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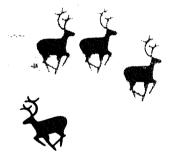
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NOTES
SCIENTIFUR
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We thank the majority of our members, subscribers and supporters for their prompt payment of the very delayed invoices we sent out in April. The few of you who have not yet paid your invoices, will in August receive a reminder instead of the usual issue of SCIENTIFUR.

The present issue of SCIENTIFUR is of "summer size", mainly because too many of you, whom we have been asking for contributions, have not yet responded to our request. Hopefully many of you will be more active after a good summer holiday.

In the meantime we are grateful to the efficient contributors both with regard to abstracts and original reports, of which we receive an increasing number. Some of our members would find it positive if a referee system could be established to guarantee the scientific value of the original scientific reports. This idea is not new to us, but we have to realise that more than 50% of our readers are mainly interested in the more applied side of the information given in SCIENTIFUR. Whether it will be possible to bring reviewed scientific reports, will depend on willing "reviewers" within the different scientific disciplines we are dealing with in the scientific information on fur animals.

The Board of Directors will discuss the matter, but we should like to receive some suggestions from YOU – in your capacity as user of the information given. Please send your suggestions to the editor. Especially we should be pleased to receive a message from

those of you "experts" who might be willing to act as reviewers on a honorary basis.

The vice-president of IFASA, Dr. Bruce D. Murphy, is still trying to solve the question in which form IFASA/SCIENTIFUR should go on the Internet. The Board of Directors will also deal with this matter. Hopefully the time is near, when THE SCIENTIFUR IN-DEX covering more than 7,000 titles of scientific or technical reports on fur animal science and production can be found on the Internet. This INDEX and the complete collection of the 201/2 volumes of SCIENTIFUR are the most complete basis of information on fur animal production and science. Surely the Internet connection will widen the group of users considerably and thereby give rise to a growing number of subscribers and contributors who form the entire basis for SCIENTIFUR.

In the present issue of SCIENTIFUR we bring you abstracts from most of the scientific reports given in the TECHNICAL YEAR REPORT 1996 published by the Research and Advisory Units of the Danish Fur Breeders Association in April 1997. The report consists of a total of 29 reports dealing with a broad spectrum of subjects within the production of mink and foxes.

Hopefully we will receive abstracts of all reports in the year report in English, so that everybody may evaluate whether they wish to order the report which is written in Danish. The Technical year Report 1996 with a total of 236 pages and ISSN No. 1395-198X can be ordered at: PFR, Herningvej 112C,

DK-7500 Holstebro, Denmark, Fax No. +45 97 43 52 77.

If some of our readers, for instance in connection with the arrangement of an exhibition or a technical/scientific meeting, might need some information material on IFASA and SCIENTIFUR as well as on the SCIEN-TIFUR INDEX and BOOKS PUBLISHED ON FUR ANIMAL PRODUCTION, please contact the secretariat. We shall be pleased to send you a few copies of our information material as well as sample copies of SCIEN-TIFUR. Please bear in mind though that your secretary and editor is not in the office every day, so you should order the material in due time before the actual occasion.

One of the important objectives of IFASA is the arrangement of international scientific congresses and other international meetings (symposia etc.) within the field of fur animal science.

Should a group somewhere be discussing

in need of e.g. names and addresses of colleagues within the individual working groups of IFASA or perhaps need assistance in connection with such an arrangement, it might be a good idea to contact IFASA, the secretariat or one of the board members. Their names and addresses can be found on the inside of the first cover page.

One of the strong sides IFASA/SCIENTIFUR is the focus on international co-operation and communication within fur animal production.

Wherever you are - in the real or scientific world - we wish our readers a good summer /winter at the northern/southern hemisphere.

the relevance of such arrangements and be Gunnar Vørgensen

Your Editor

FAGLIG ÅRSBERETNING 1996

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ERRATUM

SCIENTIFUR regret very much the linguistic mistakes made in the papers of the dissertation report of Dr. Randi Oppermann Moe in SCIENTIFUR VOL. 21, NO. 2, PP. 106-108.

You are therefore kindly asked to place this erratum in the actual issue of SCIENTIFUR, so the misunderstandings can be corrected. In the SCIENTIFUR index, the correct word "hyperthermia" will be used.

Paper III: Physiological, mechanisms involved in stress-induced **hyperthermia**. *Page 106, title and text line 2 and 3*.

Paper IV: Effect of indomethacin on LPS-induced fever and **hyperthermia** induced by physical restraint in the silver fox (*Vulpes vulpes*). *Page 106, title and text, point 1 line 2, and point 4 last line*.

Paper V: Effects of handling physical restraint on rectal temperature, cortisol, glucose and leukocyte counts in the silver fox (*Vulpes vulpes*). Hyperthermia on *Page 108*, line 12 and 28.

Paper VI: Anxiolytic drugs inhibit **hyperthermia** induced by handling in farmed silver foxes (*Vulpes vulpes*). *Page 108, title and text line 5*.

Paper VII: Effects of putative environmental stressors on deep body temperature and behaviour in silver fox vixens (*Vulpes vulpes*). **Hyperthermia** on *Page 109, line 20*.

Please correct these mistakes and keep thereby SCIENTIFUR as your "correct" partner in the fur animal science.

With excuses to the author and the readers,

The Editor



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Quantitative measurement and analysis of fur density in mink skins

Kaj Thorhauge

Well known nuclear technology has been used to determine mink pelt density. The equipment and procedure are described. Properties of wool and guard hairs are related to measuring geometry. Transmitted and attenuated particles emitted from Kr-85, beta-sources, are detected at room temperature in micro-sensors equipped with Si-diodes. From three levels and eight areas of the pelt, 240 non-destructive and automated measurements are transformed using the empiric attenuation law. Specific densities (mg/cm³) of wool and guard hairs and their distribution on the pelt are calculated. Function was tested and nearly 1000 pelts were measured. General pelt properties are reported and classification suggested.

Thesis, 82 pp. 25 figs., 4 tables, 35 refs. In DANH. Author's abstract.

Pelage cycle and hair bundle structure in the young and adult ferret, Mustela putorius

Leena Blomstedt

The pelage growth cycle and hair bundle structure of three male ferrets, Mustela putorius, as young animals and adults were examined histologically. The follow-up period was 17 months. The growth phase of guard hairs and underfur hairs in follicular bundles was analysed. Skin samples from the hip were prepared for light microscopy; paraffin sections were cut parallel to the skin surface and stained with a modified SACPIC method. Guard hairs started to develop earlier than underfur hairs. Young ferrets shed hairs between August and mid-November, the guard hairs in three waves and the underfur hairs in one period. In adult ferrets, guard hair growth peaked three times between June and October. There were individual differences in the summer underfur: growth occurred in one period or in two separate waves. Moulting of the summer coat ended late in October, coinciding with the maximum number of growing winter underfur hairs. In the winter pelage the two hair types matured simultaneously. There was a guard hair in all mature winter coat hair bundles. In addition, all hairs had a medulla.

Can. J. Zool. 73: 1937-1944, 1995. 3 tables, 7 figs., 29 refs. Only abstract received. Author's abstract.

