alone/single in the cage. One percent of the animals were selected for breeding animals and rest were pelted as production animals. Breeding animals are subjected to a restricted feeding regime after weaning, because this improves fertility through longer-term control of BCS (fatness) and body weight (Kempe 2018). The foxes were evaluated twice; first at the time of weaning and for the second time at grading. There were 5 evaluators, who assessed always the same foxes. Age at the time of evaluation was classified into six classes (Table 2).

The studied traits are presented in Table 2. LAXITY, VALGUS, MOVE and BCS were evaluated 1st time on days July 28 to August 23 in 2018 and on days October 29 to November 19 in 2018. The thickness of subcutaneous fat was assessed by a subjective body condition scoring method (Kempe 2018) on a scale of 1 to 5, where 1=very thin and 5=extremely fat. LEG was evaluated on a scale of 1 to 5 (best) (Kempe 2018) and VALGUS on a three-point scale based on the poorer of the forelegs, where VALGUS scores were: 1=toes pointing straight ahead, 2 = toes slightly (0-45^o) rotated outwards and 3=toes strongly (>45^o) rotated outwards. Four point scoring system of MOVE, where score 4 was the best, was modified from the five point scoring system published by Kempe (2018) (WelFur 2014).

Statistical analysis

Preliminary analyses were performed using PROC GLM in the SAS Studio 5.1 software (SAS Institute Inc., Cary, NC, USA). (Co)variance components for the different traits were estimated using the restricted maximum likelihood (REML) method in the DMU program (Madsen and Jensen 2012). Both single- and multiple-trait analyses were carried out. The following linear animal model was used for variance component estimation:

$\mathbf{y} = \mathbf{Xb} + \mathbf{Wc} + \mathbf{Za} + \mathbf{e}$, where **y** is a vector of observations, **b** is a vector of fixed effects, **c**, **a** and **e** are vectors of random litter, animal and residual effects, respectively. Matrices **X**, **W** ja **Z** are the corresponding incidence matrices. In the single-trait analyses, the litter (**c**), animal (**a**) and residual (**e**) effects were assumed to be independent, normally distributed random effects with mean zero and var(**c**)=$\mathbf{I}_q \sigma_c^2$, var(**a**)=$\mathbf{A}\ \sigma_a^2$ and var(**e**)=$\mathbf{I}_n\ \sigma_e^2$, where $\mathbf{I}_n$ is the identity matrix of size n, n is the number of animals with an observation, I_q is the identity matrix of size q, q is the number of litter effects, **A** is the additive genetic relationship matrix, σ_c^2 is the common litter environment variance, σ_a^2 is the additive genetic variance and σ_e^2 is the residual variance. Genetic correlations between traits were estimated with the multiple- trait animal model for all the analysed traits, three traits at a time. In the multiple-trait animal model, the litter, animal, and residual effects were assumed to be

independent, normally distributed random effects with zero mean and var(**c**)=**C** $\otimes$ $\mathbf{I}_q$, var(**a**)=**G** $\otimes$ **A** and var(**e**)=**R** $\otimes$ $\mathbf{I}_n$, where $\otimes$ is the Kronecker product, **C** is the variance-covariance matrix for litter effects among traits, **G** is the genetic variance-covariance matrix among traits and **R** is the variance-covariance matrix for residual effects among traits.

Table 1. *Fixed effects in single and multiple trait analyses by trait.*

	Farm	Sex	Assessor	Age at EVA	Time of birth	Pair type	Status
LAXITY1	x	x	x	x			
LAXITY2	x	x	x	x		x	x
MOVE1	x	x	x				
MOVE2	x	x	x		x		
VALGUS1	x	x	x	x	x	x	
VALGUS2	x	x	x	x	x	x	
BCS1	x	x		x	x		
BCS2	x	x		x	x	x	

1=evaluation at weaning, 2=evaluation at the end of growth period
EVA=evaluation, Status =breeding or production animal, LAXITY=carpal hyperlaxity, MOVE=ability to move, VALGUS=valgus deformity, BCS=body condition score.

Results and discussion

Carpal hyperlaxity is common, but valgus deformity quite rare (6-7% in the data) among the farmed Finnish blue fox population. In the first evaluation 56% of puppies had sufficient to very good LEG1 and most of them had no valgus deformity. Situation changed quite drastically during growth period. In the second evaluation, most of the blue foxes (97.4%) were evaluated as heavy or extremely fat, and 96.9% of them had carpal laxity. Despite the foxes' poor foreleg structure, MOVE1 and MOVE2 were estimated to be fairly good.

Table 2. *Number of observations (n), mean, standard deviation (SD), minimum and maximum values followed by estimated phenotypic variance (σ^2_P), proportion of litter variance (c^2) and heritability (h^2) with standard errors (SE) for the studied traits.*

	n	Mean	SD	Min	Max	σ^2_p	c^2 ±SE	h^2 ±SE
LAXITY1	3288	2.56	0.69	1	5	0.40	0.10 ±0.03	0.20 ±0.06
LAXITY2	3226	1.36	0.56	1	4	0.25	0.10 ±0.02	0.06 ±0.04
MOVE1	3276	3.99	0.08	2	4	0.01	0.00 ±0.02	0.03 ±0.02
MOVE2	3227	3.93	0.30	1	4	0.09	0.06 ±0.02	0.00 ±0.03
VALGUS1	3288	1.07	0.25	1	3	0.06	0.08 ±0.02	0.01 ±0.02
VALGUS2	3227	1.07	0.26	1	3	0.06	0.03 ±0.02	0.11 ±0.04
BCS1	3287	1.46	0.50	1	2	0.01	0.29 ±0.04	0.20 ±0.08
BCS2	3227	4.43	0.55	3	5	0.24	0.104±0.03	0.23 ±0.07

The genetic parameters estimated for LAXITY and VALGUS in this study indicate that genetic improvement through selection may allow to reduce leg weakness in the blue fox and improves the foxes' ability to move.

The heritability estimate of LAXITY at weaning was higher than at the end of growth period, which makes early selection of LAXITY more effective (Table 2). The two traits have favorable genetic correlation with each other, thus a good leg conformation at weaning seems to be linked to better leg conformation later in growth. (Table 3). In the case of carpal hyperlaxity, breeding evaluation and culling can be done already at weaning time.

The valgus posture of forelegs can either improve or worsen as growth progresses. Therefore, valgus deformity needs to be followed carefully during the growth period and assessed preferably twice: at weaning and at the end of growth. Results indicate that environmental factors are main cause behind VALGUS1 as the heritability was close to zero (Table 2). Yet, VALGUS2, which develops during the fast growth period, have genetic background. Although the heritability estimate is low, selection against VALGUS2 is possible.

Table 3. *Estimated genetic correlations with their standard errors (upper triangle) and phenotypic correlations (lower triangle) between the studied traits in the Finnish blue fox* [a].

	BCS1	BCS2	LAXITY1	LAXITY2	MOVE1	VALGUS1	VALGUS2
BCS1		0.33±.23	0.05±.24	0.27±.36	-0.06±.36	**-0.81±.40**	0.01±.28
BCS2	0.00		**0.57±.19**	-0.02±.31	0.66±.35	0.83±.47	**-0.50±.22**
LAXITY1	0.01	-0.02		0.42±.28	**0.93±.30**	-0.52±.64	-0.11±.23
LAXITY2	-0.02	-0.33	0.21		0.06±.52	0.00±.83	0.32±.36
MOVE1	0.00	0.01	0.06	0.01		0.04±.73	0.48±1.44
VALGUS1	0.01	0.04	-0.15	-0.02	-0.03		0.29±.68
VALGUS2	0.06	0.00	-0.02	-0.03	0.00	0.05	

[a] Genetic correlations differing more than 1.96 ×S.E. from zero are in bold.

The genetic correlation between VALGUS1 and VALGUS2 didn't differ from zero meaning that they seem to have similar phenotypes, but their etiology and genetic background may be different (Table 3). Surprisingly, VALGUS was associated with lower BCS (slimmer) in both weaning and end-of-growth measurements. The reasons behind this negative correlation requires a more extensive set of data and further studies, e.g. the effect of body weight and growth rate on the develpment of valgus posture.

Lack of genetic variation in MOVE may be due to problems in phenotypic evaluation of the trait (Table 3). In the previous studies, blue fox's ability to move about was rated on a five-grade scale and the heritability was moderate (0.22) (Kempe 2018). If blue fox is selected for breeding, its ability to move should be god and tested properly, because they are kept longer in a cage. Walking test outside the cage, on the ground could be one option to test breeding animals.

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WebSampo, a breeding program for Finnish fur farmers

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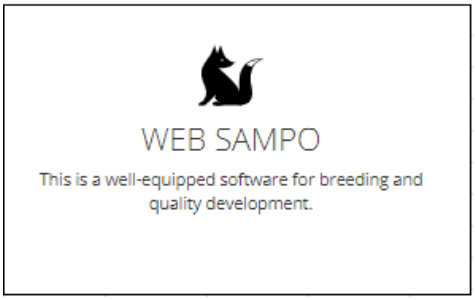
Introduction

Finland is a country with a long tradition of fur farming. In the 80´s there were over 5,500 fur farms but in the end of the 1980´s skin prizes dropped, and as a consequence, in 2013 there were less than 1000 farms, and in 2019 there were about 700 farms left. In 2019, the fur animal production in Finland was 3.1 million. The majority of them were foxes (53.1 %) and minks (33.6 %) and the rest were Finnraccoons. The size of the farms has gone up due to the current employment costs and the use of feed equipment and feeding machines. Most of the farms are nowadays located in Ostrobothnia in the Western part of Finland. The first breeding programs were introduced in the 1970's. A program called Visual Sampo, which only worked on the farms' own computers, was introduced in the 1990´s, and at that time, the first index program MIX99 was implemented. Nowadays, about 1/3 of the Finnish farmers use the breeding program in order to decide selection criteria and for mating planning, as well as to simplify and improve their production.

Figure 1.

In 2013 Saga Furs introduced a new web-based application called WebSampo designed and adapted for both foxes, mink and Finnraccoon. In 2014, the possibility to calculate national indexes for foxes with the MIX99 was implemented. It was possible to start a national breeding value calculation as the data registered by the farmers goes to a database where it can be collected and processed. The indices are standardized and there is multiple-trait evaluation of the fertility parameters. It also allows to estimate breeding value accuracies. The national value calculation increases reliability and makes is possible to compare indexes directly between fox farms. This is useful while trading with living animals and the evaluation describes the genetic value of the animal.

Figure 2.



Material and methods

The MIX99 is a Finnish value calculating 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 like cows, horses, pigs, poultry and fish. The indexes describe the breeding value of the foxes on a national basis. It also gives the possibility to compare the foxes within the farm with farm indexes.

The value calculation covers several different indexes for fertility traits, including mating success, whelping success and litter size. It involves grading traits of living individuals and pelt traits like skin size, quality, colour and even behaviour of the animal. The program also sorts the fur animals in ranking order. When the national fertility values were created, the aim was to find the best possible model. The maternal models were calculated, and it was observed how these react to skin sorting features, as breeding only for production traits may lead to unfavourable changes in other parameters.

At the beginning of the year farmers should register the most important information, i.e. breeding animals and their pedigree and grading information of the live animals. Therefore it is easier to start using a breeding program at the beginning of a year.

During the mating and whelping period it is important to register all fertility and breeding information: matings and inseminations, if the female is pregnant, whelping day, litter size at birth and after three weeks. The information is used for counting the fertility indexes. Animal cards for breeding animals and pups can be ordered through the program; they all have a barcode ticket that should be attached to the individual skin (Figure 3). The barcode ticket allows follow-up of every individual skin with quality information and skin prices.

Figure 3: *Illustration of the barcode ticket from the WebSampo program.*

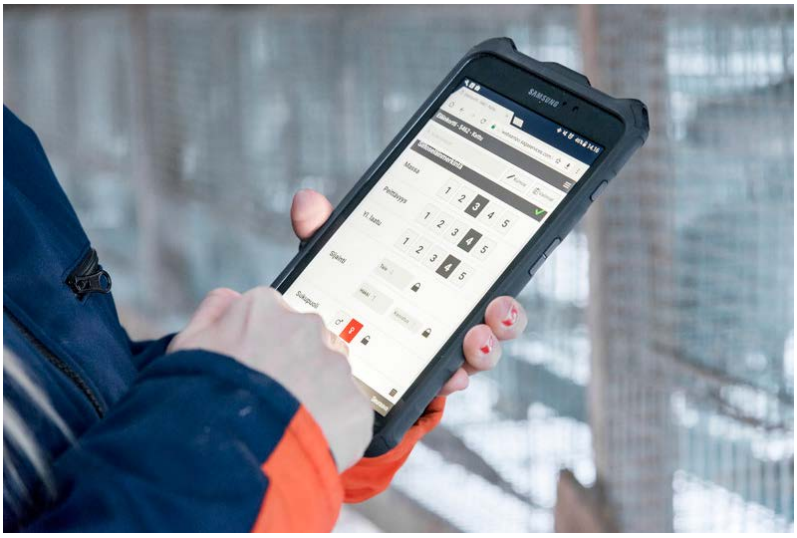
When the pelt is offered at an auction the data concerning each skin is registered in the breeding program. This information is used to calculate skin sorting indexes.

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 makes it possible to follow which breeding animals have produced high class offspring and pelts. While following the skin quality in the program it is possible to notice which animals gave the best results e.g. produced the best skins, and it helps to improve the quality.

To register the animal data there are two different programs available that can be used with mobile phones, tablets or computers (Figure 4). These applications help the farmers to do the daily registering on the farm.

Figure 4: *Illustration of an on-farm data registration WebSampo App.*



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 Web Sampo database. With these two applications it will be possible in the future to select the breeding animals that produce good offspring which grow well and produce good quality skins with a proper amount of feed (feed efficiency).

The WebSampo program has a lot of different farm statistics available. The farmer can also compare his own results with the whole country indices.

Statistics can be calculated for the whole country and WebSampo data has been used in several MSc thesis projects at the University of Helsinki.

Results

Figure 5 shows the litter size per mated silver and blue fox female from 2013-2020 and it is a comparison between farms that are using WebSampo and the other farms in Finland. The figure shows that usually farms that are using the breeding program have better result than the other farms. It also shows that the litter size dropped for all species in 2020. It was due to the challenging time because of the SARS-CoV-2 pandemia.

Figure 5. *Litter size per mated silver and blue fox female from 2013-2020 from farms using WebSampo and the other farms in Finland. Data from Fifur Finnish fur breeders´ association and from Saga Furs WebSampo database.*

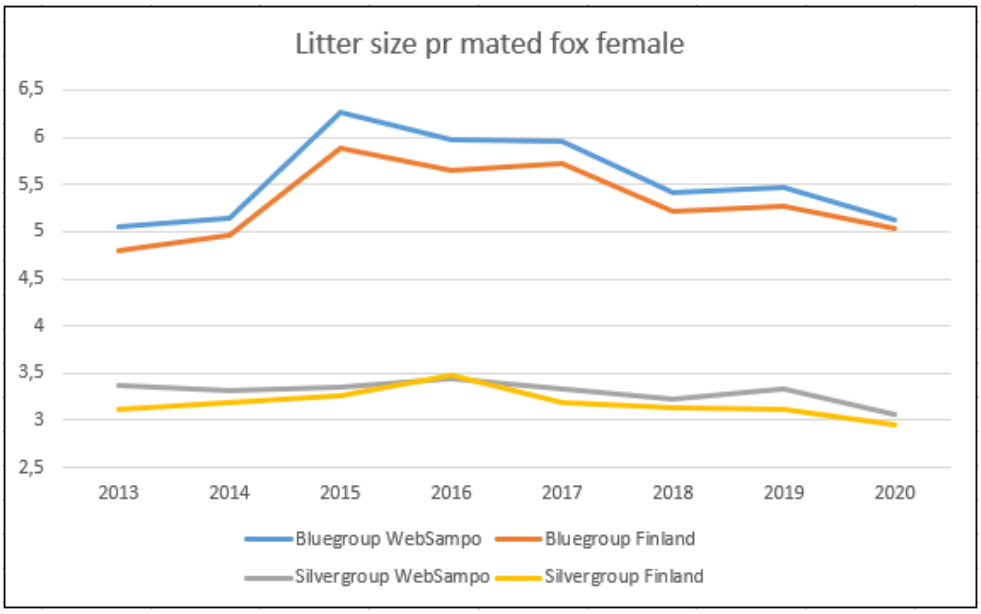
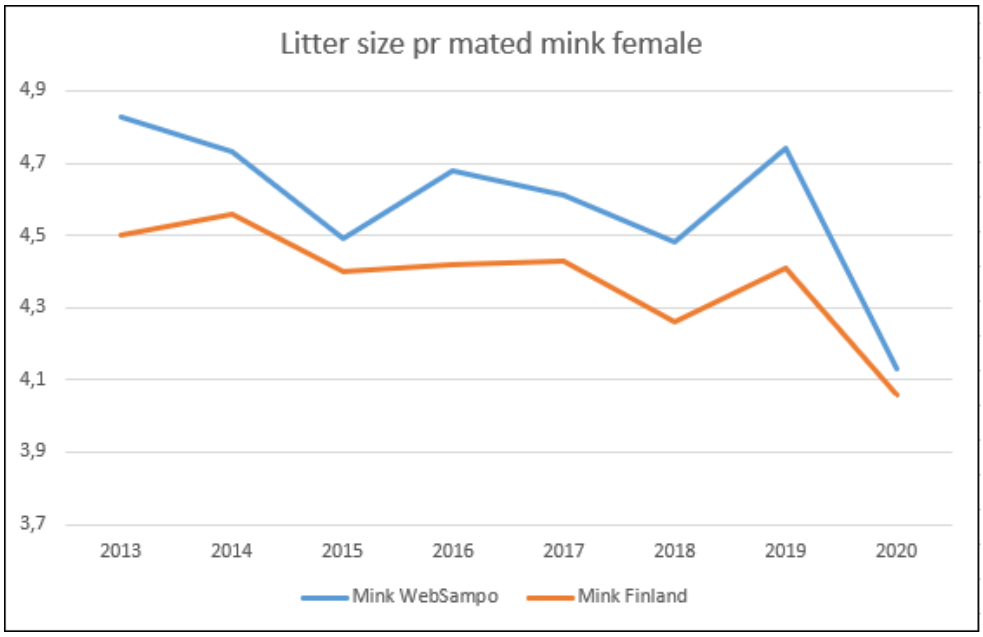


Figure 6. *Litter size per mated mink female from 2013-2020 from farms using WebSampo and the other farms in Finland. Data from Fifur Finnish fur breeders´ association and from Saga Furs WebSampo database.*



Discussion

In animal breeding, it is important to set breeding goals, evaluate the animals and then select the best individuals. The selection should be based on universal indices but it is important to keep only healthy animals. Farms can still have their own selection criteria. The data that the farmers are registering is collected in a joint

database. When the farmers are selling or buying animals, the animal data and the gradings will remain in the database and the pedigree will stays intact, which is important for the value calculation.

WebSampo animal cards include a lot of information about the pedigree and earlier matings and whelpings and there is also room for-additional individual information. If an animal card is lost, it is easy to print out a new one from the program. With the WebSampo simulation tool it is possible to divide the animals into two or three groups and print out the animal cards in different colour.

WebSampo can provide mating recommendations which are based on BLUP-indices and pedigree information of the animal. Minimizing inbreeding is important for maintaining fitness of the offspring as well as genetic diversity. Genetic diversity is essential for the genetic improvement. Breeding program efficiency can be followed up with genetic trends. These trends describe the right breeding animals. Several traits can be improved at the same time with a statistical model for several traits. WebSampo assists producers in the long-term and systematic animal breeding, which leads to higher fur quality and better litter size.