The effect of reducing day length on growth and priming of winter fur coat in blue foxes (Alopex lagopus)

Olga Szeleszczuk, Stanislaw Jarosz

The experiment carried out on a total of 60 blue foxes has shown that the keeping of animals in a room with a reduced day length according to the following time and light regimes (in terms of luminous intensity coefficient in Lx): from 2-5 August -35.71%Lx, from 5-11- August - 18.25% Lx and from 11 August to 1 November - 2.14% Lx, resulted in the speeding up of winter fur coat priming by about 3-4 weeks. In late October there were no significant differences in body weight nor fur quality. There were longer guard hairs in the side portions (59.25 mm) of the skin in the experimental animals; however, their underfur hairs were significantly shorter (ave. 27.14 mm) than in the control (29.48 mm). The guard hairs were significantly thinner (73.80 microns) while the underfur hairs thicker (17.88 microns) in animals kept in darkness than in the control animals (86.27 microns and 15.59 microns, respectively).

No significant differences were found in density distribution of the guard and underfur hairs, between experimental and control groups, although the latter, was given lower estimates in foxes kept in darkness $(16000/\text{cm}^2)$ compared to control $(17167/\text{cm}^2)$.

Zootechnika 31, pp. 89-97, 1995. 9 tables, 11 refs. In POLH, Su. ENGL. Authors' summary.

Growth and physical development of captive-raised black-footed ferrets (Mustela nigripes)

Astrid Vargas, Stanley H. Anderson

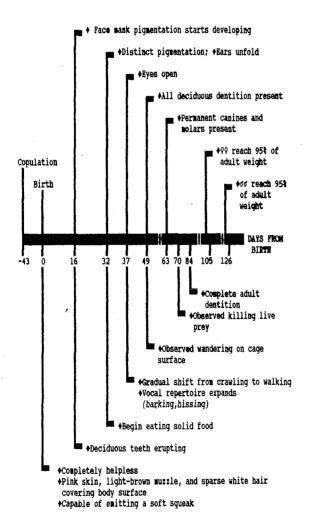


Fig. 2. Developmental landmarks for juvenile captive-raised black-footed ferrets. Postnatal days at which the various morphological and behavioural characteristics appear are approximations.

We describe postnatal development of captive-raised black-footed ferrets (Mustela nigripes) and summarise key developmental stages of growing kits. Black-footed ferret young were altricial at birth but developed rapidly. Eyes opened at approximately 35 postnatal days. Replacement of deciduous dentition began at 8 postnatal wk, and at 12 wk all permanent teeth were present. We fitted body mass data (n = $7 \, \text{d} \, \text{d}$ and $10 \, \text{PP}$) to growth curves using non-linear regression techniques. Male and female growth paralleled each other until approximately the 7th postnatal wk. Females attained 95% of adult body mass at approximately 15 wk, whereas males did not reach such mass until 18 wk after birth. Our data provide baseline information for the various zoos and breeding facilities involved in the recovery of this endangered carnivore.

Am. Midl. Nat. 135: 43-52. 2 tables, 3 figs., 42 refs. Authors' summary.

Ranging behaviour of red foxes during the mating and breeding seasons

Paolo Cavallini

Ranging behaviour (home range size, core area size, activity levels) of four red foxes (Vulpes vulpes; three males and one female) was studied by radio-tracking in a rural area of Central Italy during the mating and breeding seasons (January to May). Home range size was equal or smaller than in other areas, ranging from 47 to 320 ha (kernel analysis) or from 57 to 394 ha (minimum convex polygon), with the exception of a yearling male, who ranged over a very large area (2307 ha). Core areas ranged from 11 to 29 ha. The foxes were most active between 19:00 and 00:00 hr, then activity decreased slowly until sunrise. The foxes used about 25% of their range each night, with individually different strategies: the two resident males greatly increased their range in the second half of the female fertile period whereas the nomadic male restricted his large range during the peak of matings.

Barking bouts (indices of agonistic and contact behaviour) were at the time of births. The range expansion by males during the mating season, also reported in previous studies, was limited to the second half of the females' fertile period. The males could therefore maximise individual reproductive success by roaming only after the estrus of their mate. Because of the small number of foxes followed, these results should be verified in other studies.

Ethology Ecology & Evolution 8: 57-65, 1996. 3 tables, 2 figs., 48 refs. Author's summary.

Spatial and temporal trends and effects of population size on the frequency of colour phenotypes in the wild red fox (Vulpes vulpes)

Bradley J. Swanson, Donald R. Johnson

We analysed the hypothesised relationships of temporal, spatial, and harvest trends with frequency of red fox (Vulpes vulpes) colour morphs in 57 Hudson's Bay Company posts over a 20- to 26-year period, but found none of the strong relationships postulated to exist. A meta-analysis of each data set suggested a weak inverse relationship between latitude and frequency of the red morph. Meta-analysis further indicated a weak positive relationship with time and the frequency of the red phase, although this trend was not due to climate change. No relationship was found between harvest size and colour phase or between a 1-year lagged harvest size and colour phase, which evaluated the effects of dispersal. The data sets did not allow conclusive determination of the mechanisms behind the trends, but it is postulated that a slight selective advantage is found for the dark morphs at high latitudes, while the temporal increase in frequency of the red phenotype is probably the result of northward dispersal from, southern populations.

Can. J. Zool. 74: 1622-1631, 1996. 5 tables, 3 figs., 49 refs. Authors' abstract.

Diets of, and prey selection by, sables (Martes zibellina) in northern China

Steven W. Buskirk, Yiqing Ma, Li Xu, Zhaowen Jiang

The sable (Martes zibellina) shows close phylogenetic ties to the Eurasian pine marten (M. martes) and American marten (M. Americana), but ecological comparisons among these species are uncommon. To test for hypothesised similarities among these sibling species of martens, we studied diets of, and prey selection by, sables in northeastern China for three winters. We inferred diets from feces of sables, and availability of small mammals from snap trapping. Remains of mammals occurred in 89% of feces of sables, followed in frequency by soft and hard mast, and birds. Sables were highly selective from among small mammals; preying upon Clethrionomys rufocanus more than expected based on captures of small mammals, shrews (Sorex caecutiens) less than expected, and Clethrionomys rutilus in proportion to its availability. This pattern closely parallels that of the American marten, providing evidence for functional similarities among martens inhabiting boreal forests.

Journal of Mammalogy, 77 (3): 725-730, 1996. 1 table, 1 fig., 16 refs. Authors' summary.

Principles in fur animal breeding

K. Mandak

Introduction and basic conditions for establishing fur animal farms. Following chapters include basic information on breeding of mink, fox, coypu (nutria) and chinchilla. (Reproduction, raising the young, nutrition, fur maturation and animal binding are also covered).

Booklet 40 pp. In CZECH. Illustrations.

Investigations of hairs from silver foxes displaying the "curly hair" defect

Bent Riis

Introduction

Farmed foxes must have a perfect fur with perfect hairs to obtain maximum prices at the auctions. Unfortunately, some foxes suffer from genetic defects which impair the quality of the fur and hairs. Some of these defects are inherited as recessive genetic traits. This means that affected animals can pass the trait on to their offspring without the defect being visible on the parent animal. Ideally, breeding animals should be tested for the various genetic defects. This will require simple and inexpensive biochemical tests - but such tests are not yet available.

A hair defect, commonly known as "curly foxes" or "curly hairs", is found in silver foxes (*Vulpes vulpes*) and crosses between silver foxes and blue foxes (*Alopex lagopus*). This defect was first described in Finland over ten years ago. The exact biochemical cause remains unknown despite a large number of investigations. It is known that the defect can be expressed to various degrees and that only part of the guard hair is affected. Similar hair defects have been described in other species, including mice and man.