In the future, it is important to set national breeding goals of the organization and educate farmers and the advisors. Functional and health traits should be included into the fur animal breeding scheme.

Projects

Data from the WebSampo database has been utilized in several Master science projects (University of Helsinki)

- Genetic parameters of early pup mortality of blue foxes
- Genetic parameters of feed efficiency, BMI, size of blue foxes
- Genetic parameters of fertility, size and weight of Finnish mink
- Genetic parameters of sperm quality of blue fox
- Development of blue fox national breeding programme (Riitta Kempe, Ismo Strandén).
- Leg conformation and –health in blue fox (ProFur, Luke, University of Helsinki, Luova Oy, Saga Furs)

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

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Abstract

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.

Enhancing production and Aleutian disease resilience in mink through advanced genomics

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Abstract

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 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 five color-types were assembled to identify the most homozygous individual as the reference animal for genome 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.

Keywords: Aleutian disease, American mink, Genomics, Production

Introduction

Mink are semi-aquatic, primarily carnivorous mammals belonging to the weasel (Mustelidae) family (1). Canadian 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 (2). Genomic studies can be used to understand the genetic architecture of economically important traits in mink.

Today, high throughput technologies have been used to generate dense panels of single nucleotide polymorphisms (SNPs) for many animal species (3). Advances in next-generation sequencing have driven the costs of DNA sequencing down to the point that sequencing is feasible for high diversity of species including mink. The domestic goat reference genome is the most continuous *de novo* mammalian assembly to date (4). Thus, an accurate reference genome of mink can now be developed, relatively cost-effectively, using the same method for genomic studies in mink. This will yield a wealth of information on the mink genome including a very large number of SNPs that will be used to better understand the genetic architecture of economically important traits in mink. Therefore, this research will seek to 1) create the first accurate whole-genome sequence assembly of mink, 2) design a robust and informative SNP assay for genomic 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 important traits

including feed efficiency, Aleutian disease resilience, fur quality, reproductive performance, growth rate and pelt size.

Materials and Methods

Animals and traits

The commercial mink that were used in this study, are a combination of full and half sib progeny groups representing a multi-generation family structure drawn from the Canadian Centre for Fur Animal Research (CCFAR) at Dalhousie Agriculture Campus (Truro, Nova Scotia), and one breeding population (Millbank Fur Farm Limited, Rockwood, Ontario). We collected the following traits on 3,323 commercial mink: birth weight, 3-week weight, weaning weight, growing and furring weights and body size, grading weight, harvest weight, body size, feed efficiency, total number born, Number born alive after 24 hours, grading of pelt quality in live animals and dried skin including quality, purity, under wool density, silky appearance, guard hair length, and guard hair thickness. DNA were also collected from all animals using the tongue tissue samples.

Genome assembly and SNP assay development

One hundred mink DNA samples from five color-types (black, demi buff, pastel, mahogany and stardust) were sequenced using high coverage next-generation sequencing (>30x) to create the first SNP assay for American mink. This reference animal has been sequenced using a combination of three technologies: single-molecule real-time sequencing (PacBio RSII), paired-end sequencing (Illumina HiSeq), and Hi-C (Phase Genomics, Inc.). The developed mink reference genome then will be used to develop a custom genotyping assay. This SNP panel will create approximately 50-60,000 SNPs, which is enough for conducting genomic studies in mink. This developed SNP panel will be used for genotyping of 4,000 mink with phenotypic data.

Results and Discussion

A DNA panel composed of 100 American mink from five color-types were assembled to identify the most homozygous individual as the reference animal, which was determined to be a black healthy mink from the Millbank Fur Farm Limited (Rockwood, Ontario). The DNA of this reference mink was sent for high quality *de novo* assembly sequencing, which is still under process to improve the quality of the current reference genome (5). The genome assembly then will be used for development of high-density SNP panel (50-60k). The phenotypic data and DNA samples from 3,323 commercial mink and their parents were collected and these animals will be genotyped using the customized assay. All of the studied traits had enough variation showing the potential of improvement using genetic/genomic selection. This research will seek to discover genome regions, markers and genetic networks underlying these important traits, which will contribute novel insights into the genetic architecture of these traits that can be used for application in mink breeding. This approach will help improve the overall performance of the North American mink industry.

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

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Summary
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 over-dominance 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 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 the pathogenesis, e.g. African swine fever.

The raw data were deposited at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE163677 under Series GSE163677**.**

Running title
Genes enhancing resistance to Aleutian mink disease

Key words: Genetic resistance, virus, immune complex disease, total-IgG hyper-gammaglobulinemia

Introduction
Aleutian Mink disease (AMD), also known as Mink plasmacytosis, arises in farmed American mink (*Neovison vison*) due to Carnivore Amdoparvovirus 1 (Porter et al., 1977, Bloom at al., 1980). The Aleutian mink disease virus (AMDV) was named after the Aleutian mink autosomal recessive (*aa*) Aleutian (blue) coat color mink mutation (Hartsough and Gorham, 1956), in which the previously unseen outbreaks of the devastating disease were first identified. The Aleutian mink coat color is linked with a mutation of a lysosomal trafficking regulator protein (LYST) gene (Anistoroaei *et al*., 2013), resulting in Chediak-Higashi syndrome. In addition to albinism

(silvery hair), secretion of lytic secretory granules by cytotoxic T cells and neutrophils is affected, which impairs clearance of viruses and bacteria (Kaplan *et al.*, 2008). Subsequently, other novel mink color strains were produced through the selective breeding of Aleutian mink, among which the Sapphire and Violet mink are homozygous for the Aleutian coat color allele (aa), and these are likewise severely affected by the disease of high morbidity and mortality. While the homozygous (aa) animals are most severely affected, the disease also occurs with clearly varied frequencies among all color mink strains, including those lacking the Aleutian allele.

Aleutian disease (AD) is accompanied by the restricted virus replication predominantly in lymph node macrophages, and virus DNA replication, RNA transcription, protein expression, with low level production of infectious progeny (Best and Bloom, 2005). The disease is clinically characterized by poor reproduction, gradual weight loss, oral and GI bleeding, renal failure and uremia, and high mortality. The hallmarks of the histopathology in the clinically affected mink are: perivascular infiltration of mononuclear leukocytes (lymphocytes, plasma cells and macrophages) in the lungs, bile duct proliferation and/or infiltration of mononuclear cells in the portal areas of the liver, infiltration of mononuclear cells in lamina propria and/or around blood vessels in the intestines, interstitial nephritis with infiltration of mononuclear cells, mononuclear cell infiltration in plexus choroideus and/or perivascular cuffing of mononuclear cells in the meninges or brain parenchyma (Mori *et al*., 1994).

The AD affected mink develop immune complex deposition in various organs, with accompanied tissue injury mediated through complement activation (Tsokos and Fleming, 2004). In the context of this study, it is important to emphasize that all mink of all color strains are susceptible to AMDV infection, but only certain proportions of animals among various color strains develop the disease. The degrees of pathology among individuals, and the mortality rates among the color strains vary significantly, which strongly indicates the role of the host genetic factor/s in the disease pathogenesis (Johnson *et al.*, 1975). Aasted and Hauch (1988) followed mink with respect to the development of progressive versus non-progressive Aleutian disease following natural AMDV-infection. This was done by plasmaelectrophoresis, detection of antiviral antibodies, as well as by macroscopical examination of various organs at pelting time. They concluded that the progression of Aleutian disease was under the genetic influence.

Currently, there is no treatment for the disease, and the virus eradication efforts widely practiced since late 1970s (Cho and Greenfield, 1978) have only led to short term successes (Cepica & Iwamoto, 2012a, Farid *et al.*, 2012). The reasons for the failures have been: (i) high environmental resistance of the virus, ii) virus shedding during the incubation period, making the removal of infected animals based on seroconversion ineffective; (iii) the persistent nature of the ADV infections accompanied by intermittent chronic virus shedding; and (iv) the existence of virus reservoirs among feral mink, as well as wild species of the Mustelidae family (ferrets, otters, polecats, stone and pine martens), other carnivores such as skunks, genets, foxes and raccoons (Canuti *et al.*, 2015). Importantly, infections with lower pathogenicity virus strains, as well as immunizations, enhance the immune complex mediated component of the disease (Porter *et al.*, 1972, Aested *et al.*, 1998). The failures of the AD control measures are the reason behind the search for the host DNA markers associated with the Aleutian mink disease susceptibility (ADS) vs. resistance (ADR). These are then to lead to the genetic test to be used in breeding for disease resistance. Such a test would represent a major advance in the efforts to reduce the severe economic impact the AD has been having on the mink farming industries worldwide. The only currently available phenotypic "*disease resistance marker*" (not the infection resistance) (Cepica *et al.*, 2012 a, b; Cepica 2014; Cepica 2016), is based on the disturbed MALDI-TOF albumin: IgG (A: IgG) ratio. This marker can only be applied in AMDV infected animals, as the uninfected

animals show as falsely disease resistant. Therefore, this phenotypic test can only be performed only on the farms with high serologically determined prevalence of the infection, and not on the farms and in regions experiencing temporary freedom from the virus. Furthermore, if identified, the genetic marker/s would likely be an important reference for elucidation of the factors participating in the pathogenesis of several important animal and human chronic viral infections, similarly accompanied by the immune complex disease (Trautwein, 1992).

The most significant pathophysiological finding of the diseased adult mink is the progressive polyclonal IgG hypergammaglobulinemia (Obel, 1956). Initially, because of the persistent nature of AMDV infection, the ensuing hypergammaglobulinemia was assumed to be constituted only by antiviral specificities. However, evidence of the significant participation of the AMDV-induced autoimmune antibody has been accumulating since 1980s (Hahn and Hahn 1983, Miyazawa et al., 1994, Babkina 1996, Cepica, 2014). This progressive hypergammaglobulinemia leads to tissue damage through immune complex (I.C.) mediated disease, characterized by glomerulonephritis, arteritis, and death (Porter & and Larsen 1967; Porter *et al.* 1969.; Porter *et al.*, 1973; Porter *et al.*, 1980.; Porter *et al.* 1984).

Association studies of SNP markers have shown useful in establishing association with mink production traits (Cai *et al.*, 2018). While SNP marker associations with resistance against viral disease have been reported in plants (Chung *et al.*, 2014) and lower animals (Rosani *et al.*, 2019), such reports are rare in mammalian species (Bowles *et al.*, 2014). Interestingly, the genetic basis of the susceptibility/resistance to African swine fever (ASF), also an immune complex disease, has been established (Palgrave *et al.*, 2011, Lillico *et al.*, 2017). In view of the chronic development, the presence of extremely varied clinical symptomatology ranging from subclinical to terminal, and of the intermittent virus shedding (Cepica, 2016), sensitive and specific diagnosis of AD among AMDV infected individual animals has been virtually impossible (Jensen *et al.*, 2016). This has been the principal reason behind the previously reported failed AD-SNP association studies (Farid et al., 2014). The sensitive and quantitative test for the AD-associated hypergammaglobulinemia was introduced by Cepica et al., 2012 a, b. This test uniquely allows highly sensitive and specific determination of the immune complex *disease* onset through the decline of MALDI-TOF albumin: IgG ratio. Such sensitive discrimination of hypergammaglobulinemia related homeostatic disturbance was previously not possible, whether by quantification of total, or virus specific antibody. This novel methodological tool has been a significant contribution to an individualized, a sensitive detection of hypergammaglobulinemia-associated homeostatic disturbance/disease due to its capacity to indicate the disease onset over a large range of total, and virus specific antibody levels (Cepica A., 2016). The authors demonstrated that the MALDI-TOF A: IgG ratio is a powerful tool in phenotypic selection of AD resistant individuals among the AMDV infected mink. In this paper, we apply this unique tool to investigate the association of the AD resistance with SNP markers.

Materials and Methods

MALDI-TOF for Albumin/IgG

Blood plasma albumin/IgG profiling was performed as initially described (Cepica *et al.*, 2012 b), with the following adjustments; 0.5 µL of the 1st matrix (sinapinic acid in 85% ethanol at the concentration of 25 mg/mL, Sigma Aldrich, Germany) was applied onto the target polished stainless steel target (MSP 96, Bruker Daltonics, Germany), 1 µL of plasma was diluted 1:200 with proteomic grade water (Milli-Q, Millipore Corp.), and then 1 µL of the mixture of the diluted plasma with equal volume of the 2nd matrix (sinapinic acid in Milli-Q water: acetonitril: trifluroacetic acid) was applied over the first matrix. Matrix assisted laser desorption/ionization-time of flight instrument (Microflex, Bruker Daltonics) was used in the automatic spot selection mode, applying Flexcontrol 3.4 software. Setting of accumulation of 360 satisfactory shots, or early

termination was applied if the intensity of the highest peak reached 5000. Measuring raster was set at 5. Data analysis was performed by Flexcontrol 3.3 (Bruker Daltonics, Germany). Albumin: γ-globulin peak heights were recorded, and the ratios were calculated. As previously validated, the ratios >8 were considered associated with health, and for this purpose, more stringent ratios <5 were considered to indicate hyper-IgG, and by extension Aleutian disease (Cepica *et al.*, 2012 a, b).

Animals

All mink included in the experiments were first proven infected by AMDV VP2-Ab ELISA, as previously described (Cepica, 2016). The animals were then placed into the disease *susceptible* (ADS) and disease *resistant* (ADR) cohorts based on the results of the MALDI-TOF A/IgG. While the A: IgG ratios of <8 were previously demonstrated to indicate disturbance of homeostasis (Cepica & Iwamoto 2012a, Cepica *et all*. 2012b, b, Cepica 2016), to minimize the probability of inclusion of the animals with borderline values to either of the cohorts, only the animals with the A: IgG below 5 were considered disease susceptible in this study, while the animals ranked with the highest A: IgG ratios over 8 were included in the resistant cohort.

By establishing MALDI-TOF A: IgG (A: IgG <5), 230 individuals from a total of 572 (40.5%) of AMDV infected mink were classified as diseased (ADS). This group was designated as group A. The second group (B) was constituted by AMDV infected but AD resistant (ADR) animals (A: IgG >8). Animals were from two different farms, farm 1 with black mink and farm 2 with mahogany color type mink. History of previous selection, if any (e.g., by iodine precipitation test, ELISA) was unknown to us. The percentage of the group A among the animals from farm 1 was 67.5% (3 uninfected mink were excluded /uninfected/), and on farm 2 it was 30% (9 mink were excluded /uninfected/). Twelve mink were excluded as they were antibody negative (not infected), and thus could be either resistant of falsely resistant. However, the number of the uninfected animals was very low (2% of the total population).

DNA preparation and sequencing

The DNA was extracted from buffy coats by QIAmp DNA Mini Kit (QIAGEN) according to the manufacturer's instruction, and DNA quantification was performed on a NanoDrop One (Thermo Fisher Scientific). Finally, DNA samples from 97 animals of the group A (MALDI-TOF A/IgG <5) and 97 animals of the group B (MALDI-TOF A/IgG >8) were double digested and end labelled by restriction-site-associated DNA methodology, (Miller et al. 2007). For each end labelled product 66 bp were sequenced. Approximately 7 million sequences could be localized on the mink genome for each animal, using BOWTIE and SAMTOOLS (Langmead 2010).

Results

Data analyses

The produced sequences were ordered for each animal with reference to the mink sequence from the first draft reference genome of the American mink (Cai *et al.*, 2017) in a sorted bam file by the freeware SAMTOOLS. For group A and group B, the bam files were genotyped by the freeware samtools mpileup command, and finally the number counted for each genotype. Positions with more than one new variant as well as lines with more than 15 missing in one of the groups were discarded. The results from the two groups were merged yielding more than 42 thousand of information lines. In this respect our methodology is slightly different from the standard methodology. More precisely, in Table 1 the data is very comparable to a Manhattan plot, but only the extreme lines (differences between the two groups) are shown. There are markers on 867 assembled mink pieces out of total 931 pieces. There are 18 cases where the distances between the markers are more than 750,000 bp, so there is a good likelihood that all the mink genes were comprehended. The standard deviation

of the differences of the numbers in group A and group B is 7.9 for old variant dominant, with a mean value of –1.1, so the difference more than 40 was considered highly significant which must also be valid for the 5 most extremes in the 3 classes which are presented in our Table 1.

Table 1. *The results indicating the most extreme differences between the two groups of animals.*

		Variant		**A animals**				**B animals**				**difference**			
Mink sequence	*Position*	*old*	*new*	*old ho*	*het*	*new ho*	*missing*	*old ho*	*het*	*new ho*	*missing*	*A-B*	*Canis fam gene*	*Position*	*Chromo -some*
New variant dominant															
FNWR01000007.1	19671970	C	T	38	43	10	6	5	7	80	4	-70	Protocadherin Fat 3 **		
FNWR01000727.1	177545	A	T	35	43	18	1	6	10	81	0	-63	*PRRC2A* *	2304731	12
FNWR01000002.1	13593979	G	T	4	59	23	11	65	25	0	6	-61	ZNF396	54715354	7
FNWR01000315.1	1775906	G	T	28	55	2	12	79	14	0	3	-51	Zinc finger		
Old variant dominant															
FNWR01000253.1	567945	A	C	68	26	0	3	14	65	14	3	54	No neighboring genes		
FNWR01000002.1	2106329	A	C	55	33	1	8	4	57	20	15	51	GATA-6	65935744	7
FNWR01000439.1	319399	C	T	75	19	3	0	25	53	18	0	50	PRRC2A		
FNWR01000721.1	13966	A	G	0	12	80	5	6	54	30	6	50	Zinc finger protein 852		23
FNWR01000624.1	46073	G	A	2	29	66	0	29	43	19	5	47	APT *	2407397	12
FNWR01000439.1	580174	C	T	6	26	65	0	27	50	18	1	47	PRRC2A	58982732	16
Over dominance in one of the groups															
FNWR01000112.1	345419	A	C	8	86	3	0	0	14	80	2	72	NPRL3	40695613	6
FNWR01000147.1	1713060	AAG	A	1	57	39	0	0	13	81	2	44	No neighboring genes		
FNWR01000601.1	144294	T	C	0	14	72	11	1	57	33	5	-43	HLA class II *	2243494	12
FNWR01000106.1	9577216	T	C	5	69	21	2	0	27	56	13	42	No neighboring genes		

* The findings from the 25 next highest differences the following are close to HLA and APT

- complement C2 isoform X2
- natural cytotoxicity triggering receptor 3
- allograft inflammatory factor 1

** found by human homology only

Table 1. The results indicating the most extreme differences between the two groups of animals, under the assumptions that the *original* allele was dominant, the *new* was dominant in one of the groups, and in one case *over dominance* is considered. The mink sequence name and position are given, new and old alleles as well as the number of homozygotes (ho) and heterozygote (het) for group A and group B. In addition, the number missing and the difference between the group A and B are indicated. In the last columns, the dog gene name and genomic position are provided.

As the magnitude of the differences of allele occurrence between the group A and B is abundant, the most extremes variations for candidate genes associated with the immune response were further analyzed. Five positions for each of the three cases, new variant dominant, old variant dominant and over dominance are shown in table 1. Intermediate inheritance was not considered, as its occurrence in cell to cell interaction was extremely rare. For each of the most extreme positions a flanking area of 2000 bp was considered for the position in the mink assembly. These sequences were used to blast the dog genome, to find the corresponding genes either within, or in the proximity. The genes are ordered according to the difference between group A and B. Since no homology to dog could be found for the position FNWR01000007.1 19671970, human homology was used. The genes in this area are very scattered, with the Protocadherin Fat 3 being the closest, however still 130k bp downstream.

Discussion

The genetic nature of the susceptibility/resistance of the individual mink to the disease had been strongly indicated already from the field observations of consistently significant differences in morbidities and mortalities of the outbreaks AMDV infection outbreaks among diverse color strains of mink (Johnson *et al.*, 1975, Hadlow *et al.*, 1983), well prior to the experimental validation by Aested and Houch, 1988.

Obviously, the specific and sensitive phenotypic determination of the disease vs. health in AMDV infected animals is critical for the accuracy of the SNPs association studies. The clinically consequential hypergammaglobulinemia is the most consistent and important clinical feature of the disease, and its determination requires that the total IgG, comprising of the antiviral and the autoimmune idiotypes, is assessed (Cepica, 2014, 2016). However, there is a large range of reported normal reference Ig levels among various species (Weiss et al., 1994), and this makes simple quantification of Ig unsuitable for the purpose of sensitive detection of clinically consequential elevation of serum immunoglobulins triggering the disease among individuals (Cepica, 2016). In MALDI-TOF determination of the clinically relevant hypergammaglobulinemia in an individual, the total IgG is expressed as a ratio with the albumin level (Cepica et al., 2012 a, b). This is necessary, because the total IgG levels, and their degree of participation in inducing immune complex disease do not correlate with the levels of the specific antiviral response. The lowered A: IgG ratio (<5) by MALDI-TOF spectrometry discerns homeostatic disturbance of increased blood viscosity in a highly individualized manner, as the decrease of blood viscosity may or may not occur over a relatively large range of IgG values. The relative signal intensities within the mass spectrum of a complex sample are constant. If the interest is the ratio of two proteins within complex sample like serum, such ratios are quantitatively reproducible (Duncan *et al.*, 2008). In addition, regardless of the subject's degree of dehydration, or the accuracy of the sample dilution, both albumin and IgG signals are acquired in the single MALDI-TOF spectrum, thus yielding the same A: IgG ratio.

In this case, the value of the MALDI-TOF determination of the A: IgG for the purpose of the determination of the AMDV-associated disease vs. health lies in the fact that if the ratio was calculated through independent determinations of albumin and IgG by other methods, rather than by MALDI-TOF, the yielded A: IgG ratio values would be highly irreproducible because of the large standard errors in quantification of both components. Considering the range of the reference mink albumin and IgG values (Weiss et al., 1994), the resulting "normal" reference A/IgG ratios determined through independent measurement of albumin and IgG would lie between 11.3 and 4.1, with the majority of the values being below the MALDI-TOF A/IgG minimal value of eight. This means that many animals deemed diseased, would in fact be clinically healthy, and thus would be improperly eliminated from breeding. The MALDI-TOF A: IgG phenotypic resistance value (>8), used in this study, had been previously validated in the breeding for A. *disease* resistance trial. Breeding of

these animals led to the increase in the birth rates and a decrease in the mortality after the first year of the selection, with the productivity returning to the levels comparable to the periods before AD outbreaks after the second selection season (Cepica, 2016). In contrast, the control (disease susceptible) animals with the A/IgG <5 invariably died in a matter of days or weeks. Thus, in this project the A. disease resistant cohorts was constituted by mink of A: IgG >8. For the additional measure of confidence, and to eliminate the animals with possibly transiently lowered ratio, only the mink with the A: IgG <5 were placed into the disease susceptible cohort.

The accurate, previously unavailable phenotypic assignment of ADV infected animals into "the diseased vs. the healthy cohorts" by MALDI-TOF A: IgG (Cepica *et. al.*, 2012b) was the essential prerequisite to the accuracy of this study. Past research efforts in this direction relied erroneously on the quantification of the ADV specific Ab (ELISA) or on virus presence, as phenotypic disease/health markers (Cepica, 2014; Cepica, 2016), and consequently no meaningful association of Aleutian disease resistance-with SNP markers has been so far reported (Kowalczyk *et al.*, 2019, Farid *et al.*, 2014). The use of only the most extreme allele associations increased the probability of identifying the real effect on the disease resistance. By the comparison to the dog genome, which is relatively well annotated, the associated dog genes might point at the real mink genes implicated in the ADV disease resistance in American mink.

GATOR complex protein NPRL3

In the case of GATOR complex protein NPRL3 isoform X6, there is clearly over dominance, with 72 more heterozygotes in group A than group B (9 standard deviations away from the mean). Since one of the functions of this protein is to remove the excess of antibodies, it seems logical that it shows the strongest effect on the differences between the two groups. The GATOR subcomplex GATOR2 indirectly activates mTORC1 and the TORC1 signaling pathway through the inhibition of the GATOR1 subcomplex (Bar-Peled et al., 2013). It is negatively regulated by the upstream amino acid sensors SESN2 and CASTOR1 (Wolfson et al, 2016; Saxton et al., 2016a; Saxton et al., 2016b). In addition to its role in regulation of the TORC1 complex, it promotes the acidification of lysosomes and facilitates autophagic flux (Cai et al., 2016). By these statements it is regarded as a strong candidate gene.

PRRC2A gene

The PRRC2A gene (Banerji *et al.* 1990) codes for the proline-rich coiled coil 2A, also known as BAT2. The BAT cluster of genes is found near the genes for TNF α and β. PRRCA can be found in the human major histocompatibility complex class III region. The major histocompatibility complex, class II, DQ β and antigen peptide transporter 2 isoform X1 are closely linked, which can be seen from both the dog and the mink position. Using the next most 25 extremes revealed more in the vicinity of the two, the complement C2 isoform X2, the natural cytotoxicity triggering receptor 3 and the allograft inflammatory factor 1. They should all be regarded as one linkage group and genetically they should be treated as one gene, which appears to have influence on the AMDV-associated disease resistance. Although it is difficult to assess in which combination they exert an influence, the MHC region is still a candidate area. From our extremes the chromosomal areas around the MHC indicate that genes in this region play a major role in the development of the AMDV associated disease, as indicated by the lowered MALDI-TOF A: IgG.

Protocadherin Fat 3 (FAT3)

The next most extreme difference between group A and B has been found at FNWR01000007.1 position 19671970 hitting the dog genome approximately 144 k bp downstream of the Protocadherin Fat 3 gene. Due to this distance, FAT3 is considered a weak candidate gene. However, FAT3 should not be completely

excluded as in this region appears to be one of the strongest differences between the two groups of animals. The gene regulates cell to cell interaction.
The rest of the positions indicated in Table 1, are considered to be random variations with no real effect. The FAT3 or some genes in the neighboring region would have a strong effect only if there is strong linkage disequilibrium to the GATOR gene which might be maintained by the higher fertility among females not affected by the disease.

Conclusions
The results of this study show the association of a variant GATOR 6 gene, as well as an area around the HLA complex, with the development of total IgG hypergammaglobulinemia followed by immune complex disease, in some ADV infected animals. The results of this study can provide the base for the future development of a genomic test for assisted selection in breeding for A. disease resistance, where the phenotypic A: IgG selection is not possible because of the temporary absence of the virus on a farm. The finding may also contribute to understanding of other established models of the chronic progressive viral infections caused by members from a variety of virus families, associated with autoimmune disorders, e.g., lymphocytic choriomeningitis virus (LCMV), 2003) and African swine fever (Palgrave *et al.*, 2011, Lillico *et al.*, 2017, Takamatsu *et al.*, 1999), as well as several important human viral infections that belong in this category also, e.g., varicella zoster, Hantavirus, Dengue, B19 parvovirus, hepatitis A, B, C, E, and HIV (Michalska *et al.*, 2001, Kunin, 2017a).

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Conflicts of interests
The authors declare that they have no conflicts of interests.

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Effect of FSH β and NCOA1 gene polymorphisms and expression on pink eyed white mink reproductive traits

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Abstract

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 FSHβ 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 FSHβ and NCOA1 genes, including g.1228G>A, g.1866T>C, g.151536T>C, and g.185162C>T. The g.1228G>A polymorphism of FSHβ 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 ($P<0.01$). FSHβ 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 FSHβ and NCOA1 might be involved in the regulation of embryo attachment mechanisms in pink eyed white mink breeding.

Keywords: Mink, FSHβ Gene, NCOA1 Gene, SNP, Expression

Poster session

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

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Summary
Milk proteins comprise a non-uniform group of compounds different in terms of composition 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). was conducted with our modification. In comparison to the other species, milk of silver fox females is characterized by the profile of casein proteins. The fraction of α-casein was not revealed in fox females milk, whereas, in raccoon dogs, this fraction was stated in trace amounts in the 1st and 2nd phase of lactation. In the milk of both canids, β-casein prevails between casein proteins.. Higher content of particular fractions of whey proteins can point to the presence of inflammation taking place in a milk gland. The content of lysozyme in milk of foxes and finraccoon remains on a relatively high level in contrast to milk of the other females of domestic animals.

Key words, Silver fox, finraccoon, milk, protein, polymorphism

Introduction
Mammals are the only group of animals having a mammary gland that have evolved the mechanism of milk production. Secretion produced in that gland is the only source of food for the offspring in the first weeks after birth. In most mammals, milk excreted only in the period of lactation contains nutrients necessary for proper functioning of young organisms in the first days of life. In monoestrus breeding females of *Canidae* lactation takes place only once a year and this process lasts from 6 to 8 weeks (Ahlstrøm Ø., 1992). Malfunctioning mammary glands cause disorders in milk production and secretion which can be a persistent problem in feeding their pups. As indicates Szeleszczuk et al. (2007), the perinatal period and the first week of pups' life are the most crucial for breeding Canidae. In that period, 30-48% of offspring deaths are recorded and the only food consumed by pups is the female milk (Ahlstrøm Ø. and Wamberg S., 2000).

Milk is biological solution containing approximately 12.8% of dry matter. Milk dry matter consists of proteins, carbohydrates, fats, minerals and vitamins (Szeleszczuk et al., 2017). Depending on animal breed, genotype,

phase in lactation, animal health status, environmental conditions, animal age as well as other factors milk composition may change (Ruska & Jonkus, 2014)

Milk proteins comprise a heterogeneous group of compounds that vary in composition and physical features. Nitrogenous protein compounds of milk are divided into two basic fractions: casein and whey ones. Particular groups are different in terms of amino acids composition, molecular mass and properties. Caseins that belong to the group of phosphoproteins are included in the most important milk proteins. In milk, casein is a colloidal solution appearing in the form of spherical, porous clusters called micelles. Casein fractions perform various functions starting from nutritional to immunological effects. On the other hand, whey proteins appear in the form of molecular dispersion. They develop in the milk gland but a certain amount of them filters from blood plasma. Albumins ordered according to their content in milk: β-lactoglobulin, α-lactalbumin and whey albumin form about 75% of whey proteins. Whey albumins differ from casein in molecular structure, they do not contain phosphorus, however, greater amounts of cysteine, lysine and cystine are stated. Beside numerous albumins, immunoglobulins (Ig) are also included in whey proteins. They are macromolecular globulins which are selectively transported from blood plasma to the milk gland. In relation to the physicochemical structure and biological activity, there are three main classes: IgG, IgM and IgA. The main point is the importance of these molecules as they decide about the level of organism immunity, being responsible for humoral immunity and showing antibacterial activity.

The profile and polymorphism of casein and whey proteins of milk is thoroughly known in most species of farm animals. However, there is lack of information in local and global literature on protein sequence in carnivorous fur animals.

The aim of the paper was to try to determine the profile of milk proteins of farm representatives of Canidae. For this purpose, preliminary methodological surveys aimed at adapting parameters of electrophoresis by SDS-PAGE method to the milk of farm Canidae became essential.

Methods and materials

The analyses were carried out on milk samples collected from 12 females of silver fox (*Vulpes vulpes*) and finnraccoon (*Nyctereutes procyonoides*) in various stages of lactation. Farm animals were in good health and kept in conditions providing their welfare. Feeding was also adapted for the species, age and breeding period. From among collected samples, 9 were selected from every female trying to choose representative ones for particular phases of lactation. To separate milk proteins, denaturing polyacrylamide gel electrophoresis (PAGE) in the presence of SDS was used. In milk electrophoretic separation, whole cow's milk was used as a point of reference. 4% stacking gel and 12% separating gel were used to separate proteins according to their size. The proteins were separated in the Bio-Rad's MINI-PROTEAN system with constant voltage initially set at 100 V and increased after 40 min to 150 V. To determine the moment of completing electrophoretic separation of proteins, the electrophoresis front was observed. The gels and required for their preparing 10% APS, 10% SDS and buffers as well as the electrode buffer were made *ex tempore* of Sigma reagents. To estimate molecular masses of separated proteins, the Prestained Precision Protein Standards marker (Bio-Rad, catalogue no. 161-0372) was used as after separation it gives bands equivalent to proteins of molecular masses in the range from 10 to 250 kDa.

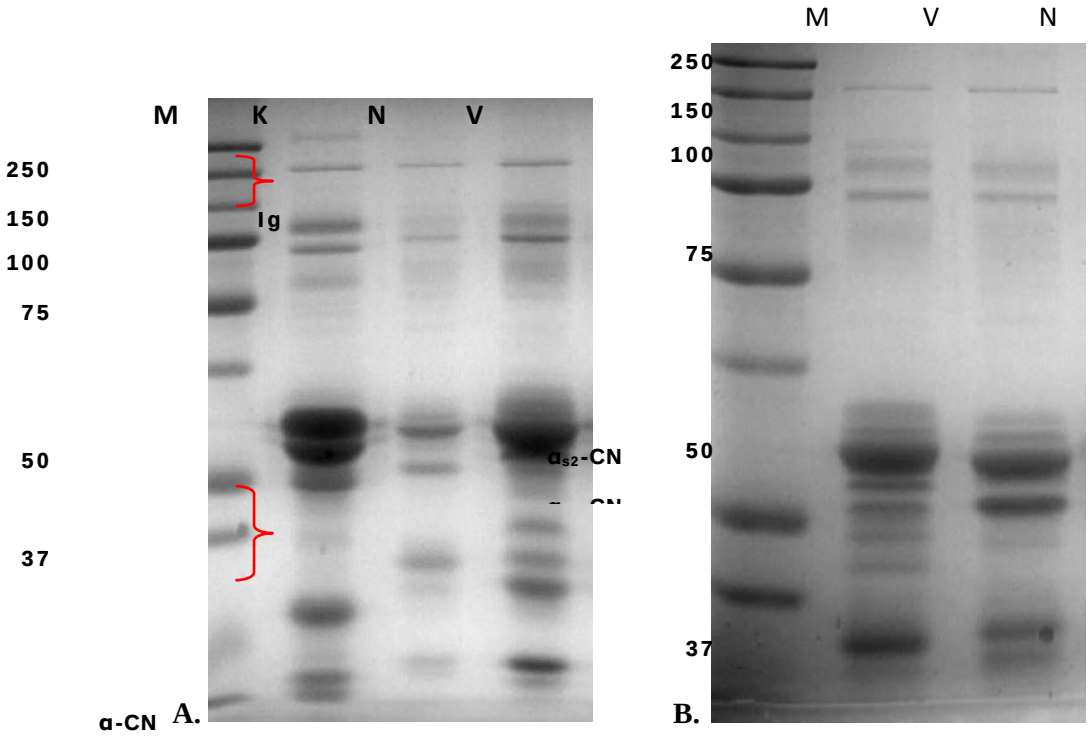
Results and discussion

Comparing milk proteins of silver foxes and Finnraccoons separated as a result of denaturing electrophoresis with cow's milk proteins illustrates great diversity of milk content between species that are so distant from

each other phylogenetically. And it is not just in terms of the amount of proteins included in them but also their molecular mass. In the light of the results of this analysis, it is no surprise that efforts to raise orphaned fox kits on cow milk usually failed [Young and Grant 1931]. Contrary to cow, Canidae milk contains less α-caseins and the one of silver fox contains also less κ-casein. Canidae α- and β-caseins have greater molecular mass. κ-caseins with the smallest molecular mass were present in the milk of cows but also their mass was significantly lower in finraccoons than in foxes. In the milk of Canidae, γ-caseins are present in much greater amount than in cow. On the other hand, the fraction of γ-casein was not observed in Finraccoon. The content of β-lactoglobulins in Canidae milk was lower than in the analysed cow milk, contrary to the observed content of α-lactalbumin which, additionally, turned out to be higher in the milk of Finraccoon. Both these whey proteins have higher molecular masses in the analysed species of carnivorous fur animals.

The analysis of milk samples coming from various periods of lactation in particular species revealed that changes within proteins are marginal or even absent. In silver fox, the level of lactoferrin slightly increases and the content of β-lactoglobulin decreases. In Finraccoon, the amounts of serum albumins and α_{s1}-caseins were slightly higher and the number of immunoglobulins was decreasing along with the course of lactation. In electrophoretic separation of milk proteins of analysed females of silver fox and Finraccoon, larger inter-individual differences were not observed. In silver foxes, one of the samples had significantly increased concentration of proteins, especially whey ones, and one additional band was observed in the milk of V5 individual which, however, requires further analyses.

Fig. 1. *Comparative electrophoresis in milk proteins of different animal species.* ***A*** *– cow, finraccoon, silver fox* ***B*** *– silver fox and finraccoon. Designations: M - marker, K - cow, V - fox, N - finraccoon, IG - immunoglobulin, LTF - lactopherine, ALB- albumin serum, CN-casein, αLA - α-lactoalbumin, βLG - β-lactoglubulin, # - low molecular peptides.*



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Estimation of genetic parameters of growth curve parameters in mink

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Abstract

Understanding the genetics underlying growth curve is important for selection of animals with better growth potential. 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, Bridges and Janoscheck) and then estimated the heritabilities of parameters derived from the best-fitted model and the genetic correlations among them. 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. Sex had significant effect ($P < 0.05$) on asymptotic weight (α), constant growth rate (β), growth rate at mature (k), shape parameter (m), weight at inflection point (WIP), age at inflection point (AIP) and absolute growth rate (AGR) while colour had a significant effect on k, m and AIP. The estimated heritabilities (±SE) for α, β, k, m, WIP, AIP and AGR were 0.21±0.08, 0.15±0.08, 0.07±0.07, 0.20±0.08, 0.33±0.09, 0.10±0.08 and 0.15±0.07, respectively. Significant ($P < 0.05$) positive genetic correlations were estimated between several growth parameters. 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.

Keywords: Genetic parameters, Mink, Nonlinear models

Introduction

Growth is an economically important trait in animal farming; therefore, better knowledge of animal growth is necessary for optimized management and feeding practices and genetic improvement of the species. Among different approaches to understand the animal growth, mathematically modelling which allows to characterize the growth patterns and visualize the shape of growth over time, is a particularly useful approach. In livestock species, growth rate and other growth parameters were shown to be heritable and responsive to the selection programs. Mink is a major animal used for fur industry. Price of pelt is the most important factor for mink farmers, and it is known to have a strong correlation with body weight at pelting. Previous studies suggested that growth parameters can be used in mink breeding programs (Liu, et al. 2011, Do, D.N. and Miar, Y., 2020). Therefore, this study was performed to determine the best growth models for different color types in mink and then to estimate the genetic parameters for growth traits derived from the best performance model.

Materials and methods

Mink were all raised individually in each cage under standard farming conditions at the Canadian Centre for Fur Animal Research (CCFAR) at Dalhousie University. Body weights were reported in there week interval from week 13 (August 1) to week 31 (December 6) of life. A total of 516 females and 510 males with five different colours (black, demi buff, pastel, mahogany and stardust) were used in the current study.