Materials and methods

Silver foxes, both normal animals and foxes displaying the "curly hair" defect, were used in this project. All animals were from the Fur Animal Farm situated at the Danish Institute of Agricultural Science, Research Centre Foulum, Denmark and fed according to standard for the farm. Summer coat hairs were cut from the animal. Scanning electron microscopy (SEM) was performed according to the manufacturer of the SEM equipment (Philips, Holland) after coating of the specimens. All amino acid analyses were carried out after acidic hydrolysis at 110°C for 16 hours, followed by HPLC analysis as de-

scribed by the producer of the equipment (Hewlet Packard, USA) with standard amino acids plus Citrullin included in parallel runs.

Results

Scanning electron microscopy analyses clearly show a collapse in some parts of the affected hairs (fig. 1). The reason for this collapse is not known, but a defect in the build-up of the guard hair medulla is very likely.

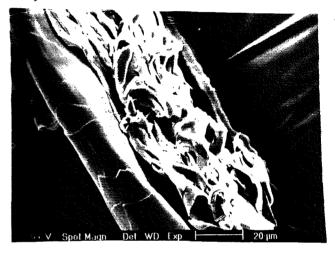


Figure 1. Part of a summer fur guard hair from a silver fox affected by the "curly hair" syndrome. The collapse of the hair and the non-sheaded IRS is shown (magnification x 618).

Amino acid analysis carried out on affected and normal guard hairs shows a difference in the content of Citrulline, an amino acid derived from Arginine. Citrulline is mainly found in the medulla and in the Inner Root Sheet, IRS. It was found that the affected hairs had a significantly higher content of Citrulline compared to unaffected hairs.

Whether this finding is a biochemical marker for curly hairs or because the IRS is stuck in the crevices on affected hairs (fig. 1.) is not clear at present.

Conclusion

These results indicate that the defect "curly hair" is caused by a defect in the affected

hairs' medulla. Unfortunately, the proteins and hence the genes involved in the synthesis of the hairs medulla are not characterized at present. This means it is not possible to develop a genetic test able to reveal the carrier animals at the present time and such tests must await identification of the genes and gene products involved in the medulla. A biochemical test for the defect "curly hairs" in farmed foxes will therefore take some time to develop and will require a large research effort.

Acknowledgment

The project was carried out at the University of Adelaide, Australia, and financed under the OECD project "Use of transgenic animals as a model for studying protein biosynthesis of keratins in hair follicles".

Technical Year Report 1996, pp. 175-180. In DANH, Su. ENGL., 1 table, 4 pictures, 6 refs. Author's summary

Fur development and quality in pastel mink fed at varying protein levels during the growth period.

Palle V. Rasmussen, Christian F. Børsting

The development of the winter coat during the period 24 Aug. to 21 Nov. was studied in 3 groups of pastel male mink fed a normal (35% of ME) or a low (20% of ME) protein level in different phases of the growth period from 27 June until 21 Nov.. Group 1 (N=14) was fed the low protein level and group 3 (N=15) was fed the normal level throughout the experiment. For group 2 (N=11) dietary protein level was changed from the normal to the low level on 24 Aug. Skin biopsies were taken on six dates from 24 Aug. to 21 Nov.. Histo-morphological, sensorial and physical methods were used to characterise hair quantity and fur quality.

From the beginning of the experiment and until 24 Aug. animals of group 1 consumed

more energy than the other two groups. Despite this difference the mean weight of group 1 was only 1596 g compared to 1673 g and 1642 g in groups 2 and 3, respectively. At pelting the weight of group 2 (1963 g) was only slightly higher compared to group 1 (1927 g), whereas the weight of group 3 was the highest (2114 g).

Histological examinations showed that underfur growth in group 2 was different from the other two groups. On 21 Sept. the highest ROA (ratio of activity = (number of underfur fibres in anagen stage)/(underfur fibres in anagen + telogen stage), %) was observed in group 1. On 5 Oct. ROA was significantly lowest in group 2. On 19 Oct. the highest ROA (85.9 %) was observed in group 3. However, at all six dates of sampling during the experiment ROA was consistently lowest in group 2. Results of the sensorial judgment of pelts showed, that the hair density and general fur quality were significantly highest in group 3. Furthermore, the pelts were significantly longer in this group. In all these respects group 1 and 2 differed only slightly from each other. The sensorial results were documented by a physical method, which also indicated shorter guard hair and underfur fibres in group 1 and 2. However, no correlation was found between the number of underfur fibres per area of fresh skin at pelting time (which only varied a little between groups) and the hair density judged on subsequently scraped, stretched and dried pelts. It was also found that especially the hair length was influenced by the different protein levels. ROA and underfur length were correlated significantly (r = 0.53; P = 0.002). In all probability the fibre thickness was influenced, too. This was, however, not examined. It was concluded that both a constantly low and a reduced protein level from 24 Aug. influenced the final hair quantity and fur quality negatively and to the same degree. It appeared that the length and probably the thickness of underfur fibres were most affected.

vinterpelsens udvikling (1) relativt antal uldhår i vækstfase (%)

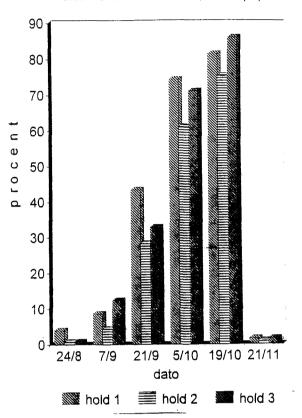


Fig. 1. The relative (%) number of underfur fibres in anagen phase (the growth ratio of the underfur) for groups 1, 2 and 3 during the development of the winter oat on the hip area of mink.

Technical Year Report 1996, pp. 181-189. In DANH. 2 tables, 2 figs., 5 refs. Authors' summary.

Lipid extraction from milk using supercritical fluid extraction (SFE)

Steen Buskov, Hilmer Sørensen, Jens Christian Sørensen

The composition of mink milk is a vital factor for the growth and health status for mink kits at the time of weaning owing to the

correlation of status at this point to the production result. Total contents of lipid, protein and carbohydrates are important but there is not sufficient information for detection of the effects feed and environmental factors have on mink milk composition or quality and thus the value for the kids. Therefore a new method is wanted for analyses of mink milk constituents, giving the opportunity to work on small quantities and with a more gentle and selective separation of the milk constituents than possible with traditional techniques based on initial lipid extraction by use of organic solvents. A supercritical fluid extraction method using carbon dioxide has been developed allowing small amounts of milk (less than 100 mg) to be fat extracted prior to extraction of amphiphilic lipids in a more selective way than what can be accomplished with the Soxhlet method.

The carotenoids are detectable (4 - 10 ng/g fat) in the extracted lipid whereas phospholipids are kept more effectively in the residue after fat extraction. The SFE method also has the advantage of being a more environmentally safe procedure and about 40 times as fast as traditional Soxhlet extraction.

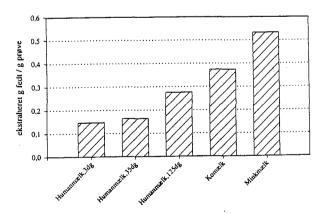


Fig. 1. Lipid extraction of milk from humans, cows and mink by means of supercritical fluid extraction (SFE).

Technical Year Report 1996, pp. 151-158. In DANH. 6 figs., 8 refs. Authors' summary.

Quantitation of fat and oilcontent with supercritical fluid extraction (SFE)

Steen Buskov, Hilmer Sørensen, Jens Christian Sørensen

Lipids, especially fat and plant oils, are important constituents of mink feed. Therefore, fast and quantitative extraction and analysis of these compounds are necessary in connection with production and analytical quality control of mink feed. Traditional methods normally uses the Soxhlet procedure and petroleum ether as solvent, but a promising alternative is the relatively new and fast supercritical fluid extraction (SFE) method which uses compressed CO² as solvent. The effectiveness of SFE compared to the traditional Soxhlet procedure for quantitation of fat and oil content in various plant products was studied. The quantitative data obtained with SFE were equal to those obtained with the Soxhlet procedure with respect to oil and fat contents. The results showed that it was possible to make quantitative and reproducible oil extractions on 1-2 g of sample in less than 30 minutes with SFE compared with up to 24 hours with Soxhlet. However, the oil obtained by SFE had a much lower phospholipid content than oil extracted by the Soxhlet procedure; lower than about 0.04 wt. % compared to 0.6 - 1.5 wt. % in the oil obtained with Soxhlet. Therefore use of compressed carbon dioxide

(SFE) instead of petroleums ether (Soxhlet) seems much more selective and therefore preferable, when it is important to have separate extractions and methods of analyses for phospholipids and other amphiphilic compounds in the matrix.

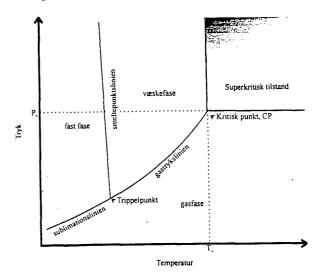


Fig. 1. Definition of the supercritical condition of a substance. CP indicates the critical point when $T = T_c$ (critical temperature) and $P = P_c$ (critical pressure). Further, the triple point is stated when the solid phase is in equilibrium with the fluid and gas phases.

Technical Year Report 1996, pp. 159-162. In DANH. 1 table, 5 figs., 20 refs. Authors' summary.

God sommer...



Genetic-control of PI and GC variants in the American mink

G.P. Borodin, A.V. Perelygin, T.I. Axenovich, O.V. Trapesov, O.L. Serov

Genetic polymorphism of the serum α -protease inhibitor (PI) and group-specific component (GC) in mink was revealed using one-dimentional polyacrylamide gel electrophoresis and immunoblotting. Two codominant alleles were identified at each of the two loci.

The data ruled out the possibility of any linkage between the *PI*, *GC* and the coat colour gene Crystal (*Cr*).

Animal Genetics, 26: 435-437, 1995. 1 table, 2 figs., 5 refs. Authors' summary.

The structure of os malleus in palaearctic mustelidae (carnivore)

A.V. Abramov, G.F. Baryshnikov

The size and structure of mallei in 20 species of Mustelidae from the Palaearctic were studied. The results enable us to discriminate 8 groups of genera:

1) Enhydra, 2) Lutra, 3) Mellivora, 4) Meles, 5) Culo, 6) Charronia, Martes, 7) Vormela, 8) Mustela. These groups correspond to the subfamilies of Mustelidae proposed by Pocock (1921). The considerable differences between M. putorium and M. eversmanni and isolated position of M. erminea are noted.

The mallei of *M. lutreola* and *M. sibirica* are very similar. The main paths of the evolution of the malleus in Mustelidae are as follows: collium broadens and shortens, therefore, the angle between the base of manubrium and collium decreases; lamina becomes narrow (from *Charronia* to *Martes*,

from *Gulo* to *Meles*) and decreases; the medial excision of lamina closes (from *Lutra* to *Enhydra*), its size decreases; the position of manibrium straightens (*Mustela* + *Vormela*); the caput of malleus increases. The increase of caput in *Mustela* and *Vormela*, as compared to *Lutra* and *Enhydra* and especially to *Gulo*, *Meles* and *Martinae*, is correlated with the increase of the articular surface and, probably, with the increase of incus.

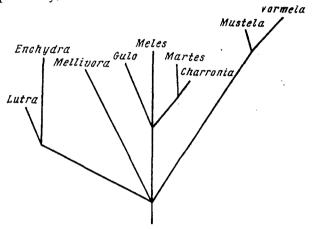


Рис. 5. Основные паправления морфологической специализации молоточка у Mustelidae

Zoologichesky Zhurnal 74 (6): 129-142, 1995. In RUSS, Su. ENGL. 2 tables, 5 figs., 37 refs. Authors' summary.

On systematic position of Canis ekloni

G.F. Baryshnikov, A.V. Abramov

The Russian traveller Przhevalski proposed the name *Canis ekloni* (=*Vulpes ekloni*) for a fox which he found in northern Tibet (Kukunor). Different authors used this name synonymously for *V. ferrilata* or *V. corsac*, and sometimes considered it as nomen nudum. The picture from Przhevalski's book (1883: 218) made the name *Canis ekloni* known. A study of the Tibetan fox skull was carried out for the first time, and it revealed conspecificity of *V. ekloni* (=*V. ekloni*) and *V. ferrilata*. The Tibetan fox is distinguished from other Asian foxes by size (the least breadth between orbits) and also by morph-

ology of the upper incisors and premolar P^1 . V. ferrilata is a specialised species, separated, apparently, on the superspecies level, within the genus Vulpes.

Zoologichesky Zhurnal 71 (11): 121-127, 1992. In RUSS, Su. ENGL. 2 tables, 3 figs., 27 refs. Authors' summary.

Correlation between different production data in mink

Ulla Lund Nielsen

When choosing breeding animals with a desired trait, it is important what correlation

the trait in question has to other traits. In other words, in which direction do the other traits move? Various calculations of correlation between different traits are often missed when one needs them. The aim of this article is to collect them all in one table.

The article includes the following traits: birth date, litter index, live animal quality, clarity, colour, and weight, pelt nibbled, pelt nibbler, pelt quality, clarity and colour, neck chip, body chip, tail chip, silkiness, pelt length and increase in pelt size from before and after pelting.

Technical Year Report 1996, pp. 19-25. In DANH. 1 table. Author's summary.



"The obstetrician delivered you."



The lactating mink (Mustela vison) Genetic and metabolic aspects

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P.O. Box 50
DK-8830 Tjele, Denmark

New doctor in the family. We congratulate Dr. Bente Krogh Hansen with the title and the fine work which is the basis for it all.

The thesis is based on four papers aiming at providing genetic and metabolic information of importance for lactation performance in mink. Two studies each performed on scanblack mink kits and mink dams, respectively, are presented. Effects with influence on mink kit and dam performance in the lactation period were investigated. The material originated from a selection experiment in the period form 1989 to 1994 with divergent selection lines for high and low kit weight gain from two to four weeks of age, and a control line. The production line was used as control with regard to reproduction results. The control line for weight development consisted of production line dams with five to eight kits from 1991 to 1994. A part of this control line was used in the metabolic studies in 1991 to 1994.