A selection of six different non-linear growth models including Logistic, Gompertz, Brody, Richards, Bridges, and Janoscheck were used for describing the growth models in mink [1,2]. In each model, the body weight

was fitted as a function of measurement week. The Akaike's information criterion (AIC) was chosen to determine the most optimal model. Sex was used as a fixed effect in the models for estimating the genetic parameters for asymptotic weight (**α**), growth rate at mature (**k**), shape parameter (**m**), weight at inflection point (**WIP**), age at inflection points (**AIP**) and absolute growth rate (**AGR),** while color was also used as another fixed effect in the models used for genetic parameters estimation of **k**, **m** and **AIP.** Heritabilities and genetic parameters were estimated under univariate and bivariate models using the DMU package (https://dmu.ghpc.au.dk/), respectively.

Results

The Richards model had the lowest AIC values in the black, demi buff, mahogany and pastel mink while Logistic model had lowest AIC value in the stardust mink. Overall, the Richards was the best model to describe the growth curve in mink. The estimated heritabilities (±SE) for **α, k, m, AGR, WIP, AIP** and **AGR** were 0.21±0.08, 0.07±0.07, 0.20±0.08, 0.33±0.09, 0.10±0.08 and 0.15±0.07, respectively (Table 1).

Table 1. *Estimates of genetic (below diagonal) and phenotypic (above diagonal) correlations, heritabilities (diagonal), and their standard error of estimates for growth parameters.*

Growth parameters	**α**	**k**	**m**	**WIP**	**AIP**	**AGR**
α	***0.21±0.08***	*-0.17±0.03******	*-0.17±0.03*	*0.42±0.03*	-0.06±0.04	*0.26±0.03*
k	0.29±0.20	**0.07±0.07**	*0.91±0.01*	*0.58±0.02*	*0.94±0*	*-0.11±0.03*
m	0.14±0.20	*0.89±0.04*	***0.20±0.08***	*0.55±0.02*	*0.93±0*	*-0.26±0.03*
WIP	*0.76±0.10*	*0.78±0.09*	*0.70±0.11*	***0.33±0.09***	0.64±0.02	*-0.21±0.03*
AIP	0.36±0.19	*0.94±0.03*	*0.91±0.03*	*0.82±0.08*	**0.10±0.08**	*-0.18±0.03*
AGR	0.33±0.20	-0.08±0.24	*-0.58±0.20*	0.05±0.20	-0.31±0.23	***0.15±0.07***

* Significant values were highlighted in Italics.

Significant (P < 0.05) positive genetic correlations were estimated between several parameters in growth models such as **α** and **WIP** (0.76±0.10), between **k** and **m** (0.89±0.04), **k** and **WIP** (0.78±0.09), and **k** and **AIP** (0.94±0.03) (Table 1).

Discussion

Significant (P < 0.05) effects of sex and week of body weight measurement found in the current study were also reported in the previous studies (Liu, et al. 2011, Do, D.N. and Miar, Y., 2020). Similar to the previous reports (Liu, et al. 2011, Do, D.N. and Miar, Y., 2020), we also reported that Richards was the best model in four different colour types (black, demi buff, mahogany and pastel) and Logistic was the best model for stardust mink. The differences in the model performance might be either from the variation in the genetic makeup of animals or from the differences in the samples size. It is important to note that we had only very few individuals for stardust mink which might reduce the accuracy of assessment of the model performance. The low-to-moderate heritabilities for growth parameters in the current study indicated the low-to-moderate genetic variability for these traits. The magnitude of estimated heritabilities and genetic correlations for growth parameters varies in the other species. Overall, the results suggested that the selection program to improve the slope of the growth curve of mink may be feasible, but further studies required to determine the effectiveness of reshaping growth curve in mink via a breeding program.

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Skeletons morphometrics of the farmed types of blue fox (*Alopex lagopus*) – preliminary research

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Abstract
Breeding work carried out over the years in the Polish breeding of the blue fox, involving the introduction of new types from the Polish type, through Norwegian, to Finnish, has contributed to increase in the body weight of animals. This increase automatically caused anatomical changes in the fox organism, especially in the bone system. 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 during fur slaughter. The bones were treated with 3% water solution of NaHCO3 and bleached in perhydrol solution and then dried. Among other things, the length of the skull, 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. 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.

Introduction
The breeding of polar foxes in Poland was carried out on a large scale since the fifties of the 20th century. The fox bred at that time was similar in size and shape to a wild Arctic fox. In order to improve the quality of fur in particular, polar foxes of the Norwegian type were imported to Poland in the 80s, and of the Finnish type in the 90s (Cholewa 1988). Breeding changes resulted in a significant growth of animal weight. The sudden increase in the body weight of animals sometimes even several times, entails anatomical changes, especially within the foxes' skeletal system.

The aim of the study was to determine the morphometry of the skeletal system in the blue fox, Polish, Norwegian and Finnish type.

Material and Methods
Preliminary studies were carried out on 8 individuals of polar fox of different breeding type came from farms in Poland. One fox of Polish type, two animals of Finnish type and 5 animals of Norwegian type were examined in basic morphometric tests.

The bodies were cleaned of muscles and then prepared in 3% water solution of sodium bicarbonate (NaHCO3). Cleaned bones were bleached in perhydrol solution (H_2O_2) and dried at room temperature (16 to 20°C) for 24 to 48 hours. Size measurements were taken with a slide caliper or tape with an accuracy of 0.1 mm.

The following indicators were measured: length of individual sections of the spine; length of long bones; measurements of the skull; measurements of the pelvis and scapula.

Results

The collected measurements showed that Finnish foxes were characterized by the highest body weight (26.5 kg), Norwegian foxes (9.4 kg) were only slightly heavier than Polish type foxes (7.4 kg).

On the basis of skull measurements, clear differences in absolute skull length between fox types were found. The value of this parameter ranged from 0.168 m in Finnish type foxes, through 0.154 m in Norwegian type to 0.145 in Polish type. In the case of the length of the facial part, the skull of the Norwegian and Polish type reached a similar value, and a significant longer part was characterized by the skull of the Finnish type. An inverse relationship was found for the length of the brain part of the skull, where similar length was found in the Finnish and Norwegian type, and much shorter in the Polish type. The skull of the Polish type, despite being similar in size to the skull of the Norwegian fox, was significantly wider and more massive.

The length of individual sections of the spine between fox types was compared. The length of the cervical, thoracic, lumbar, sacral and caudal sections was analysed. It was observed that the length of the cervical section of the spine increased respectively with an increase in the body weight of the examined animals. The average length of the thoracic section of Norwegian foxes (0.184m) was slightly longer than that of the Polish type (0.18m), but significantly shorter than that of the Finnish type (0.227m). A similar relationship was observed in the mean length of the lumbar section (0.125m - 0.157m) and in the sacral section (0.275m - 0.335m). In these four sections of the spine, the length of individual sections increased between the types of foxes (Polish - Norwegian - Finnish) with an increase in their body weight. The longest caudal section was characterized by the Norwegian fox type.

In the case of scapula measurements, the highest values of all parameters were obtained for the Finnish type of fox, and the lowest for the Polish type of fox. The analysis of mean length of the bones of the long thoracic limb: humerus, elbow and radius showed the same correlation. The longest bones were characterized by the fox in the Norwegian type, and the shortest by the fox in the Polish type. The values of these parameters for the fox in the Finnish type were slightly lower than for the Norwegian type.

In case of pelvic measurements, the highest values of all parameters were obtained for fox of Norwegian type and the lowest for fox of Polish type. The analysis of the length of long bones of the pelvic leg: femur, tibia and fibula was performed. The average length of femur of foxes in the Norwegian type was slightly longer than of foxes in the Finnish type. The shortest femur was found in foxes of the Polish type. The average length of tibial and fibula was similar in Finnish and Norwegian type foxes, but slightly longer in Finnish type foxes. Significantly shorter tibial and fibula were found in foxes of Polish type.

Discussion

Zoometric measurements are used to objectively assess the animal's shape and provide information about its structure. They allow the animals to compare with each other and with their pattern, and monitor the growth of the animal and changes in body structure over generations.

The first zoometric measurements of foxes of the Polish type were carried out in the 1950s (Frindt, 1960). There are no morphometric data on Finnish and Norwegian foxes.

Due to extensive breeding work, the knowledge of anatomical changes in the skeleton of foxes is important for their proper breeding.

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DNA specific repeats in mink and some related species.

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Abstract

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 coat color. The material investigated are genomic DNA from 6 domestic mink, 2 trapped mink from North Minnesota, a ferret an otter and an ermine. DNA from some of the animals was sequenced on the Illumina HighSeq 2000 platform. 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 ermelin, 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 study 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.

Data

Table 1. *Number of repeat found in mink and related species*

-	b-lokus	b-lokus*	m-repeat	virus	virus*	mil.reads	
minnesota1	10	0*	1664	5078	78*	303	
minnesota2	98	0*	393	539	9*	44	
cross	581	275*	3874	5278	518*	136	
brown	6	6*	294	378	97*	33	
US-brown	1134	286*	7770	8958	900*	530	
NCBI.mink.seq	3	0*	86	111	6*	4.8***	
ferret**	0	-	0	5	-		
hermelin	0	-	0	2	0	39	
odder	0	-	0	0	0	29	

* together with m-repeat found by blast

** from NCBI genomic assembly

*** an extract of NCBI seq with a "paired read" 150 bp for every 1000 bp

Table 2. *The repeat in percentage*

-	b-lokus*	m-repeat	virus	per.mill.reads
minnesota1	0.14	24.64	75.2	22.3
minnesota2	9.51	38.15	52.3	23.4
cross	5.96	39.80	54.2	71.6
brown	0.90	43.00	56.1	20.3
US-brown	6.34	43.49	50.1	33.7
NCBI.mink.seq	1.50	43.00	55.5	40.4***
ferret**	0	0	100	-
hermelin	0	0	100	-
odder	0	0	0	-

* together with m-repeat found by blast

** from genomic assembly

*** an extract of NCBI seq with a "paired read" 150 bp for every 1000 bp

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

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Abstract

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 ± 0. 10) and the other two groups (G-1: 0. 28 ± 0. 16; G-2: 0. 27 ± 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.

Keywords: Chinchilla, melanogenesis, tyrosine, phenyloalanine, behaviour

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

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Abstract

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 2,198 mink 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) and counterimmunoelectrophoresis (CIEP), non-specific AD test of iodine agglutination test (IAT), health test of packed cell volume (PCV), reproductive performance of number of live kits at birth (LB), overall fur quality (LQ) and guard hair length (LN). Significance ($P<0.05$) of fixed and random effects were determined by univariate analyses while 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 qELISA, 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 LQ, and 0.52±0.06 for LN. The qELISA had significant ($P<0.05$) positive genetic correlations with CIEP (0.41±0.11) and IAT (0.61±0.08), and negative genetic correlations with PCV (-0.49±0.08), LB (-0.24±0.12), and LN (-0.18±0.09). High heritability of qELISA and its significant genetic correlations with IAT, PCV, LB, and LN suggested that qELISA can be used as an indicator trait for AD resilience in mink genetic/genomic selection programs.

Key words: American mink, Aleutian disease, reproductive performance, fur quality, genetic correlation, heritability.

Introduction

The serious economic losses caused by Aleutian diseases (AD) make it difficult for mink farmers to maintain their business. The unsatisfactory outcome of AD test based culling strategy has urged the fur industry to select for AD resilient mink (1). Selection for disease resiliency requires a heritable indicator trait that is easy to measure and genetically correlated with disease resilience traits. Several AD related tests have the potential to be used for genetic selection of AD resilient mink, but little is known about their genetic and phenotypic parameters. Therefore, we aimed to 1) estimate the heritabilities of AD related tests, fur quality, and reproductive performance traits, and 2) estimate the genetic and phenotypic correlations among AD related tests, fur quality, and reproductive performance traits.

Materials and Methods

Mink were raised under standard farming conditions at the Canadian Centre for Fur Animal Research (CCFAR) at Dalhousie Faculty of Agriculture (Nova Scotia, Canada). The data included 2,198 mink which were the progeny of 281 sires and 671 dams. The measured traits were quantitative enzyme-linked immunosorbent assay test (**qELISA**), iodine agglutination (**IAT**), counterimmunoelectrophoresis (**CIEP**), packed cell volume (**PCV**), the number of newborn kits survived 24 hours after birth (**LB**), and overall fur quality (**LQ**) and guard hair length (**LN**) in live animal.

The significance of the fixed (sex, year, age, colour, and test month) and random effects (permanent environmental, dam, and common litter) were tested for each trait using REML procedure in ASReml 4.1 (2). Only significant (P<0.05) effects were kept in the following bivariate model to estimate the genetic and phenotypic parameters for each trait:

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X_1} & 0 \\ 0 & \boldsymbol{X_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_{a1}} & 0 \\ 0 & \boldsymbol{Z_{a2}} \end{bmatrix} \begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_{pe1}} & 0 \\ 0 & \boldsymbol{Z_{pe2}} \end{bmatrix} \begin{bmatrix} \boldsymbol{pe_1} \\ \boldsymbol{pe_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_{d1}} & 0 \\ 0 & \boldsymbol{Z_{d2}} \end{bmatrix} \begin{bmatrix} \boldsymbol{d_1} \\ \boldsymbol{d_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_{c1}} & 0 \\ 0 & \boldsymbol{Z_{c2}} \end{bmatrix} \begin{bmatrix} \boldsymbol{c_1} \\ \boldsymbol{c_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix},$$

where $\boldsymbol{y_1}$ and $\boldsymbol{y_2}$ are the vectors of observations for trait 1 and 2, respectively; $\boldsymbol{b_1}$, $\boldsymbol{b_2}$, $\boldsymbol{a_1}$, $\boldsymbol{a_2}$, $\boldsymbol{pe_1}$, $\boldsymbol{pe_2}$, $\boldsymbol{d_1}$, $\boldsymbol{d_2}$, $\boldsymbol{c_1}$, $\boldsymbol{c_2}$, $\boldsymbol{e_1}$, and $\mathbf{e_2}$ are the vectors of fixed, additive genetic, permanent environmental, dam, common litter, and residual effects for trait 1 and 2, respectively; $\boldsymbol{X_1}$, $\boldsymbol{X_2}$, $\boldsymbol{Z_{a1}}$, $\boldsymbol{Z_{a2}}$, $\boldsymbol{Z_{pe1}}$, $\boldsymbol{Z_{pe2}}$, $\boldsymbol{Z_{d1}}$, $\boldsymbol{Z_{d2}}$, $\boldsymbol{Z_{c1}}$, and $\boldsymbol{Z_{c2}}$ are the incidence matrices relating the observations to fixed, random additive genetic, permanent environmental, dam, and common litter effects for trait 1 and 2, respectively. The final heritability for each trait was obtained by averaging the estimates of multiple corresponding pairwise bivariate analyses.

Results

The genetic and phenotypic correlations, heritability, and their standard error of estimates among the studied traits were presented in Table 1. The qELISA had the highest heritability among all AD related tests (0.43±0.05 for qELISA, 0.22±0.06 for CIEP, 0.19±0.05 for IAT, 0.22±0.05 for PCV). The qELISA showed significant (P<0.05) genetic correlations with CIEP (0.41±0.11), IAT (0.61±0.08), PCV (-0.49±0.08), LB (-0.24±0.12), and LN (-0.18±0.09).

Table 1. *Estimates of genetic (below diagonal) and phenotypic (above diagonal) correlations, heritabilities (diagonal), and their standard error of estimates among the studied traits.*

Trait	qELISA	CIEP	IAT	PCV	LB	LQ	LN
qELISA	**0.43±0.05**[1]	**0.26±0.03**	**0.40±0.03**	**-0.32±0.03**	**-0.10±0.03**	0.02±0.04	-0.04±0.04
CIEP	**0.41±0.11**	**0.22±0.06**	**0.17±0.03**	**-0.10±0.03**	**-0.56±0.03**	-0.01±0.04	-0.05±0.04
IAT	**0.61±0.08**	0.28±0.15	**0.19±0.05**	**-0.30±0.03**	-0.07±0.04	0.05±0.04	**-0.09±0.04**
PCV	**-0.49±0.08**	-0.03±0.17	**-0.56±0.11**	**0.22±0.05**	0.07±0.04	**-0.11±0.04**	0.03±0.04
LB	**-0.24±0.12**	-0.21±0.22	-0.16±0.21	0.20±0.21	**0.08±0.02**	0.05±0.05	-0.02±0.05
LQ	-0.05±0.11	-0.09±0.17	-0.22±0.14	**-0.40±0.14**	0.25±0.20	**0.35±0.07**	**0.24±0.03**
LN	**-0.18±0.09**	-0.19±0.14	**-0.25±0.12**	-0.03±0.13	0.19±0.17	**0.38±0.12**	**0.52±0.06**

[1]*The significant (P < 0.05) estimates were bolded.*

Discussion

The appearance of AD can decrease reproductive performance and degrade fur quality of mink (3,4). Selecting AD resilient mink based on a heritable AD related test trait, which is genetically correlated with disease resilience traits, is a potential method to effectively control AD. To the best of our knowledge, there is no previous study conducted on the estimation of genetic parameters for qELISA test in mink. However, the estimations of genetic parameters for qELISA were reported for other diseases in other species. For instance, the heritability of qELISA test for Johne's disease in dairy cattle was estimated to be 0.07-0.16 using different models (5) and 0.02 for Newcastle disease in chicken (6), which were lower than our estimate of 0.43. The qELISA had low-to-moderate genetic correlations with LB (-0.24) and LN (-0.18), which were similar to the genetic correlations of qELISA for Johne's disease with production traits (-0.11 with protein yields in milk and -0.29 with productive life) in dairy cattle (5), but much lower than the genetic correlations of qELISA for porcine reproductive and respiratory syndrome with the number of piglets alive at 24 hours (0.73) in swine

(7). The inconsistency of our genetic parameters estimations with other studies can be attributed to several factors including the nature of diseases, genetic makeup of species, statistical models, and the different transformations of qELISA scores. In conclusion, the high heritability of qELISA and its favorable genetic correlations with LB and LN showed the potential of qELISA test as a good indicator trait for AD resilience in mink.

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

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Abstract:
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 fifty 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 IV: Environmental impact of fur farms

Environmental impact of fur farms

Oral presentations

Changes and megatrends in global fur business

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Summary
In recent decades, the international trade pattern in both raw fur skins and fur garment has changed. Fur garment industry has to a high degree moved from Europe to Asia – in particular China. However, the picture of Asia as very important importer of raw fur skins, and as manufacturers of fur garment is also changing. Hong Kong was a transit country, but now China is the direct market. Within recent years new Asian countries have become more important within dressing and tanning of raw fur skins, but also production and export of fur clothing in these new Asian countries has increased. The income level seems to be significant drivers behind the international specialisation in global fur industry.

The purpose of this article is to identify, explain, verify and empirically document selected trends and changes in the global fur business. Such trends and changes may be important for the long-term and strategic development of the entire fur industry.

Keywords: Wave, international specialisation, garment, China

Introduction
The fur industry is a part of very global value chains: The production of raw fur skins 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 low-wage countries. The international trade and specialisation 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 skins and fur garment has changed. 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 skins, 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 skins, while Hong Kong lost importance.

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.

On this basis, the purpose of this article is to identify, explain, verify and empirically document selected trends and changes in the global fur business. Such trends and changes may be important for the long-term and strategic development of the entire fur industry.