Development of kit body weight and body length from parturition to pelting was studied, together with dam weight performance during lactation. Growth performance of kits and body weight changes of dams were negatively influenced by litter size and litter weight. Kit growth was positively influenced by ad libitium feeding and age of dam. The dam weight changes were positively influenced by ad libitum feeding, but in adult dams the weight loss was more severe than in yearling dams.

The direct heritability estimate on kit body

weight $(h_a^2 \approx 0.06 \text{ to } 0.54)$ and body length $(h_a^2 \approx 0.07 \text{ to } 0.46)$ was found throughout the growth period. The maternal heritability estimate (h_m^2) in the suckling period was 0.21 to 0.31. Maternal effect was important during suckling, but decreased from weaning to September. However, the results indicate that there is a potential for improving the maternal traits to provide, e.g. increased milk production. The direct heritability estimate was found for dam body weight $(h_a^2 \approx 0.42)$ and weight changes $(h_a^2 \approx 0.16 \text{ to } 0.28)$ during lactation.

Lactating dams lost around 15% of their body weight, and the main part of this loss occurred during the fifth and sixth weeks of lactation, even though the energy consumption increased throughout the lactation period. The energy required for milk production to sustain the growth of the litter in relation to the total energy consumption confirmed that the dams were in negative energy balance during most of the lactation period. Energy consumption in relation to dam weight changes and litter weight performance and concentrations of triiodothryronine and thyroxine are discussed.

Publications included in the thesis

This thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Hansen, B.K. 1997. Mink kit growth performance in the suckling period. I. Environmental factors affecting body size of kits. Acta Agriculturae Scandinavica, Section A, Animal Science. 46. In press.
- II. Hansen, B.K & Berg, P. 1997. Mink kit growth performance in the suckling period. II. Estimates of sources of variation. Acta Agriculturae Scandinavica, Section A, Animal Science. In press.
- III. Hansen, B.K. & Berg, P. 1997. Mink dam weight changes during the lactation period. I. Genetic and environmental effects. Acta Agriculturae Scandinavica, Section A, Animal Science. Accepted.
- IV. Hansen, B.K. 1997. Mink dam weight changes during the lactation period. II. Energy consumption and plasma concentrations of thyroid hormones and insulin. Acta Agriculturae Scandinavica, Section A, Animal Science. In manus.

Reprints and preprints are published with kind permission of Acta Agriculturae Scandinavica, Section A, Animal Science.

All papers are abstracted in this issue of SCI-ENTIFUR. Ph.D. thesis 1997, pp. 1-27. 2 tables, 4 figs., 32 refs. Dept. Of Breeding & Genetics, Research Centre Foulum, P.O. Boks 50, DK-8830 Tjele, Denmark.

I. Mink kit growth performance in the suckling period. I. Environmental factors affecting body size of kits

Bente Krogh Hansen

Growth performance was studied in 2918 standard black mink kits. The kits originated from a divergent selection experiment for body growth between two and four weeks. Growth was influenced by sex, litter size, age of dam, and production year. Sex dimorphism was found already at birth. Litter size had a negative effect on body weight and body length from birth to pelting. The

age of the dam (yearlings vs. adults) had a positive influence on body weight, especially during the suckling period, and after weaning until September (age about 4.5 months) and even longer in female kits. The effect on body length was significant for female kits until July (age about 3 months).

Ph.D. thesis 1997, pp. 29-37. 5 tables, 33 refs. Author's abstract. Dept. Of Breeding & Genetics, Research Centre Foulum, P.O. Boks 50, DK-8830 Tjele, Denmark

II. Mink kit growth performance in the suckling period. II. Estimates of sources of variation

Bente Krogh Hansen, Peer Berg

Records of 4103 scanblack mink kits born in 1989-94 were used to estimate genetic parameters for body weight and body length during the growth season with special emphasis on the suckling period. Maternal additive and direct additive effects and effects of permanent maternal and specific litter environment were estimated, using restricted maximum likelihood (REML) in univariate and bivariate animal models. The maternal additive effect was constant during the first 4 weeks after parturition at $h_m^2 \approx 0.30$ for weight, and decreased thereafter. The heritability estimate for body weight decreased from $h_{a}^{2}\approx0.19$ at birth to $h_{a}^{2}\approx0.12$ at 4 weeks but increased to h² ≈ 0.50 in August to December. The maternal permanent environment was important only at 2 and 4 weeks, $c_p^2 \approx 0.10$. The specific environment, common within the litter, increased from $c_s^2 \approx 0.24$ at birth to $c_s^2 \approx 0.40$ at 2 and 4 weeks, but decreased thereafter to $c_s^2 \approx 0.10$ in December. Estimates for body length showed a slightly different pattern, h² varying from 0.07 to 0.17 in the lactation period, but increased to 0.46 at pelting. The heritability of maternal additive effect (h²_m) for body length varied from 0.17 to 0.24 during the lactation period and decreased thereafter to 0.06 at pelting.

The results indicate that selection for maternal traits should be based on kit body weight, or body length within 4 weeks after birth, or on growth from 2 to 4 weeks. The largest direct response for body weight would be obtained by selection on body weight in August-December.

Ph.D. thesis 1997, pp. 39-45. 3 tables, 30 refs. Author's abstract. Dept. Of Breeding & Genetics, Research Centre Foulum, P.O. Boks 50, DK-8830 Tjele, Denmark

III. Mink dam weight changes during the lactation period. I. Genetic and environmental effects

Bente Krogh Hansen, Peer Berg

Records from 570 scanblack mink dams with 786 lactations in the period 1989-94 were used to estimate genetic parameters for body weight and weight changes during the lactation period from parturition to six weeks post partum. Direct additive effects and effects of permanent environment were estimated using REML in univariate and bivariate models.

During the first six weeks of lactation, the dam lost around 15% (169 g) of the body weight at parturition, the main part, 10% (112 g), during the last two weeks. Older dams lost more weight than yearling dams, especially during the late part of the lactation period. Dams fed ad libitum had a higher body weight during the last part of the lactation period. The litter size, the sex of the kits and the litter weight influenced weight loss of the dam, especially in the late part of the lactation period.

The heritability of the direct additive effect was intermediate to high for body weight $(h_a^2 \approx 0.39 \text{ to } 0.58)$, but lower for weight changes $(h_a^2 \approx 0.15 \text{ to } 0.38)$. The permanent

environmental effect was important for the total body weight ($c^2\approx0.23$ to 0.30), less important for weight changes ($c^2\approx0.13$), but however, significant. The repeatability for weight changes between parities was intermediate to high ($r\approx0.10$ to 0.52), indicating that a dam with a high weight loss in the first lactation has corresponding weight loss in the following lactation period.

Ph.D. thesis 1997, pp. 47-61. 4 tables, 29 refs. Authors' abstract. Dept. Of Breeding & Genetics, Research Centre Foulum, P.O. Boks 50, DK-8830 Tjele, Denmark

IV. Mink dam weight changes during the lactation period. II. Energy consumption, plasma concentrations of thyroid hormones and insulin

Bente Krogh Hansen

Consumption of metabolizable energy (ME) and plasma concentrations of triiodothyronine (T_a) and thyroxine (T_a) were studied in lactating scanblack mink in the period from 1991 to 1994. The dam body weight and kit growth performance were recorded to evaluate factors with influence on ME consumption and concentration of T_3 and T_4 . The daily ME consumption increased steadily from 1095 kJ in the first week to 1796 kJ in the fourth week post partum, while the concentration of T₃ decreased and T₄ increased during lactation. The concentration of insulin during lactation was also investigated, but no clear pattern was found. In adult dams, ME consumption and concentration of T, were lower than in yearlings. Litter weight had more influence on dam ME consumption than the number of kits.