From Europe to Asia

In the early 1960s, the U.K. and Germany were the dominant raw fur skin importing countries in the world. Together they accounted for 70 per cent of total imports. Subsequently, the pattern of trade has changed.

A new international division of labor has taken place over the past 50 years or so. The world center for raw fur skin trade and demand has shifted from Western Europe and North America to Asia, particularly China. Countries like the UK, Germany, France, Belgium, USA and Canada were all major importing countries for decades, but their role has declined significantly and has been taken over by China in particular. The shift from Europe to Asia is evident from figure 1 and 2.

Figure 1. *Europe's and Asia's import of raw fur skins (per cent of world total import)*

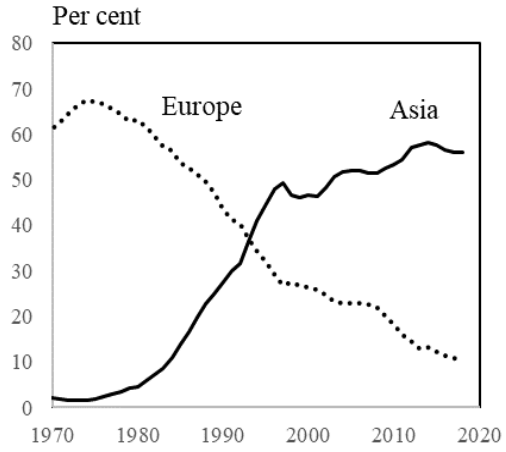
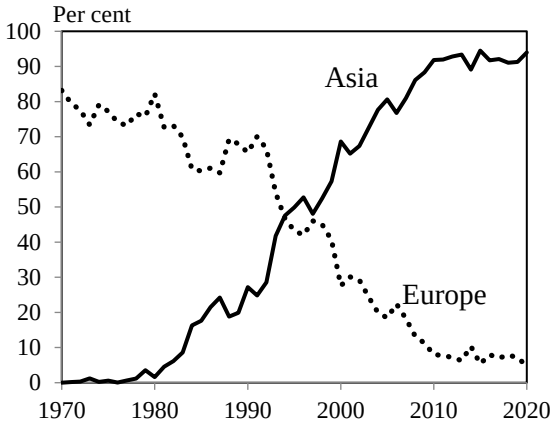


Figure 2. *Major markets for Denmark's export of raw fur skins (per cent of Denmark's total export of raw fur skins)*



Note: 3 years moving average

Source: Own calculations based on UN (2020), Statistics Denmark (2020) and Danish Agricultural Council (several issues)

Figures 1 and 2 show the same trends, but the database and trade flows are different:

Figure 1 shows two continents' imports of raw fur skins. In this case, import is proxy for the economic activity in the fur processing industry and the fur garment industry etc. By using imports as a proxy for economic activity in the industry, domestic production of raw fur skins used in the processing industry is not included.

However, quite few countries produce both raw fur skins and fur garments at a significant level, so the use of import as a proxy is realistic in this case.

Import includes also intra-Europe and intra-Asia trade, but re-export is excluded. Import to Denmark and Finland is characterized as re-export aiming at fur auction sales in Copenhagen and Helsinki.

The figure shows that an increasing share of world import of raw fur skins goes to Asia, while Europe's share of total world import has decreased significantly. Figure 1 illustrates that in the years1980-2010 a major part of European fur processing industry and fur garment industry has moved to Asia.

Figure 1 builds on import data from many countries collected in UN COMTRADE database. Such data can be uncertain and unreliable – especially given the countries included in this analysis – so the trends shown should preferably be confirmed by other data and studies.

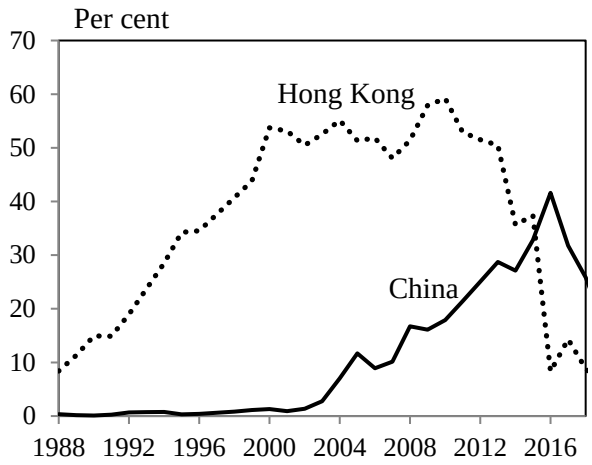
Figure 2 shows the same basic trend as figure 1, but differs by using relatively reliable trade statistics. The figure is based on Denmark and Danish exports of raw fur skins to Europe and Asia respectively. As Denmark is a major producer and exporter of raw fur skins during the period, and as export data comes from Statistics Denmark, the results can be assumed to be both representative and reliable. The figure shows that an increasing part of Danish export of raw fur skins has been targeting Asia, while export to other European countries has had a decreasing role. This confirms clearly that a major part of European fur processing industry and fur garment industry has moved to Asia.

From Hong Kong China to Mainland China

Hong Kong has been the clear market leader for a long time when it comes to international trade in fur skins and fur products. From 2000 to 2010, more than half of total world exports of raw skins went to Hong Kong. It is very unusual for one country to account for more than half of total world imports of one product. Hong Kong was typically transit country, with China being the final destination market. Subsequently, China became the direct export market, while Hong Kong lost importance.

Hong Kong's role as the dominant marketplace for raw fur skins emerged during the 1990s (See figure 3).

Figure 3. *The significance of Hong Kong and China for the four main exporting countries: Raw fur skins. 1988-2018*



Note: The four largest exporting countries during the years were (in addition to Hong Kong): Denmark, Finland, Canada and the United States.

Source: Own production based on UN (2020).

The figure shows the percentage share of exports from the four largest exporting countries, which has gone to Hong Kong and China respectively. For the years presented, these four countries accounted for more than 95 percent of total raw fur exports to Hong Kong from all countries.

The figure shows a very sharp increase in exports to Hong Kong from the late 1980s to around 2000, with Hong Kong accounting for 50-60 percent of all imports of raw skins in the world. After 2013 the share of export has fallen significantly. Since 2003, China has become increasingly significant as an export market for raw fur skins, and China was few years ago the world's largest export market for the four largest exporting countries combined. There are clear indications of export market substitution from Hong Kong to mainland China.

There are no fur farms in Hong Kong, but fur processing takes place in Hong Kong, although the number of companies is decreasing. Most of Hong Kong's fur industry and processing is now located in China at plants, which are owned by Hong Kong Chinese.

From China to "New Asia"

Asia as an export market is also changing in other ways. As shown in figure 3, in recent years export to both China and Hong Kong decreased. Fur processing industry near costal areas in Eastern China is under pressure from other industries and from authorities, and to some extend the industry is moving to other parts of China and to other countries. Also increasing wages and lack of sufficient labour is driving this development. An increasing share of exports to Asia is now going to a number of countries, which can be called "New Asia". These countries consist primarily of Cambodia, Vietnam, Malaysia and Thailand, cf. figure 4 and 5.

Figure 4. *Import of raw fur skins (per cent of world total import)*

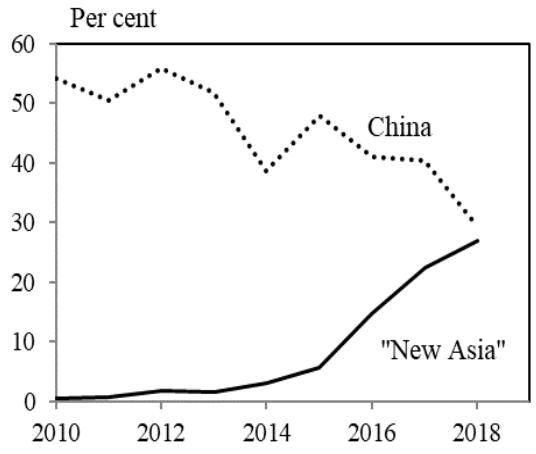
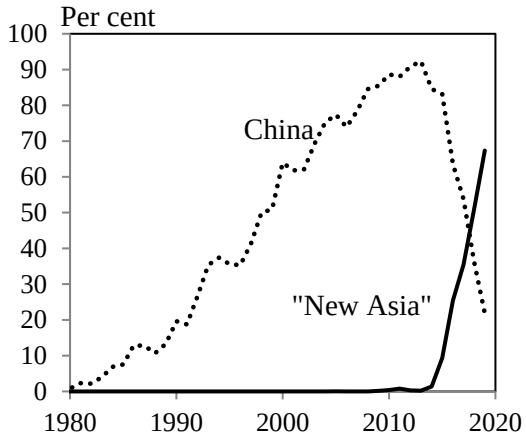


Figure 5. *Denmark's export of raw fur skins (per cent of Denmark's total export of raw fur skins)*



Source: Own calculations based on UN (2020), Statistics Denmark (2020) and Danish Agricultural Council (several issues)

Figure 4 clearly shows that in recent years an increasing share of raw fur skin import from Asian countries goes to "New Asia".

As was the case with figure 1, figure 4 is also based on import statistics which can be uncertain and which may need verification. For that reason figure 5 shows the development of Denmark's export of raw fur skins to China and to New Asia. Figure 5 confirms the new trend towards more focus on fur industry in New Asia. 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 own processing companies, which now increasingly have moved from China to these new Asian countries. What has moved from China to New Asia is the mostly tanning and dressing industry, while the fur garment and fashion industry has moved less. However, New Asia also exports finished products like fur jackets and coats, and Europe and North America are important markets for this export. The export of fur garment from New Asia doubled from 2013 to 2018 and now amounts to more than USD 50 millions – equivalent to almost half of, for example, France's equivalent export.

Change in geographical value chain

China's new position, where part of the tanning and dressing industry has been offshored to other low-cost Asian countries, changes the geographical value chain, as China is a major player in the market. Figure 4 illustrates this development.

Figure 4. *China's import of raw fur skins and tanned, dressed fur skins*

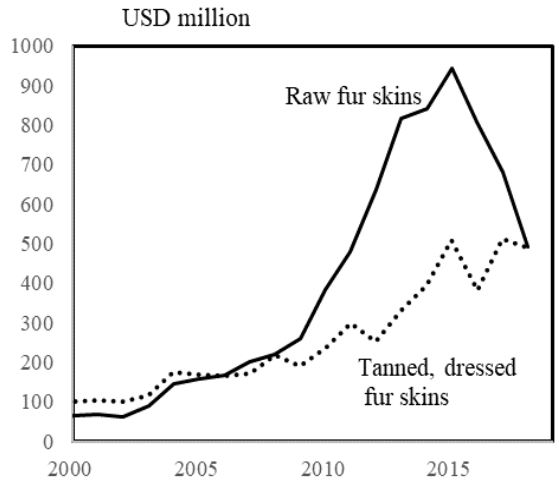
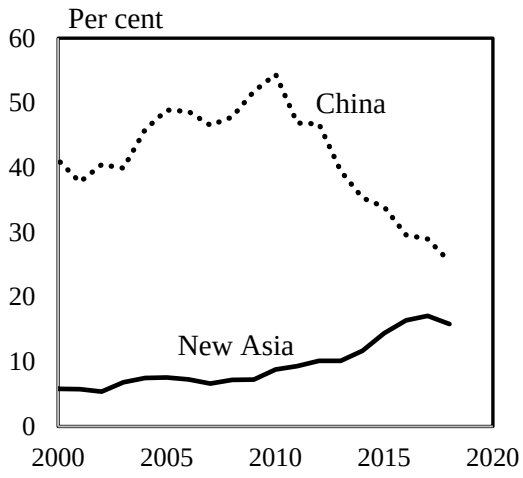


Figure 5. *Europe's import of fur clothing, accessories etc. from Asia*



Notes to figure 5:
Europe = France, Italy, Greece, Spain and UK being the biggest European importers
China = (China mainland + Hong Kong): Europe's import from China as a share of Europe's total import
New Asia: (Thailand, Vietnam, Indonesia, Malaysia, India, Pakistan, Philippines, Cambodia, Myanmar and Singapore). Europe's export to New Asia as a share of Europe's export to Asia (China, Hong Kong + New Asia). 3 years moving average.

Source: Source: Own calculations based on UN (2020)

Figure 5 shows that during the recent decade a declining share of Europe's import of fur clothes etc. comes from China. An increasing share is imported from European countries (intra-EU import). The figure also shows, that an increasing share of Europe's import from Asia now comes from New Asia.

All in all, the trends verify that New Asia is gaining an increasing role in dressing and dying of raw fur skins - mostly at the expense of China. However, China is still a very large manufacturer and exporter of fur clothing, but part of the dressing and tanning has left the national value chain and has been offshored. New Asia also takes over part of China's production of fur clothing, but to a lesser extent than dressing and tanning.

Economic welfare level and fur business

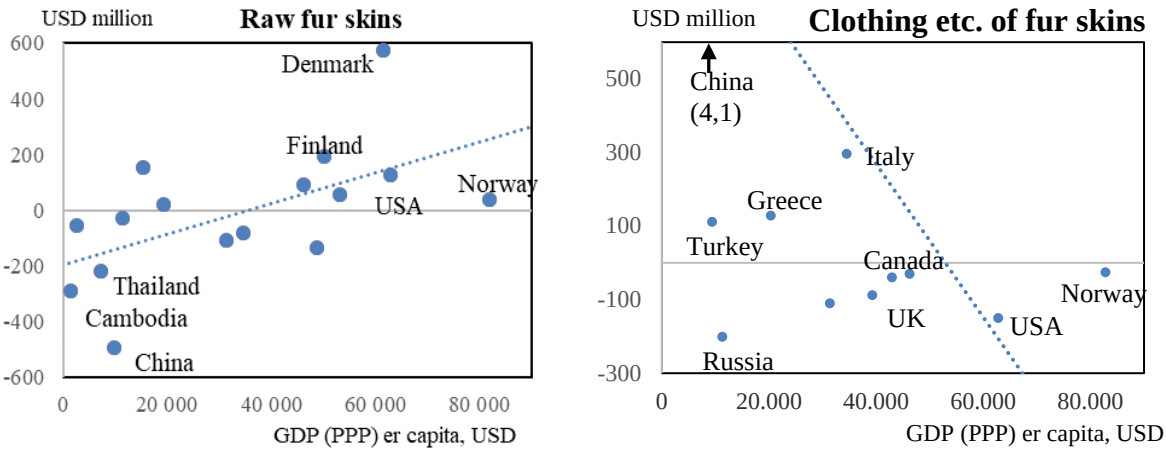
The level of economic welfare – measured as gross domestic product per capita, GDP – is an important driver in fur business: First, increasing GDP stimulates demand for fur garments, as demand is rather income elastic. Second, GDP to a certain degree determines the international specialisation of fur business, as production of the raw fur skins (fur farms) takes place in developed countries, while value is added in developing low-cost countries. The significant difference in wage costs, increasing globalisation and intense international competition means that low technological and labour-intensive production moves to countries with low costs.

At the same time, fur animal production has become very advanced, and, in reality, the production of high quality fur requires a range of skills. Knowledge in the fields of disease prevention, breeding, advice, vertical integration, access to feed, etc. are critical areas, which not all countries are able to handle optimally.

The climate also clearly plays an important role as it limits the number of countries, which can produce fur competitively.

In general, there is a relatively clear pattern globally: Raw fur skins are predominantly produced in high-income countries, while production of fur clothing mainly takes place in countries with low costs and low GDP per capita. These connections are illustrated in figure 6.

Figure 6. *Net export of raw fur skins and fur clothing: Various countries in relation to per capita GDP*



Note: Net export and GDP per capita: 2018. All countries with net export or net import > 20 million USD are included.

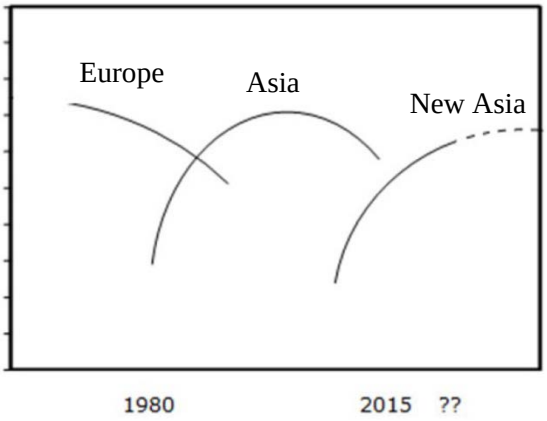
Source: Own calculations based on UN (2020) and World Bank (2020) and Hansen (2016 a+b)

The figure illustrates the correlation between, on the one hand, the level of economic development of the countries (GDP per capita), and on the other, the net export of raw fur skins and fur clothing. The general picture is that the high-income countries produce and export raw fur skin, while less developed countries produce and export fur clothing.

Global waves

From an overall and historical perspective, three significant global waves can be identified: In the first wave, the fur clothing industry was located in Europe. In the next wave, it moved to China. In an upcoming wave, it will move to other Asian countries (New Asia), cf. figure 7.

Figure 7. *Global centers of fur processing and fur garment industry (Schematically)*



Source: Own production

To some extend the fur industry seems to follow 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 not fur product customers.

The fur farms have primarily been located in Europe and North America throughout the period. China has tried to produce raw fur skins from its own fur farms, but it has been a very fluctuating production and of relatively low quality.

Conclusions

In this article several trends and changes in fur business are identified explained, verified and empirically documented:

- Over the past 50 years the world center for raw fur skin demand has shifted from Western Europe and North America to Asia, particularly China.
- Hong Kong was market leader for a long time when it comes to international trade in fur and fur products. However, since 2003, China has become increasingly significant as an export market for raw fur skins, and a market substitution from Hong Kong to mainland China has appeared.
- In recent years an increasing share of raw fur skin import from Asian countries goes to New Asia.
- New Asia is gaining an increasing role in dressing and dying of raw fur skins - mostly at the expense of China.
- New Asia also takes over part of China's production of fur clothing, but to a lesser extent than dressing and tanning.

- The general picture is that the high-income countries produce and export raw fur skin, while less developed countries produce and export fur clothing. The level of economic welfare is an important driver for the international specialisation in global fur business.
- The fur industry seems to follow the same pattern and waves as the textile industry, which has also moved to countries with lower labor costs.

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Pyrolysis of fur animal manure into fertilizer product

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Abstract
Finnish fur animal production is concentrated in the region, where manure phosphorus (P) reallocation is needed due to the regional P surplus. 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 oxygen free atmosphere and char, liquid and gas fractions are produced. Slow pyrolysis at two different temperatures as a processing method for fox (FM) and mink manure (MM) was studied and suitability of char as P fertilizer and liquid for biogas production was evaluated. Also energy content of gas and liquids was investigated. Regarding to MM, P availability in char to plants was comparable to superphosphate and energy contents of gas and liquids higher than from FM. Results are promising for the suitability of pyrolysis for fur animal manures although further research is needed.

Keywords: biochar, phosphorus recycling, pyrolysis liquid, biogas production

Background
Finnish fur animal production is concentrated in the region, where manure P is produced over the regional crop production need (Ylivainio et al. 2014). Thus local use of fur animal manure as fertilizer is often restricted and manure P should be transported to the areas in need of P fertilization. However, dry and P-rich fur animal manure is suitable for various manure processing technologies that could enhance its transportation and more sustainable use. One alternative could be slow pyrolysis, where manure is heated in oxygen free atmosphere producing char, liquid and gas fractions. Process parameters and feedstock affects the properties of the different fractions, but in general, pyrolysis reduces both manure volume and mass and concentrates non-volatile elements like P into the char. To be feasible and sustainable manure processing technology, all pyrolysis by-products should be used sustainably and energy needed in the process be covered as much as possible by renewable energy. Thus in addition to investigate fertilizing properties of the char fraction, we aimed also evaluate the energy content of gas and liquid fractions as well as suitability of the liquid for biogas production. The research was funded by EU's Rural Development Programme for Mainland Finland 2014 – 2020.

Methods and materials
FM and MM were collected under the roofed housing units in spring 2017 from the single farm, pre-dried and pyrolysed at 340 and 470°C using batch-type laboratory-scale equipment. The effect of pyrolysis on fertilizing value compared to fresh and dried FM and MM was investigated by analyses such as pH, ash content, total P and P solubility (Hedley fractionation; Sharpley and Moyer 2000). Plant availability of P was assessed with pot experiment (completely randomized block design, n=4) 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 the detected gas main components. From the pyrolysis liquids, LHV was analyzed for unseparated pyrolysis liquids (470°C), whereas methane production potential (37 °C, 37 d, n=3) was tested after tar separation.

Results

FM and MM were suitable for pyrolysis after drying. About half of the dried manure ended up to the alkaline (pH 8–10) char fraction with high ash (45–70%) and P (65–95 g/kg DM) content. P availability for ryegrass in fresh and pre-dried manures was almost comparable to SP. Although pyrolysis decreased P solubility in both manures (Table 1.), P plant availability decreased only with pyrolysed FM compared to SP. The energy contents of pyrolysis gas from MM were higher than from FM. Furthermore, the pyrolysis liquid from MM had higher LHV and methane production potential than from FM.

Table 1. *P solubility (%) according to the Hedley fractionation in the fresh and pyrolysed mink (MM) and fox manures (FM). Results for the pyrolysed manures represents ranges of pyrolysis runs in two different temperatures. Water- and $NaHCO_3$ -extractable P represents bioavailable, whereas NaOH- and HCl-fractions are less bioavailable.*

	MM	Pyrolysed MM	FM	Pyrolysed FM
Water	33	6-7	15	3-5
$NaHCO_3$	19	5	14	4
NaOH	4	1-2	3	1
HCl	43	86-87	69	90-92

Discussion

Pyrolysis produced alkaline char fraction rich in P, while it decreased P solubility. To our knowledge, pyrolysis as a processing technology for fur animal manure and its effects on fertilizing value has not been studied, but our results are in line with studies done with other manures (Cantrell et al. 2012, Cely et al. 2015, Keskinen et al. 2019). Compared to Ylivainio et al. (2008) P availability of pyrolysed FM and MM was only slightly lower than in pelletized FM compost. The results suggest that energy from gas and liquid fractions could cover the energy needed for manure pre-drying. Furthermore, liquids might be suitable for biogas production as a co-feedstock. The pyrolysis seems promising technology to process fur animal manure into organic fertilizer products.

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

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Summary
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 annual 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.

Keywords: N, P, K, Mink waste, Mink slurry, Annual Mink Dam Production

Introduction
The Danish regulations of the main plant nutrients N, P and K from farm animal production have been based on calculations for each farmed species for more than 30 years. For most farm animals, the calculation unit have been 'a full annual production' depending on the type of production, e.g. on heifers or milking cows for cattle, on sows or finishing pigs for swine and on broilers or laying hens for poultry. For mink, the calculations have been based on one year of production from a mink dam on a mink farm, i.e. the concept of an 'annual mink dam production' (AMDP) including the kits delivered and breeder males needed for mating. In the first calculations, this was modelled for a hypothetical farm, but since 1999, the Danish regulations of mink waste have been based on regular 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, i.e. the number of AMDP, in order to calculate the content in the total mink waste from the production. As feed consumption and food content may vary between farms and feed kitchens, farmers have been given an opportunity to adjust their calculations to the actual situation.

Materials and Methods

The calculation has been based on the total feed production on all the Danish feed kitchens throughout the year and the analysed content of N, P and K from daily samples. The analysis have been sampled as part of the feed quality assurance and has been analysed and made available for calculations by the company 'Dansk Pelsdyr Foder A.M.B.A.' (Danish Fur Animal Feed) and Kopenhagen Fur. In the calculations, the total feed consumption is distributed on the total number of mink dams in Denmark on April 1st and this calculation of an AMDP is the basis for the regulation of mink waste in Denmark. The content of N, P, and K in the manure is calculated as the content in the feed minus the content deposited in the bodies of the animals produced within a year. Based on the average digestibility of the protein, the distribution of N between faeces and urine has been calculated in order to estimate the potential for evaporation, while the undigested and wasted content is found in the manure. In order to calculate the amount of nutrients in the urine and manure that will be available as fertilizers in the field, the figures from excretion from the mink are used to calculate the loss/evaporation of nutrients on the farm and during storage. These norms are calculated for an AMDP including the kits delivered and the males needed for mating. In order to adjust the calculations in case a farm has a very different feed consumption or a very different content of N or P than the average, formulas for correction have been developed.

Until 1999 the calculations were based on a 'model' mink farm with 1000 successfully mated breeding dams, 200 males and 5 500 kits born, while the calculation of nutrient content was based on the typical feed composition from the feed kitchens in Denmark. As the analyses of the feed production gradually intensified and expanded, the calculations also improved in precision over the years. As part of the obligation of Aarhus University to provide information for the Danish Competent Authorities, we have been calculating these figures for more than 35 years. Now that the Danish production have ended, this good opportunity to pass on the results and experience might be of value to others as well.

Results

Traditionally N has been the key focus in the Danish regulations and the developments in the calculations from 1999 to 2019, including a number of other key figures, are presented in Table 1. The outcome of the similar calculations for an AMDP regarding P and K have been included as well.

Table 1. *Key figures from the calculation of nutrient content of mink waste in Denmark from 1999 to 2019 for an "annual mink dam production" (AMDP) which is a dam, her litter and the male used for mating.*

Excretion of N, from an annual mink dam and per pelt produced	**1999**	**2003**	**2005**	**2007**	**2009**	**2011**	**2013**	**2015**	**2017**	**2019**
g delivered in feed	4923	5420	5749	5581	5924	6063	6251	6262	6449	6437
g in feed loss	394	434	460	446	474	485	500	501	516	515
g eaten	4529	4986	5289	5135	5450	5578	5751	5761	5933	5922
Deposited in percent of eaten	*7.33*	*7.04*	*7.29*	*7.59*	*7.60*	*7.66*	*7.74*	*8.07*	*7.81*	*7.96*
g deposited in body and pelt	332	351	386	390	414	427	445	465	463	471
g excreted in faces and urine	4197	4635	4903	4745	5036	5151	5306	5296	5470	5451
Deposited in percent of delivered	*6.75*	*6.48*	*6.71*	*6.99*	*6.99*	*7.05*	*7.12*	*7.43*	*7.18*	*7.32*
g excreted in faces	679	748	899	873	927	948	978	979	1009	1007
g excreted in urine	3518	3887	4004	3872	4109	4203	4328	4316	4461	4444
g N from an annual mink dam	**4591**	**5069**	**5363**	**5191**	**5510**	**5636**	**5806**	**5797**	**5985**	**5965**
g P from an annual mink dam	**1029**	**907**	**1141**	**801**	**859**	**1000**	**1060**	**1027**	**957**	**943**
g K from an annual mink dam	**544**	**-**	**527**	**494**	**486**	**467**	**562**	**546**	**609**	**531**
Food production in Tonnes	418416	506137	571392	640417	655449	64965	801891	877480	874849	632407
Thousands of dams 01/04	2137	2512	2662	2933	2865	2835	3161	3396	3394	2464
Average litter size	5.36	5.29	5.45	5.34	5.52	5.53	5.61	5.55	5.38	5.34
Kg feed per annual mink dam	196	201	215	218	229	245	254	258	258	257
Kg feed per kit (pelt)	37	38	39	41	42	44	45	47	48	48
g N per pelt produced	857	959	983	971	998	1020	1036	1045	1113	1118
Average body weight for males	2500	2700	2900	3000	3100	3200	3300	3500	3600	3700
Average body weight for females	1300	1400	1500	1550	1600	1650	1700	1800	1850	1900

Over the 20 years from 1999 to 2019, the amount of N, P and K per AMDP has increased gradually by 29.9% 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. In the same time span, the amount of feed per AMDP has increased by 31.1%.

In the latest calculations based on data from the 2019 production, the available amount of N, P and K as plant fertilizer after storage was 3620 g, 777 g and 438 g from the slurry tank and 599 g, 173 g and 219 g from the bedding material and waste from under the cages.
The following two correction factors for farms that differed from the norm was set to:

- For feed consumption and protein content in the feed, the correction can be calculated as:
  - ((kg feed pr. annual mink dam * g crude protein pr. kg of feed / 6250) – 0.471) / 5.97.
- For feed consumption and phosphorus content in the feed, the correction can be calculated as:
  - ((kg feed pr. annual mink dam * g phosphorus pr. kg of feed / 1000) – 0.070) / 0.944.

Discussion

From 1999 to 2019, the protein content in the feed has decreased slightly, and therefore increased a little less than the increase in feed consumption. Simultaneously the body weight at time of pelting has increased by 47 %. The amount of P and K per AMDP decreased illustrating that the content in the feed is in surplus and depends mainly on the feed ingredients available and thus the content of e.g. fish bones and other indigestible parts.

The increase in the parts of the nutrients deposited in the mink indicates that the feed efficiency increases with feed consumption and body weight. This is the same as in other animal species although the potential is less in mink as the length of the growth season depends on the priming of the winter fur rather on the weight development.

Due to the closing of mink production as we know it in Denmark, these calculations will not be continued. I hope that others might find them useful or inspiring.

Acknowledgements

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https://anis.au.dk/forskning/sektioner/husdyrernaering-og-fysiologi/normtal/

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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Summary
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.

Keywords: Model, retail, weight, area, sustainability, recommendation

Introduction

In order to calculate the fur environmental footprint in Europe, the exact amount of fur skins on the European consumer market must be calculated or estimated. The amount must be presented in number of skins, weight or area. However, in general, the availability of data to quantify the consumer market on country levels is insufficient. National statistics do not include these data, and statistics from business organisations etc. are in general not sufficient and comparable.

At the same time, fur garments are produced and purchased in many different countries. International trade is significant, as China is a major producing and exporting country, while Russia and Western countries are major importing countries. Such globalized markets and global value chains make it even more difficult to uncover production and trade flows and to estimate final consumption.

Several previous studies have been based on data from few countries with available data, which made it possible to build models that can be used to estimate the retail value of fur products in other similar countries. However, this method and this approach had several weaknesses: First of all, not all countries fit so well in such a model. Secondly, the method estimated the value of fur coats and fur garments at consumer level, but fur accessories etc. were not included, and coats etc. with only minor fur parts were included at full value. Thirdly, transforming consumer market value of all kind of fur garments to area or weight of fur skins is difficult and uncertain.

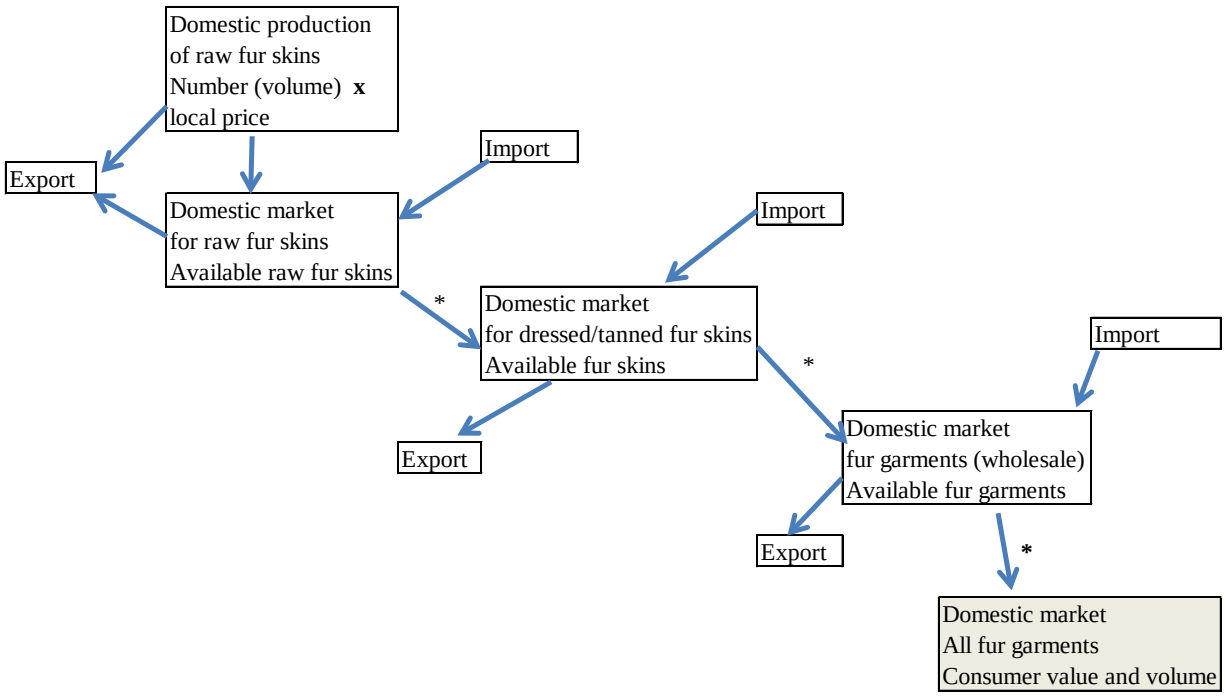
Based on this experience, a new way or model to collect data and to estimate the consumer market volume can be considered: Calculating the fur volume and value downstream from farmer to consumer based on raw skin production, import and export data along the value chain and mark-ups. Mark-ups are factors or coefficients that measure the value of fur skins. A mark-up of 7 means, that the value a raw fur skin is multiplied with 7 from auction house to a fur coat in a retail store. Mark-ups can also be used from fur manufacturing sale to retail sale, from import value to retail sale etc. Mark-ups can then be used to calculate the fur retail value in each country, each region and globally.

This model starts with raw fur skin, where national data are available - or can be available - except for countries, where data are less reliable. Based on mark-ups in the value chain from raw skin to final consumer products, and based on import and export of both raw and tanned skin and fur clothing, the total final consumer volume and value for each individual country, group of countries and for all countries in total can be estimated.

Model description

The model, value chain, trade flows, inter-relations and coefficients are shown in figure 1.

Figure 1. *The fur consumer model: Value chain, trade flows, inter-relations and coefficients*



*Note: * shows where mark-ups are used*

Source: Own presentation

The model takes production of raw fur skin as a starting point – and then we move downstream in the value chain. This "forward integration approach" ensures some consistency of both model, data and results.

The model must be used for all individual countries, and finally all results can be collected in a global or regional database. The model can be updated each year, by use of constant mark-ups, or by updating mark-ups year by year.

Mark-ups are determined by local reporters from each (major or significant) country. Special countries (with no local data supplier, with reliable fur garment consumer value or volume, or countries with non-transparent markets like e.g. China) can be treated separately by using data from other sources and add data directly to the bottom line.

Coefficients (mark-ups) are expected to be rather identical for similar countries. However, methods to estimate coefficients can be described. Calculation of fur skins per fur garment can be used: If one fur coat demands for example 25 fur skins, and if the price of both coat and raw (or dressed) fur skin is available, then the factor or mark-up can easily be calculated. See also Hansen (2016a) with examples of calculation mark-ups.

Mark-ups have so for been estimated based on knowledge from several European fur organisations and companies. The mark-up is expected to be valid for all raw (or dressed) skins, and then the consumer value of all skins – regardless of its final use – is included. In that way the results of the mark-up-model show the value at consumer/retail level that raw fur skins have generated regardless of outlet, product etc.

The model is based on exogenous data, where international databases (UN databases) can be used to extract import and export data, while production data can be identified from national statistics, local key persons, auction houses etc. Mark-ups are endogenous (internal) data, where local reporters have the knowledge to collect data. Mark-ups will probably be rather constant over time, if prices of raw fur skin are constant. However, when raw fur skin prices are high, then mark-ups are expected to be relatively low - and high when raw fur skin prices are low. Mark-ups are expected to be rather identical among similar countries. However, in rich countries mark-ups are expected to be relatively high, as labour costs and other costs are higher. If some countries have extreme mark-ups, action have to be taken in order to ensure comparability.

For some few countries fur retail sales are registered and published by the national statistical authorities or similar institutions. In these cases, these official statistics are used.

An evaluation of the model is presented in Hansen (2017).

Production of raw fur skins

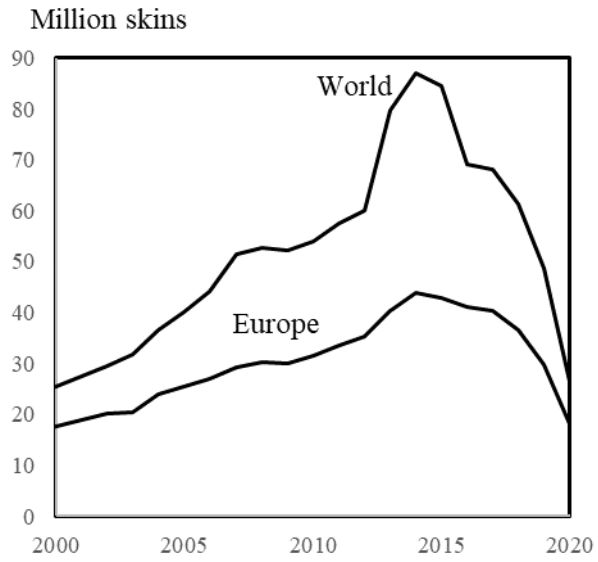
In order to calculate the fur consumer volume, it is crucial to estimate the production of raw fur skins. All natural fur consumer sales originate from raw fur skins, so the number and the value of produced raw fur skins are important information generating the value and volume of fur skins further along the value chain.

In the vast majority of cases, national statistical institutes and industry organisations do not calculate the value of fur skins produced on fur farms. Often there is no comprehensive market price that can be used. When no figures for production value exist, not even at the national level, it becomes even more difficult and uncertain to make calculations at the international level.

In the following, data collection will initially focus on mink skin production, which is by far the most important fur type. The figures are based on information from official statistics, trade associations, companies, scientific

papers and reports, interviews with experts, etc. In some cases, estimates have been calculated due to a lack of information, cf. figure 2.

Figure 2. *Production of raw mink skins 2000-2020*



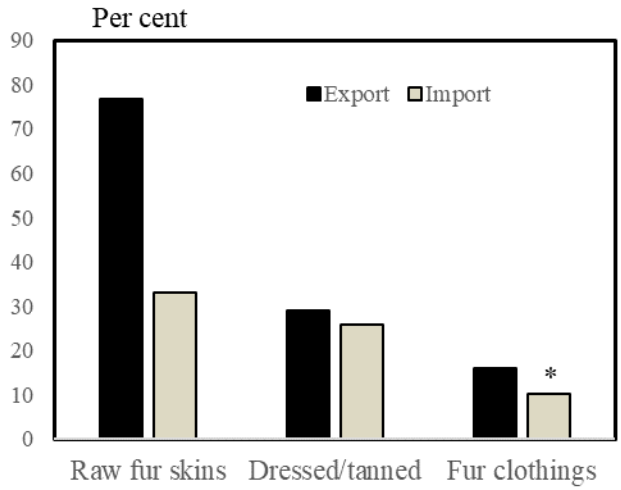
Note: 2020: Preliminary data and forecasts
Sources: Hansen (2016a) and updated figures

Figure 2 shows, that Europe is a major producer of mink skins, and in recent years Europe's share has increased. The figure also demonstrates significant changes in production from year to year. As consumption is based on production, the level of production is important and must represent a long term average or an equilibrium level in order to be used to estimate a reliable level of consumption.