Ph.D. thesis 1997, pp. 63-79. 4 tables, 1 fig., 33 refs. Author's abstract. Dept. Of Breeding & Genetics, Research Centre Foulum, P.O. Boks 50, DK-8830 Tjele, Denmark



Study on fox reproduction in Poland

Dr. Marian Brzozowski Warsaw Agricultural University – SGGW Dept. Of Animal Breeding 05-840 Brwinow ul. Przejazd 4 Poland

New doctor in the family. We congratulate Dr. Marian Brzozowski with the title and the scientific work it is based on.

The aim of this study was to analyse the reproduction indices in silver and blue foxes, since beginning their farming in Poland up to the end of the 80-ties. The material was a query-sheet, prepared and elaborated by the author. The questionnaires were obtained from 27 silver fox farms and from 27 blue fox farms from different parts of country, from the period 1981-1990. There were about 55.000 silver fox females (on the average about 5.500 females per year) and about 78.000 blue fox females (on average about 7.800 females per year) under observation. Any possible to rich foxes farming in Poland, were added into material.

There were 3 groups of indices under analyse:

I – mating systems: number of females per male (polygamic rate), rate of mating-active males and mating season characteristics (dates and system of mating in farms);

II – reproduction results: rate of mated and non-mated females, fertile and barren females, weaned and non-weaned females;

III – female fertility: litter size at birth, at whelping and mortality rate per female and per weaned female.

All data were collected separately for silver and blue foxes, so it was possible to compare the species in reproduction results.

The influence of some extrinsic factors on the reproduction results in fox farms were investigated in this study: A – form of ownership – private or non-private (states or cooperatives);

B – climate – mild (vegetation season over 220 days per year) and harsh (vegetation season under 220 days per year);

C – distance from a large city (over 100.000 habitants): under 50 km and over 50 km from such a city.

All obtained results were calculated statistically by variance analysis with application of an F-test to estimate statistical significant differences between the value of the same index in different groups of farms.

The results obtained in this study are as follows:

- 1. The polygamic rate in silver fox farms increased from 2.0 in the 30-ties to about 2.8 in the 80-ties. The index was higher in non-private farms. Polygamic-rate in blue fox farms was more stable it was about 2.6 in 60-ties and about 2.6 in 80-ties. The index was higher in non-private farms. The index was higher in silver fox farms than in blue fox farms, particular in non-private farms. The rate of matingactive males, estimated in the 80-ties on a group of 54 farms, was equal in both species in silver fox farms it was 93.52%, in blue fox farms 92.22%.
- The mating season did not change in both fox species under farming conditions – silver fox females are usually mated from the end of January to the

- middle of March, blue fox females are usually mated from the end of February to the middle of April
- 3. Mating system did not change in both fox species under farming conditions silver fox females are usually mated in the second day of heat and again the next day, blue fox females are usually mated in the second day of heat and mated at least two times in the next days.
- 4. Reproduction results in silver fox farms were estimated since the end of the 40ties to the end of the 80-ties by literature studies. It was found that the rate of mated females was over 95% in all this time, the rate of fertile females increased from 85% to about 90%, the rate of weaned females decreased from 80% to about 75%. The reproduction results estimated on 27 silver fox farms in the 80ties were as follows: rate of mated females - 98.67%, rate of fertile females -92.25%, rate of weaned females - 83.29%. The losses in reproduction were due to the effect of a high rate of non-weaned females (over 50% of losses) and the rate of barren females. In estimated silver fox farms were found 1.38% non-mated females, 6.43% barren females, 8.95% nonweaned females. Reproduction results in blue fox farms, in literature studies, were estimated since the beginning of the 60ties to the end of the 80-ties. The level of indices in the 60-ties was as follows: mated females - over 95%, fertile females - about 85%, weaned females about 80%. In the 70-ties and 80-ties no progression in the indices level was found. The level of reproduction indices, in 27 estimated blue fox farms in the 80ties was as follows: mated females -98.75%, fertile females - 86.75%, weaned females - 79.94%. The losses in reproduction were due to the high rate of barren females (over 50% losses) and the rate of non-weaned females. In the 80ties on 27 estimated farms were found
- 1.97% non-mated females, 11.28% barren females, 6.81% non-weaned females. A higher level of reproduction indices was found in silver fox farms than in polar fox farms (the rate of mated, fertile and weaned females. The lower rate of non-mated and barren females and higher rate of non-weaned females were found in estimated silver fox farms compared to blue fox farms.
- 5. Fertility indices from literature studies show that in silver fox farms fertility was on the increase, when counted per weaned female and fertility did not increased when counted per female on the farm. Fertility per female in the 30-ties and in 80-ties was as follows: about 4.5 pups at birth, about 3.5 pups at weaning, mortality rate - about 20%. Fertility indices estimated in 27 silver fox farms in the 80-ties, per weaned female was as follows: 4.92 pups in birth, 4.35 pups at weaning, mortality rate – 11.50%. A high level of fertility indices was found in blue fox farms in the 60-ties, per female – over 9.5 pups at birth, 8.0 pups at weaning, mortality rate - 15%. In the 70ties and 80-ties fertility indices decreased, probably as a result of a very high increase in the number of blue foxes per farm. Fertility indices estimated in the 80-ties in 27 farms per weaned female were as follows: litter size at birth - 8.78, litter size at weaning - 7.72, mortality rate - 12.07%. Litter size at birth in both fox species was higher on private farms. Pup mortality on private farms was also higher, but there was no difference in litter size at weaning between private and non-private farms in both species. Litter size at weaning was also a bit higher in silver fox farms from milder climate and in blue fox farms from harsher climate.
- 6. The influence of farm property was found as the most important extrinsic factor for reproduction results: in private farms of both silver and blue fox, the

level of reproduction indices was higher than in non-private farms. The influence of climate and distance from a large city was not so significant.

Thesis, 60 pages. In POLH, Su. ENGL. 27 tables, 8 figs., 85 refs. Author's summary.

Cortisol production in fetal adrenals of the silver fox

L.V. Osadchuk

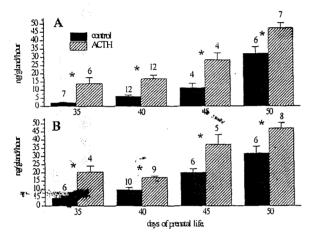


Figure 5. Control and ACTH-stimulated (50 mlU/sample) in vitro production of cortisol in silver fox fetuses aged from 35 to 50 days of fetal life. The animals were killed at ages indicated on the x-axis. Asterisks designate significant differences (p at least < 0.05) between control and ACTH groups. Values are mean ± SEM. The different numbers above the bars indicate the numbers of foxes in a group. A - females, B - males.

The present study was designed to examine cortisol production by the silver fox fetal adrenals and their response to ACTH at different periods of prenatal life. Serum levels of cortisol were determined on days 35, 40, 45 and 50 of gestation (term on day 52) in embryos of both sexes. Cortisol content in adrenal tissue homogenates and its in vitro adrenal production were also investigated at the same time points. Hormones were measured by RIA. The levels of cortisol changed slightly during embryonic life. The adrenal content and the in vitro production of cortisol increased sharply and progressively (by 4-10 times from days 35 to 50 of gestation). The rises in cortisol content and in vitro production were associated with a

rapid growth of the fetal adrenals. There were no sex differences in cortisol level and its adrenal content. ACTH (50 mlU per sample) increased the *in vitro* cortisol production by the adrenal in the two sexes on all the studied days (by 3-4 times on day 35 and by 1.4-1.7 times on day 50). These results indicate that 1) silver fox fetal adrenals are capable of synthesising cortisol; 2) ACTH is a potent activator of the *in vitro* cortisol production during embryonic life in this species.

Theriogenology 47, pp. 903-912, 1997. 5 figs., 13 refs. Author's summary.

Reproductive function of young silver fox males (*Vulpes vulpes*) under long-term selection for domestic behaviour

L.V. Osadchuk, L. Jalkanen, A.A. Philimonenko, V.V. Gultjaeva

Sperm morphology, plasma testosterone level and sexual activity of young silver fox males selected for domestic behaviour has been studied. It is established that the number of spermatozoa in the semen of males from the selected population was lower compared with the control, whereas abnormal spermatogenesis level was significantly increased. The plasma testosterone level in males after a female introduction increased to the same extent in both groups. Sexual activity in males from the selected population was lower than in the control during the first mating season. The data obtained suggest a depressing effect of selection for domestic behaviour on spermatogenesis and sexual activity in the young silver fox males and point to hereditary changes of some chains of reproductive functions of males under the selection for domestic behaviour.

Journal of Evolutionary Biochemistry and Physiology, Vol. 31, No. 5, 6: 662-667, 1995. In RUSS, Su. ENGL. 3 tables, 18 refs. Authors' summary.

Biosynthesis of cortisol and its control by adrenocorticotropic hormone in adrenals of silver fox embryos

L.V. Osadchuk

We studied the cortisol level in blood serum and adrenal homogenates, as well as the *in vitro* production of cortisol in adrenals of silver fox embryos of both sexes in response to exogenous adrenocorticotropic hormone (ACTH) stimulation. The level of cortisol in blood serum did not show any significant changes during the embryo development, while its level in adrenals and production by adrenals *in vitro* increased progressively from Day 35 to Day 50 of prenatal life. We found that ACTH is capable of stimulating cortisol biosynthesis and production *in vitro* during all studied periods of embryogenesis.

Russian Journal of Developmental Biology, Vol. 27, No. 3, pp. 218-221, 1996. In RUSS, Su. ENGL. 3 tables, 14 refs. Author's summary.

Developmental changes in testicles during the postnatal period in raccoon dogs

Piotr Niedbala, Olga Szeleszczuk, Stanislaw Jarosz

Studies on developmental changes in testicles in the postnatal period in raccoon dogs were carried out on the farm Chorzelow from June 1992 to March 1993.

At the beginning of month animals were weighed and blood samples taken to determine plasma testosterone level. Testicle size and consistency were also measured.

During the period from June to January the volume of gonads as well as their weight increased (from 0.16 to 5.33 cm³) and (from 0.3 g to 5.6 g), respectively.

The consistency of raccoon testicles, as the mating season approached, changed from soft and moderate to hard and very hard.

Single spermatocytes were already noticed in August, however, a true increase of spermatogenesis was found in September. A full process of spermatogenesis occurred in the testicles of raccoon dogs before the mating season.

The highest plasma testosterone level (16 mmol/l) was attained in January i.e. a month before the mating season.

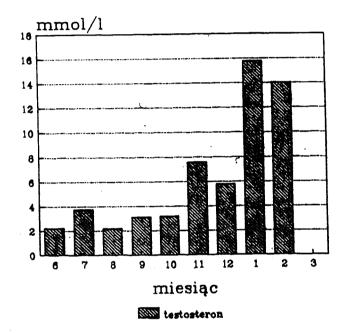


Fig. 4. Plasma testosterone level in raccoon dogs during postnatal period.

Zeszyty Naukowe 15, pp. 171-176, 1994. In POLH, Su. ENGL. 1 table, 4 figs., 9 refs. Authors' summary.

Studies on the dynamic structure of mink ovaries during estrus

Liu Yutang, Zhou Qiping, Zhang Shuyun, Qin Pengchun

Serial sections of mink ovaries during estrus were made and morphological structure and histochemical changes of follicular growth, atresia, ovulation, and interstitial gland cells were studied. The mink follicular growth is in the form of wave upon wave, and follicular atresia appears as three types. Epithelial-type theca interna cell in the atretic antral follicles attain proliferation to form interstitial gland tissue and finally fill the remnant follicular cavity. The functional significance of the distribution of the lipid droplets that consists mainly of phosphatide and triglyceride, and the biosynthesis of steroidgenesis are discussed.

Journal of Northeast Forestry University (China), Vol. 23 (2), pp. 49-54, 1995. In CHIN, Su. ENGL. 2 tables, 1 fig., 7 refs. Authors' summary.

Map kinase activation and raf-1 synthesis in blue fox oocytes is controlled by cumulus granulosa cells

P. Kaláb, V. Srsen, W. Farstad, A. Krogenæs, J. Motlik, A-L. Hafne

The activation of MAP kinase (MAPk) and Raf-1 synthesis and phosphorylation were investigated during in vitro and in vivo maturation of blue fox oocytes. The oocyte cumulus complexes (OCCs) cultured in vitro were isolated from ovaries during proestrus and estrus. Isolated OCCs with oocyte vitelline diameter $> 100~\mu m$ were divided into two groups: OCCs with well developed multilayered compact cumulus (type A) and OCCs consisting of corona radiata only (type B).

Both types of OCCs were cultured in modified TC 199 medium at 39°C and 5% CO_2 in the absence or in the presence of 1 μ g FSH/ml. The effect of 2.5 μ M okadaic acid was tested in part of samples. Samples containing 8-9 OCCs were removed from the culture after 24, 48, 72, and 120 h, cumuli were removed by pipetting and the washed oocytes were extracted in SDS PAGE sample buffer and kept at -80°C. In separate experiments, OCCs were flushed from oviducts at similar time points (24, 48, 72 h) fol-

lowing unstimulated ovulation and cumulus free oocytes extracted as described.

All samples were analysed by SDS PAGE and immunoblotting with anti-ERK-1 (MAP kinase) and anti-Raf-1 antibodies.

In the presence of FSH, MAPk phosphorylation started after 48-72 h and never reached the same level as in the control without FSH, where the first MAPk shift was observed after 24-48 hours. In ovulated oocytes, where the estimated time of LH peak was taken as a start of meiosis the time course of MAPk phosphorylation was comparable with the FSH treated oocytes, or slower. To the contrary, in B oocytes, MAPk phosphorylation was accelerated reached higher levels as compared to oocytes with well developed cumuli. Interestingly, the extent of MAP kinase activation and the amount of detectable signal of phosphorylated Raf-1 was well correlated and reached highest levels in ovulated oocytes, indicating that Raf-1 synthesis takes place during meiosis in fox oocytes.

On the other hand, acceleration of GVBD with OA was associated with increased MAPk phosphorylation in the absence of detectable Raf-1.