International trade

International trade is another important parameter, when consumption of fur products is calculated. International trade of fur products from the whole value chain is significant, and Europe's share of world trade is also remarkable, cf. figure 3.

Figure 3. *Europe's share of world trade of fur skins at different levels of the value chain (2018)*



** European import as a share of world export (and not world import). As significant mis-match between world total import and world total export indicates, that the registered world total import figure is too low. For that reason Europe's total import is compared with world's total export.*
Includes also intra-European trade.

Source: Own calculations based on UN (2020).

Europe has a major role on international markets for fur skin products, which must be taken into account when EU consumption is to be calculated. Import and export may be a larger source for consumption that internal EU production.

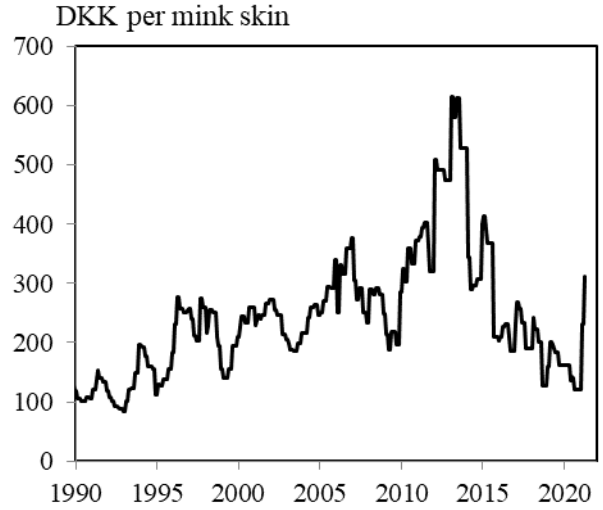
European fur consumer (retail) value

In order to calculate the value – and later the amount – of fur on the European consumer market, all inputs, factors and mark-ups described in figure 1 must be considered. The price of fur skins is also important: An increasing import value of fur garment does not necessarily mean an increasing amount of fur skins. The increasing value may be a consequence of higher prices of raw fur skins, so the influence of price variations must be eliminated.

Prices of mink skins fluctuate widely, and market support, import and export regulation for fur skins is generally not very widespread compared to other agricultural commodities. Price volatility of mink skins is thus considerably larger than on other agricultural products, cf. also Hansen (2016a + b).

Prices vary from auction to auction, and figure 4 shows the average prices per mink skin per auction in Kopenhagen Fur in the period 1990-2019.

Figure 4. *Prices of mink skins in Denmark. Average price per auction 1990-2021*



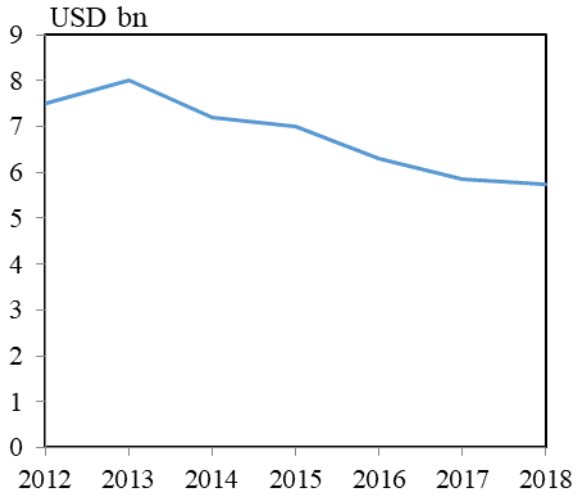
Source: Kopenhagen Fur (2021)

The figure shows large fluctuations in prices from year to year and from auction to auction.
However, seen over a number of years, there is evidence of an automatic stabilization, with supply and demand adapting and with prices fluctuating around a market equilibrium.

Falling prices mean – ceteris paribus - that the value of, for example, imported fur clothing decreases, while the quantities imported may not decrease. It is also seen that the mark-ups from auction price to consumer price are larger, the lower the auction price is. Both uncertainties can be reduced by using variable mark-ups depending on the upstream price in the value chain.

Taking into account production levels, auction prices, variable mark-ups, stocks, fur manufacturing data, international trade of raw mink skins and fur garments, lags etc. the European fur retail sale is calculated, cf., figure 5.

Figure 5. *European fur retail sale, 2012-2018*



Source: Own Calculations based on model in figure 1.

The European fur retail trade in 2018 was about 6 billion USD. During recent years Europe's share of world retail fur trade has been between 20 and 25 percent. A part of the decline is a result of lower prices of fur garments due to lower prices of raw mink skins. This means that the decline in volume is less than the decline illustrated in figure 5.

From European fur retail value to European fur retail volume

As estimated above, Europe accounts for 20-25 per cent of world fur retail sale. Assuming a 20 per cent higher retail value in Europe than the world average due to higher mark-ups, then Europe will use 16-20 (20-25 *0,8) per cent of all skins produced in the world. All raw fur skins end up as fur jackets, coats, trims etc.

To calculate from number of skins to area and weight, these assumptions are used:

Table 1. *Size, weights and world market share of mink and fox skins*

	Share of world trade (%)	Average no. of pelts in 1 m^2	Average weight (kg)
Mink	91	8,09	0,137
Fox	9	2,41	1,19
Mink/fox average		7,579	0,232

Source: Fur Europe (2020) and own calculations based on UN (2020)

The number of world production of is a key number. However, the estimated or registered number of produced raw skins has been very unstable during recent decade, and a high or a low number of produced raw skins one year does not necessarily reflect the retail sale the same years. There will be lags and stocks – a several stages

in the fur value chain. For that reason an average covering several years will be used to reduce or eliminate this uncertainty.

The analysis is based on fur retail with mink and fox skins as raw material. Annual production data for mink skins are available, while data for world production of fox skins are less available. Production of fox skins is estimated in this way: Finland produced around 2 million fox skins per year in 2010-2018, and Finland has 90 per cent of the world market for raw fox skins. As a share of fox skins is not exported and imported, it is assumed that the average annual production of fox skins is 3 million.

Based in these assumptions, the weight and area of fur skins in European fur consumer market is calculated – see table 2.

Table 2. *Estimation of weight and area of fur skins in European fur retail sale*

	World mink skin production		World mink and fox skin production	Europe's share		Weight Tonnes	Weight Tonnes	Area, m^2	Area, m^3
	Own estimat.	KF estimation	Own estimation	16% (20%)	20% (25%)	16% (20%)	20% (25%)	16% (20%)	20% (25%)
2000	25.509.189	27.540.000	28.509.189	4.561.470	5.701.838	1.058	1.323	601.856	752.321
2001	27.356.940	29.535.000	30.356.940	4.857.110	6.071.388	1.127	1.409	640.864	801.080
2002	29.452.961	31.525.000	32.452.961	5.192.474	6.490.592	1.205	1.506	685.113	856.392
2003	31.883.391	34.135.000	34.883.391	5.581.343	6.976.678	1.295	1.619	736.422	920.528
2004	36.678.290	36.250.000	39.678.290	6.348.526	7.935.658	1.473	1.841	837.647	1.047.059
2005	40.046.915	40.115.000	43.046.915	6.887.506	8.609.383	1.598	1.997	908.762	1.135.952
2006	44.263.200	44.310.000	47.263.200	7.562.112	9.452.640	1.754	2.193	997.772	1.247.215
2007	51.580.876	57.470.000	54.580.876	8.732.940	10.916.175	2.026	2.533	1.152.255	1.440.319
2008	49.649.407	51.300.000	52.649.407	8.423.905	10.529.881	1.954	2.443	1.111.480	1.389.350
2009	52.098.041	47.650.000	55.098.041	8.815.687	11.019.608	2.045	2.557	1.163.173	1.453.966
2010	53.895.546	50.585.000	56.895.546	9.103.287	11.379.109	2.112	2.640	1.201.120	1.501.400
2011	57.427.884	56.135.000	60.427.884	9.668.461	12.085.577	2.243	2.804	1.275.691	1.594.614
2012	59.937.777	59.310.000	62.937.777	10.070.044	12.587.555	2.336	2.920	1.328.677	1.660.846
2013	79.712.115	82.025.000	82.712.115	13.233.938	16.542.423	3.070	3.838	1.746.133	2.182.666
2014	87.059.779	82.480.000	90.059.779	14.409.565	18.011.956	3.343	4.179	1.901.249	2.376.561
2015	84.571.009	73.130.000	87.571.009	14.011.361	17.514.202	3.251	4.063	1.848.708	2.310.886
2016	69.198.940	74.370.000	72.198.940	11.551.830	14.439.788	2.680	3.350	1.524.189	1.905.237
2017	68.101.618	72.915.000	71.101.618	11.376.259	14.220.324	2.639	3.299	1.501.024	1.876.280
2018	62.025.519	68.755.000	65.025.519	10.404.083	13.005.104	2.414	3.017	1.372.751	1.715.939
2019	55.621.500	58.810.000	58.621.500	9.379.440	11.724.300	2.176	2.720	1.237.556	1.546.946
2020	39.871.500		42.871.500	6.859.440	8.574.300	1.591	1.989	905.059	1.131.323
2010-19	65.220.290	68.290.714	68.220.290	10.915.246	13.644.058	2.532	3.165	1.440.196	1.800.245
2010-19	65.000.000	68.000.000	68.000.000	11.100.000	13.600.000	2.500	3.200	1.400.000	1.800.000

Note: "16% (20%)" means that Europe is expected to have 20 percent of world market, but due to a 20 percent higher mark-ups in Europe compared to world average, the market share is reduced with 20 percent, which is 16 percent.

Source: Own calculations.

The average for the years 2010-19 gives the best estimate for a market in balance and for a longer term value.

The amount of fur skins on the European consumer market 11,1-13,6 million fur skins equivalent to 2.500-3.200 tonnes and 1,4-1,8 million m^2.

Sources:

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Session V: Health and disease

Health & Disease

Oral presentations

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

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Abstract
A study of four generations of mink on a Danish research farm (n= 27,511) was conducted with the objective of studying occurrence of urolithiasis and cystitis in relation to familial disease history. 221 mink were diagnosed postmortem with urinary tract disease resulting in a prevalence of 0.8 %. Using Chi square test, we found association between familial history of urolithiasis and cystitis in breeding animals and urolithiasis and cystitis in offspring ($p<0.05$). 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.

Keywords: Urinary tract disease, heredity, disease prevention

Introduction
Mink cystitis often occur simultaneously with struvite urolithiasis (Sompolinsky, 1950; Nielsen, 1956). Cystitis and urolithiasis have been reported to be most prevalent in male mink kits in the early growth season, with variation in prevalence between farms and seasons (Sompolinsky, 1950; Nielsen, 1956; Witte and Zimmermann, 1985; Clausen, 2006). The complete pathogenesis remains to be uncovered.

The color type of mink has previously been linked to susceptibility of certain diseases (Hegreberg et al., 1969; Tung et al., 1981; Jespersen et al., 2017). Reports in cats and dogs show that some breeds are predisposed to struvite urolithiasis. (Sosnar et al., 2005; Cannon et al., 2007; Houston et al., 2016, 2017). Additionally, there are reports of familial risk of urolithiasis in humans (Curhan et al., 1999; Hemminki et al., 2018).

In this study we seek to investigate familial occurrence of urolithiasis and/or cystitis on a research farm.

Material and methods
The preliminary data included all mink (n= 63,110) at Kopenhagen Research Farm in Holstebro, Denmark from 2012 to 2015. The study population was defined as a subset (n=27,511) of this population. Dead or

euthanized mink were subjected to necropsy by the farm veterinarian and mink with a post mortem diagnosis of urinary tract disease (urolithiasis, cystitis and/or nephritis) were recorded. From Kopenhagen Fur's breeding database individual mink data regarding litter, sire and dam of all mink on the research farm during the study period were extracted. Chi square test was used to investigate the association between urinary tract disease in mink kits and family history of disease. Family history was defined as urinary tract disease leading to mortality in siblings of the dam or sire. Mink without post mortem diagnosis of urinary tract disease were defined as healthy.

Results

A total of 221 mink kits of the included study population (n=27,551) were diagnosed postmortem with urinary tract disease resulting in a prevalence of 0.8 %. Chi square test at a 95 % confidence level showed a significant association between mink kit with a post mortem diagnosis of urinary tract disease and a family history of urinary tract disease ($p<0.05$).

Discussion

These results indicate an association between mink kits with urinary tract disease diagnosed postmortem and a family history of urinary tract disease diagnosed postmortem ($p<0.05$). Familial risk of urolithiasis is previously reported in other species including humans (Curhan et al., 1999; Hemminki et al., 2018). The results indicate that selective breeding may affect the prevalence of urinary tract disease on some farms. By exclusion of mink with siblings diagnosed with urinary tract disease post mortem from breeding, the farmers may be able to reduce the occurrence of urinary tract disease leading to mortality in mink kits.

Acknowledgements

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Study on mink tolerant to Aleutian mink disease virus

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Abstract
Aleutian disease (AD), caused by Aleutian mink disease virus (AMDV), is one of the most important infectious diseases of farmed mink, causing significant health and (*suggestion: to be added*) welfare problems for the animals and financial losses to the farmers. 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 on a 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-free farm. We have compared gross – and histopathology, antibody levels, 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.

Keywords: AMDV, tolerance, PCR, histology, ELISA

Background
Aleutian disease of mink (AD) can cause severe welfare problems to the animals as well as financial losses to the farmers. AD is caused by Aleutian mink disease virus (AMDV) - a parvovirus with a 4.8 kb ssDNA genome that encodes three non-structural proteins (NS1, NS2, and NS3), and two structural proteins VP1 and VP2 (reviewed by Canuti et al. 2015).

Clinical signs of AD can vary from subclinical to severe and fatal. Some mink can clear the virus, but the rest either die from it or carry it and spread it for the rest of their lives (reviewed by Canuti et al. 2015). Earlier studies have shown that it's possible to breed tolerant mink (Farid and Ferns 2017) which makes it important to clarify reasons and mechanisms behind varying virus persistence and disease severity.

Methods and materials
Samples
Mink from three farms were sampled. Rectal swaps and blood samples on filter paper were taken from farm 1, that has focused on breeding AMDV tolerant mink for decades, in four occasions between May 2017 and November 2019 starting with 10 white, brown, and grey female mink and ending with six brown, five grey, and six white mink since 13 mink died for natural causes during the follow-up. Spleen, kidney, serum, and blood samples were taken during euthanizing the mink in November 2019. They were also taken from five

thin and dehydrated white female mink from farm with an acute infection (farm 2), and seven healthy white female mink from AMDV-free farm (farm 3).

Pathology and histology

Mink from farm 1 and two mink from farm 3 were submitted for gross and histopathological examinations. Body weight and weight of the spleen were measured. Brain, hypothalamus, heart, lungs, spleen, liver, bone marrow, kidney, small intestine, colon, and tissues with possible abnormalities were taken for histology and scored from 0-3 for lesions. Tissue samples from spleen and kidney were also taken for PCR.

DNA extraction, PCR, and sequencing

DNA was extracted from blood, spleen, and kidney with NucleoSpin Tissue kit (Macherey-Nagel). DNA was extracted from stool samples with QIAamp Fast DNA Stool Mini Kit (Qiagen) or with QIAQube HT using DNeasy 96 PowerSoil Pro QIAcube HT Kit. Follow-up blood samples from farm 1 were tested with pan-AMDV-PCR that amplifies region 578-951 (Jensen et al. 2011 and Virtanen et al. 2018) and pan-AMDO-PCR that amplifies region 1662-2302 (Knuuttila et al. 2015). All pan-AMDV-products were sequenced with Sanger sequencing to be used in phylogenetic analysis. Pan-AMDO-results were analyzed based on amplification curves, melting curves, or sequencing. Those samples that were positive with at least one PCR were considered positive. Tissue samples from November 2019 collection were tested with pan-AMDV-PCR and all positive spleen samples and those kidney samples that were negative in spleen were sequenced. Virus copy numbers in spleen and kidneys were determined with quantitative NS1-probe-PCR (Virtanen et al. 2019).

Serology

All the blood on filter paper samples and serum samples were tested with VP2-ELISA (Knuuttila et al. 2009). Blood was extracted from filter paper by incubating a piece of filter paper in 200 µl of PBS o/n and undiluted liquid was used for ELISA. ~~Serums~~ Sera were diluted 1:200.

Data analysis

Statistical analysis was done with IBM SPSS statistics 27. Phylogenetic analysis was performed as described previously (Virtanen et al. 2018).

Results

In necropsy, one grey mink of farm 1 had ~~kachexia~~ cachexia, dehydration, pale, enlarged and mottled kidneys, and splenomegaly. No significant macroscopical ~~lesion~~ lesions were detected in other mink. In histopathology, the clinically sick mink had vasculitis, glomerulonephritis and increased number of plasma cells in the spleen. Accumulations of mononuclear cells on kidneys, liver and heart and gastrointestinal tract as well as perivascular cuffing in the meninges were also detected. Some clinically healthy mink of farm 1 had unspecific mild changes in one or more organs. These milder changes, however, might not be related to AMDV.

Mink from farm 2 were all ELISA- and PCR-positive and mink from farm 3 were negative. In farm 1 (Table 1), we observed frequent coinfection and mink that were ELISA-negative but PCR-positive.

Table 1: *Percentage of positive samples in PCR and ELISA in farm 1 during follow up*

	ELISA	PCR
March 2017	44	52
October 2017	71	100
October 2018	52	95
November 2019	59	100

Mink from farm 2 had significantly higher AMDV genome copy number in both spleen (p=0.006) and kidney (p=0.011) than mink from farm 1. No difference between copy numbers in spleen and kidney within either of the farms (farm 1: 0.259, farm 2: 0.421) or between color types in farm 1 (p=0.609) were detected (Fig. 2).

Figure 1: Boxplot presentations of the qPCR results from spleen and kidney sorted by tissue (A) or color type (B).

Correlation between ELISA, qPCR, and histology results was studied (Table 3). Significant positive correlation was detected between qPCR result from the spleen and ELISA absorbance as well as between ELISA absorbance and proportion of spleen weight from body weight. Non-significant positive correlation was also detected e.g. between histology score and qPCR results.

Table 3: *Spearmans correlation coefficences between ELISA, histology, and qPCR results.*

	ELISA absorbance	Histology score	Spleen weight from body weight	Spleen qPCR	Kidney qPCR
ELISA absorbance	1.000	0.256	0.575*	0.576*	0.258
Histology score	0.256	1.000	-0.001	0.226	0.255
Spleen weight from body weight	0.575*	-0.001	1.000	0.078	0.384
Spleen qPCR	0.576*	0.226	0.078	1.000	0.111
Kidney qPCR	0.258	0.255	0.384	0.111	1.000

** Correlation is significant at the 0.05 level (2-tailed).*

Strains from farm 1 located in four branches in 2017, three branches in 2018, and two branches in 2019. Strains from farm 2 were all located in one branch in phylogenetic tree (Figure 2). Sequence data indicates frequent co-infection and different sequences were acquired from tissues and blood of the same mink in several occasions.

Figure 2: Phylogenetic tree of nt 578-951. Branches only containing sequences from other studies have been collapsed and posterior probabilities above 0.9 are shown next to the nodes. Sequence ids from this study contain farm number, mink id, sample material (S=spleen, K=kidney, B=blood), sampling date of farm 1, country, and year.

Discussion

We found mink that were persistently infected with AMDV but that had no clinical signs of AD during the 2.5-year follow-up period and had low or no detectable antibodies. This supports earlier studies showing that some mink are tolerant to AMDV and that breeding may be used in disease control. In the future,

transcriptomes of tolerant, sick, and uninfected mink will be compared to get information about the gene-level mechanisms behind disease tolerance.

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Characterization of true enteric pathogens as tool to reduce antimicrobial use in fur animals in Finland

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Summary
Diarrhea is a visible sign of digestive tract disorder and probably the most common cause for prescription of antibiotic therapy to the whole animal stock in a fur farm in Finland. Every antimicrobial treatment increases the risk for antimicrobial resistance and decreasing use of antimicrobial substances is one way to keep occurrence of antimicrobial resistance as low as possible.

In this study we collected fecal samples from 21 voluntary Finnish fur farms farming mink (*Neovison vison*) and blue fox (*Vulpes lagopus*). Fecal samples were analyzed with routine diagnostic methods and *Brachyspira*-culture in National Food Safety authority's laboratory in Seinäjoki.

The original aim of the study was to find means to reduce need for antimicrobial treatment in fur farming and improve routine diagnostic methods to get accurate diagnosis more often by comparing results from routine diagnostic methods and next generation sequencing methods (NGS), but we were not able to achieve results from NGS.

Results differs between blue fox and mink. *Campylobacter* sp. and coccidia were common finding in fecal samples of diarrheic mink, and mink enteritis virus (MEV) was not detected. In blue fox parvovirus was common finding and spectrum of other possible pathogens was broader. *Salmonella* sp. was not detected in either of species.

Lack of knowledge and insufficient diagnostic possibilities increases antimicrobial use. With more knowledge of mink and blue fox enteric pathogens and fast and more accurate laboratory analyzes antibiotic treatment may be reserved only for those cases where it is obligatory.

Key words: antimicrobial substances, one health, diarrhea

Introduction

Digestive tract disorders are common in all animal species. Severity of the signs and prevalence of the pathogens vary with the species and age of the animal. Digestive tract infections may be caused by virus, bacteria, parasite, or fungi. Diarrhea samples of fur animals are routinely analyzed bacteriologically and parvovirus and endoparasite detection is performed.

Currently mink enteritis virus (MEV), coccidia (Molenaar 2016) and *Campylobacter* sp. are considered pathogenic to mink as well as pathogens causing diarrhea to blue fox are considered to be blue fox parvovirus (BFPV) (Veijalainen et al 1984), *Lawsonia intracellularis* (Kallio and Ahola 2012), coccidia (Juokslahti et al. 2010), and *Campylobacter* sp. Significance of *Campylobacter* sp. as enteric pathogen in fur animals is still under discussion (Dietz 2016).

Smura et al (2016) sampled mink and blue fox feces and proved that microbiota between species and healthy and sick animals is different. According to Finnish Food Authority reports most common findings in fox and mink necropsies are infection in digestive tract and lungs or sepsis (Ruokavirasto 2021a). Finnish Food Safety Authority's laboratory in Seinäjoki analyzes hundreds of fecal samples every year, unfortunately these results are not published.

L. intracellularis is well-established pathogen to blue fox and it has been detected with typical pathologic findings from blue fox in multiple occasions (Kallio and Ahola 2012, Ruokavirasto 2021a).

Campylobacter sp. and parvovirus are commonly detected from diagnostic samples, and Campylobacter detection in feces has been common reason to prescribe antibiotic treatment to fur animal farm and some tetracycline resistant strains have been detected over the years (personal data).

Parvoviruses are species specific, but signs of the infection are similar in mink and blue fox as both species develop watery to bloody diarrhea, other clinical signs may include anorexia, weight loss, dehydration, and death (Veijalainen et al. 1984, Turner 2021).

Pre-weaning diarrhea known also as 'Sticky kits'-syndrome is common in mink kits, and signs of diarrhea start as early as three weeks of age. *E. coli* is commonly detected as well as corona-, rota- and calici-viruses (Jorgensen 1996). Latter research suggests that there is not a single pathogen or reason to pre-weaning diarrhea, but many different factors influences the young mink kits. Veterinarians working with fur animals have suggested that there might be more than one factor to digestive tract disorders. This is supported also with Parrish (2008) as several viruses develop more severe disease with co-infections.

Coccidia is well-known pathogen to many animal species but pathogenesis and source of the infection is unknown in fur animals. There might be several infectious coccidia species even mink and blue fox seems to have different coccidia species; *Eimeria vison* have been detected in Dutch mink farms (Molenaar 2016) and several *Isospora*-species from Finnish fox farm (Juokslahti et al 2010). Toltrazuril have been used to prevent coccidial diarrhea, but toltrazuril is considered harmful to environment thus prudent use on antimicrobials should also include anticoccidial medication.

Salmonella sp. is important pathogen to multiple species, including humans. *Salmonella* sp. is seldom enteric pathogen to mink or blue fox, but gravid females and young animals may be susceptible to some *Salmonella* serovars causing severe infections in other organ systems. Because of the zoonotic potential salmonella is controlled vigorously in food animal production thus salmonella is cultured also from fur animal feces. *Brachyspira hyodysenteriae* is pathogen to pigs causing swine dysentery and *Brachyspira pilosicoli* have been reported to be pathogenic to dogs (Fellström et al. 2001) but *Bachyspira* sp. importance as enteric pathogen in fur animals in unknown. Brachyspira-culture requires specific sampling method and anaerobic culturing thus it is not analyzed routinely.

Our aim was to obtain new data on pathogens causing diarrhea to improve recommendation to antimicrobial treatments in fur animal production and discover possible need for new diagnostic tools. To achieve our aim, we planned to compare the results obtained from routine methods and next generation sequencing (NGS) methods from same samples and identify new possible pathogens. Due to unfortunate loss of samples, we were not able to perform NGS analyzes.

Material and methods

We sampled 21 voluntary fur farms year 2014 in Finland. Farms had either mink or blue fox, and some farms had both species. Fecal samples were collected at three timepoints, before diarrhea occurred, during the diarrhea and after signs had resolved.

In our study we collected samples from under the cages where clean cardboard was placed prior to sampling. In addition to conventional sampling rectal swabs were collected for brachyspira culture to Probact tubes with Amies media (Labema, Helsinki Finland). Samples were cooled and transported to Seinäjoki at the same day.

All fecal samples were analyzed in National Food Authority's laboratory in Seinäjoki with routine fecal sample protocol and rectal swabs were cultured to detect *Brachyspira* sp. Analyzes were started immediately and part of the sample was preserved into freezer for NGS analyzes.

Fur animal fecal samples are routinely analyzed for protozoa and helminths with McMaster flotation, aerobic bacteria, salmonella, and campylobacter cultures and parvovirus and *Lawsonia intracellularis* with PCR, exceptionally *L. intracellularis* is not analyzed from mink fecal samples.

Results

Results of the fecal analyzes differs between foxes and mink. In mink samples coccidia and campylobacter were isolated as a possible pathogen. In blue fox samples all studied pathogens were detected except of *Salmonella* sp. (table 1)

Table 1

Pathogen	detected in mink	detected in blue fox
Campylobacter sp.	Yes	Yes
Salmonella sp.	No	No
Brachyspira sp.	No	Yes
Lawsonia intracellularis	not analyzed*	Yes
Parvovirus	No	Yes
Coccidia sp.	Yes	Yes
Nematoda sp.**	No	Yes

* *Lawsonia intracellularis* have never been detected from mink in Ruokavirasto.

** Species specification was not available

Discussion

Smura et al. (2016) showed that difference between fur animal species gut microbiota is significant. In our study only two suspected pathogens, *Coccidia* sp. and *Campylobacter* sp. were detected from mink feces. It is possible that many of the bacteria which are detected from mink feces are not primary pathogens to mink, but they can overgrow and therefore be detected in stools during diarrhea outbreak. Results from Smura et al. (2016) supports this claim as analyzes of fecal microbiota revealed that microbiota changes while diarrhea is present. The difference in fecal microbiota between sick and healthy animals was even more prominent in blue fox Smura et al. (2016).

Mink enteritis virus was not detected in our study. In Finland most of the minks are vaccinated with so called triple-vaccine, including components against MEV, botulism and *Pseudomonas aeruginosa*. It is probable that

annual vaccination of the mink kits prevents MEV infections or clinical signs and MEV is not therefore issue in Finland. In the other hand there is not parvovirus vaccine for blue fox and parvovirus is common finding in diarrhea samples as it was in our study in blue fox. Need for BLPV-vaccine is obvious to prevent diarrhea in blue fox whelps and help reduce antimicrobial use to treat diarrhea.

Blue fox has longer digestive tract and larger variety of possible pathogens are detected from diagnostic fecal samples as well as our study material. In blue fox samples all analyzed pathogens; parvovirus, coccidia, nematode as well as *L. intracellularis*, *Campylobacter sp*., and *Brachyspira* sp. as new possible pathogen were detected, except salmonella.

Coccidia oocyst are commonly detected from diagnostic fecal samples as well they were detected from mink and blue fox samples in our study. Coccidia may be insignificant if the oocyst count is low however over 10 000 coccidial oocyst per gram (OPG) with clinical signs of diarrhea is considered significant (A. Näreaho, personal communication 2019). *Nematode* sp. are currently quite rare in blue fox probably due to cage environment and long history of annual antihelminth medication, to our knowledge mink have not had endoparasites in fur farms in Finland and antihelminth substances are not routinely administered to mink.

Necropsy, including histopathology is necessary part of the diarrhea diagnostics since some are opportunistic pathogens and may be present in feces without any signs of infection in the digestive tract (H. Nordgren, personal communication 2021). *Lawsonia intracellularis* is considered important pathogen to blue fox as gross pathological findings are pathognomonic and PCR analyzes from rectal epithelium confirms infection.

The role of the *Campylobacter* sp. as enteric pathogen should possibly be reconsidered as it is commonly detected from environmental samples without correlation to animal health (Hansson 2007). Blue fox is related to dog, and we considered the possibility that *Brachyspira* sp. may be pathogenic to fur animals as well, especially to blue fox. In our study *Brachyspira* sp. was detected in blue fox samples, but not in mink samples, which may indicate a possible pathogenic nature of this bacteria to the blue fox. However, the importance of *Brachyspira* sp. remains still unknown and requires further research to determine if it is a true pathogen or merely an innocent bystander.

Use of antimicrobials in Finland to fur animals has increased gradually since 2005 and has peaked years 2014, 2017, and year 2019 antimicrobial use have been highest during reported period. Between the years use of different active substances such as tetracyclines and trimethoprim sulfadiazine varies. In this data it is not differentiated which diagnosis the antimicrobial substance is prescribed for. Ruokavirasto (2021b).

It is possible or even likely that there are more pathogens causing digestive tract diseases than we currently know. We could have elucidated this question by comparing NGS results with results obtained with routine diagnostics methods, however, this was not possible to perform in this study as planned. Routine analyzes are limited thus there must be some evidence of pathogenicity before new pathogens are included to routine diagnostics scheme.

Often numerous pathogens are detected simultaneously from fecal sample and significance of each finding is not clear. Thus, veterinarian must consider therapy options carefully. Viral infections are treated with antimicrobials since current diagnostics is too slow, it may take up to one week to get results from parvovirus PCR. If the result were available faster, it would be possible to wait the results and then determine if antimicrobial treatment is necessary.

Even smaller coccidia oocyst count may be important as coccidia and parvovirus infections damage intestinal epithelium and impair immunity which predisposes to secondary infections. Preventing coccidia and parvovirus infections may improve animal health and immunity and prevent other pathogens to cause diarrhea.

Normal microbiome is essential to animal health and welfare. Antimicrobials might alter the normal gut microbiota and create possibilities for bacteria to become resistant to one or many different antimicrobial substances or the gut microbiome may be disturbed and become more prone to infections. Better communication with fur farmers about antimicrobial resistance and the adverse effects of unnecessary antimicrobial treatments is needed to decrease antimicrobial use.

Fear of monetary losses due to diarrhea, desire to cure animals as well as lack of knowledge of pathogenesis of digestive tract infections and possible viral pathogens results unnecessary antibiotic use. Better understanding of the alternative treatment opportunities such as addition of lactic acid bacteria, fiber, or alteration in diet could help prevent diarrhea and provide more options without antimicrobials.

As diarrhea might be seen with any kind of digestive tract disorder, signs of diarrhea or bacterial finding from feces alone should seldom justify to antimicrobial therapy. A complete necropsy including histopathology should be part of the diagnosis. Bacterial result without signs of infection is just a laboratory result and veterinarians should not treat laboratory results but clinical illness. Altering routine diagnostic and developing species-specific schemes should be discussed since change could improve quality of care to mink and blue fox and reduce antimicrobial use.

It would be very interesting to compare Finnish Food Authority's results to our data. In our study we sampled volunteer farms whereas Finnish Food Authority's laboratory analyzes samples from farms with signs of illness. In this study we concentrated to mink and blue fox but Finnraccoons and silver fox should also be studied as data is limited of diseases of these species. In future detailed DNA analyzes using (NGS) method to analyze diagnostic and random research samples and comparison between sample groups could provide more information about unknown bacterial and viral pathogens.

Our initial aim was to improve diagnostic methods and to gain tools to decrease the use of antimicrobials. The first step in the planned project was to compare the results of conventional diagnostic tools and next generation sequencing (NGS) however we were not able to carry out our initial plan because our samples were lost due to broken freezer. However, the results of this study indicate that parvovirus is important pathogen to blue fox and coccidia is important to both species as enteric pathogen. Importantly, using antimicrobials to treat parvovirus or coccidial diarrhea is inefficient and increases unnecessary antimicrobial use.

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Poster session

Autumn diarrhea project at Fin FurLab

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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 be 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 sub-cultured 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.

Pathomorphological pattern of farm mink kidneys – preliminary study

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Abstract
Chronic kidney disease affects health, productivity and welfare 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. In conclusion it can be said, that 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.
Key words: farm mink (*Neovison vison*), chronic kidney disease, pattern of histopathological lesions

Introduction and Aim
Chronic kidney disease, both in humans and in many animal species is a major life-limiting problem. In human medicine it is assorted to the group of civilization diseases along with obesity, diabetes, cardiovascular diseases and hypertension. Patients with impaired renal function in many cases require dialysis. In the aged individuals it's a common problem, both in laboratory and companion animals diagnosed as: chronic progressive nephropathy in rats and mice; hamster glomerulonephropathy, chronic glomerulonephropathy in gerbils or chronic kidney disease in dogs and cats. The aim of the study was preliminary pathomorphological assessment of kidneys in a group of farm mink.

Materials and Methods
Kidneys were collected from 25 randomly selected American farm mink (*Neovison vison*), both sexes at harvesting period. Slides were prepared routinely: collected material was fixed in 10% buffered formalin and embedded in paraffin (Paraplast). The 4μm thick sections were stained with haematoxylin and eosin (HE), Masson's method for the presence of connective tissue collagen fibres and Köss method for calcium salts.

Results
Histopathological examination revealed glomerular lesions in 17 animals (- 68%). It was noted as mostly focal, proliferative or membranoproliferative inflammation. Glomerulosclerosis with hyalinization was found in

three mink. Chronic inflammation at interstitial connective tissue were found in 13 mink (- 52%). Inflammatory cellular infiltrates were very abundant in 3 mink (- 12%); and in one case – with Mott cells (- 4%), respectively. Calcification was noted in 10 mink (- 40%), but it was exclusively localized in the medullary part of kidneys of 9 mink (- 36%). Mineralization of the glomeruli capsules and the interstitial tissue was found in one mink. Parenchymatous and lipid degeneration with necrosis of the epithelial cells were noted in the renal tubules of 17 mink (- 68%). But in 4 cases (- 16%) that kind of lesion appeared as macrovesicular lipid degeneration. It was solely parenchymatous degeneration or necrosis noted in 5 mink (- 20%).

Discussion and Conclusion

Chronic renal failure is a progressive disease, however it is the end-stage of many different pathological processes. In our cases kidneys were obtained from young mink at the end of husbandry period. The analysis revealed quite high occurrence of pathomorphological lesions of varying severity. It should be considered that lesions noted in affected mink would have resulted in their productivity. It is generally assumed that companion animals with kidneys insufficiency require low protein, but rats – also low calories diets. The urinary tract diseases may reflect different kind of diseases in farm mink. Thus the HP monitoring of the kidneys pattern in farm mink might be a useful tool for health and welfare status as well as feed quality assessment.

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Preliminary report on parasitological investigations in wild American mink (*Neovison vison*) population in Narwiański National Park

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Abstract
American mink (*Neovison vison*) living in wild are considered as an invasive in the Poland's environment. Mink as predators that belong to the Mustelidae family are particularly exposed to different local parasite infections. The aim of the study was to recognise the occurrence of parasitic infections in wild mink captured in Narwiański National Park. It was a part of the extended study concerning protection of natural resources. During the preliminary research there were gastrointestinal tracts of 79 individuals examined macroscopically and intestinal contents by the flotation method as well. The intestinal flukes were noted in 7 out of 79 mink (- prevalence 9%; intensity from 1 to 6 specimens per animal). The eggs of *Aonchotheca sp*. (nematode worms) were found in 6 mink (- prevalence 8%). Coccidian oocysts were detected in 6 mink (- prevalence 8%). But the mixed infection of flukes together with coccidia and *Aonchotheca sp*. with coccidia were noticed in 1 mink, respectively. It was concluded that gastrointestinal parasitic infections remained at a low level and occurred in less than 10% of examined population. Further investigations have been carried out and results are to be presented in press.

Key words: wild American mink, gastro-intestinal parasites, prevalence and intensity of infection

Introduction and Aim
The wild American mink living in Poland's environment originate from the East (former Soviety Union) and/or of those which escaped from farms in the past. As invasive predators may have strong impact on the endemic wildlife. That is why the extended studies are being conducted to discriminate their role in nature. One of the elements to recognize was the occurrence of parasitic infections in wild mink.

Materials and methods
There were 79 mink captured during the first year of the study in Narwiański National Park (North East of Poland). Postmortem examinations were conducted at the FVM of WULS-SGGW. Gastrointestinal tracts were investigated macroscopically and the intestinal contents were examined using the flotation method with supersaturated NaCl. Isolated parasites were tentatively identified morphologically and/or microscopically. Then prevalence and intensity of particular infections were estimated.

Results
Macroscopic test of the guts revealed intestinal flukes in 7 mink (in 1 female and 6 male mink). The prevalence was 9%, but the intensity from 1 to 6 specimens per host. The flotation test revealed coccidian oocysts in 6

mink (1 female and 5 males). The prevalence was 8%, but the intensity rather low. The *Aonchotheca sp*. eggs (intestinal nematodes) were found in 6 female mink only – the prevalence was 8% and the intensity estimated at low level. There were also found co-infected mink: 1 mink affected by coccidia and flukes; and another single mink infected with coccidia and *Aonchotheca sp.* nematodes.

Discussion and conclusion

This preliminary study showed that wild mink are host of parasite species typical for the local Mustelids. Although the prevalence and intensity were rather at the low levels, but it may suggest that mink are exposed and involved in life cycles of some parasite genera like: coccidia, flukes and nematodes. The study showed necessity to continue investigations on the occurrence and adequate (morphological and molecular) identification of the parasites involved.

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