Results of this study support our previous data documenting that an FSH-receptor dependent pathway is present in blue fox granulosa cells that are able to control the rate of meiosis progression. Moreover, our data indicate that the granulosa cell-dependent inhibitory effect on the start and progression of meiosis is correlated with a delay of MAPk activation and slower synthesis of Raf-1. The functional significance of MAPk phosphorylation and Raf-1 synthesis remains to be elucidated, however, their monitoring could facilitate development of physiological in vitro culture conditions for this species.

Theriogenology, Vol. 47, No. 1, pp. 400, 1997. Only abstract received. Authors' abstract.

Resumption of meiosis in blue fox oocytes is controlled by cumulus granulosa cells

Vlastimil Srsen, Jaroslav Kalous, Eva Nagyova, Petr Sutovsky, Jan Motlik

The meiotic competence of blue fox (Alopex lagopus) oocytes and influence of cumulus granulosa (CG) cells on timing of meiotic resumption were investigated. Oocyte cumulus complexes (OCCs) isolated from ovaries during anestrus were cultured up to 72 h in modified TC 199 medium only or supplemented with FSH, recombinant bovine somatotropin (BST) and okadaic acid (OA). The stage of nuclear maturation, oocyte and cumulus granulosa cells ultrastructure, and distribution of microfilaments (F-actin) and gap junctions (GJs) were observed using phase contrast light microscopy, immunofluorescence and transmission electron microscopy. It was found that only the oocytes larger than 100 mm in diameter were meiotically competent. These oocyte cultured in control medium without supplements ungerminal vesicle breakdown derwent (GVBD) after 48 h of culture (38%) and reached metaphase II (MII) after 72 and 96 h, respectively (20 and 27%). Both BST and OA accelerated resumption of meiosis (BST, 55% GVBD and 42% MII after 48 h; OA, 66% GVBD after 18 h). In contrast FSH in culture medium secured meiotic arrest (only 3% GVBD and MII after 72 h) and induced changes of CG cell shape and F-actin assembly typical for cumulus expansion. However, the innermost layers of CG cells (corona radiata) remained connected with oocyte via GJs until the end of culture. Cumuli of oocytes cultured in control, BST or OA supplemented medium did not expand (changes of cell shape and F-actin redistribution did not occur). Moreover, especially in media with BST and OA an increased detachment and rapid disconnection of their GJs with oocyte were observed. These result suggest that under in vitro conditions FSH stimulates expansion of the cumulus granulosa cells and the attached membrana granulosa cells but it secures heterologous gap junctions between cytoplasmic processes of the corona radiata (CR) cells and oolemma during three days of culture. Thus, in agreement with in vivo situation in which canidae oocytes are ovulated in the GV stage, the cumulus, mainly corona radiata cells, control resumption of meiosis in blue fox oocytes also under in vitro conditions.

Archiv. Für Tierzucht-Archives of Animal Breeding (special issue) 39, 1996. Only abstract received. Authors' abstract.

Morphological and biochemical changes in nutria semen during conservation with diluents at various glycerol levels

Olga Szeleszczuk, Stanislaw Jarosz, Piotr Niedala

Studies on morphological and biochemical changes in nutria semen during the process of freezing were conducted on 10 males of Greenland and Standard varieties. Semen was collected by the electroejaculation method elaborated by Jarosz-Szeleszczuk, allowing us to obtain semen not subjected to gelatinization. For diluting a diluent TRIS with various levels of glycerol was used: 4% (RI), 6% (RII) and 10% (RIII). Equilibration was performed by placing the cooled semen for 1 h in the cooler, with a subsequent freezing, using the MINITUB system. Straws were thawed by dipping them for 8 sec in water at 70°C.

The freezing and thawing processes were monitored and simultaneously the sperm motility and morphology as well as the levels of AspAT and ALAT in semen plasma were determined.

The sperm motility in fresh semen estimated at 53.72% after thawing, decreased to 40.83% in RI, 43.05% in RII and 33.29% in RIII. Statistically significant differences (P<0.001) were found between the RIII diluent (with 10% of glycerol) and both: RI and RII (with 4 and 6% glycerol).

The greatest numbers of sperm cells with a correct morphology (82.3 and 79.9 %, respectively) were found in the semen smears diluted with RI and RII.

The lowest activity of AspAT and ALAT was found after thawing semen using RI (2.112 and 1.805 μ m/ml), a higher one with RII (2.538 and 1.988 μ m/ml) and RIII (2.463 and 2.155 μ m/ml).

The TRIS diluent with 6% supplement of glycerol proved to be the most useful for freezing since it decreased the percentage of spermatozoa with progressive motility the least.

Zeszyty Naukowe 15, pp. 226, 1994. In POLH, Su. ENGL. Only summary received. Authors' summary.

Application of Spermac® staining for estimation of frozen semen morphology of arctic and common foxes

Olga Szeleszczuk, Wenche K. Farstad, Jan A. Fougner

Although the first trials with insemination in reproduction of foxes were started in 1934 it was as late as in 1980 that they were introduced on a larger scale, mostly to Scandinavian fox farms. One of the problems which was difficult to solve was the lack of diagnostic methods enabling full estimation of fresh and frozen semen.

The aim of this study was to evaluate the usefulness of the staining method with Spermac® for determination of the state of the acrosome in the process of freezing and thawing semen of polar and silver foxes.

Studies were conducted on a total of 74 ejaculates, obtained through masturbation from 16 polar and 18 silver foxes. Semen was diluted with diluent TRIS supplemented with 20% egg yolk and 5% glycerol up to the final concentration of 150 million

sperm in 1 ml. After cooling and equilibration the semen was frozen in 0.5 ml straws in liquid nitrogen (Planer Kryo 10). Straws with semen were thawed through dipping for 8 sec. in water at 70°C. Directly after thawing the spermatozoa motility was estimated and semen smears by Spermac® method (Oettle, 1977), modified by the authors, were made. In the smears stained by this method the acrosome, midpiece and tail attained a dark-green colour, the equatorial region turned pale-green while subacrosomal material and the head turned pink. The process of acrosome impairment was connected with a very distinct change in the intensity of its colour and shape.

A high correlation was found between sperm motility after thawing and degree of acrosome impairment. For sperm with intact acrosomes the correlation coefficient was 0.76.

Zeszyty Naukow 15, pp. 224, 1994. Only abstract received. Authors' abstract.

The use of gonadotropin hormones for stimulation of estrus and ovulation in nutria (*Myocastor coypus* M.)

Olga Szeleszczuk, Stanislaw Jarosz

The investigation, carried out during the autumn-winter season, involved 35 eightmonth-old Greenland nutria females divided into 5 genetically homogenous groups. Each female from groups I and II was injected intramuscularly with 200 u.i. PMSG (Serogonadotropina, Biowet Drwalew) and HG (Bioganadyl, Biomed Lublin). The females from group III were given 0.8 ml each and those from groups IV 0.4 ml each of PG 600 (Intervet, Holland).

The control group (V) was injected with physiological salt solution. Estral phase was estimated on the basis of vaginal mucus electric resistance, colpocytogram and appearance of internal reproductive organs. oviduct and corners of the uterus. A higher effectiveness in stimulation of estral phase in nutria was achieved after the application of PG 600.

Acta Agraria et Silvestria, Series Zootechnica, Vol. XXXIII, pp. 103-109, 1995. In POLH, Su. ENGL. 2 tables, 4 figs., 17 refs. Authors' summary.

Timing of reproduction in the red fox, Vulpes vulpes

P. Cavallini, Simona Santini

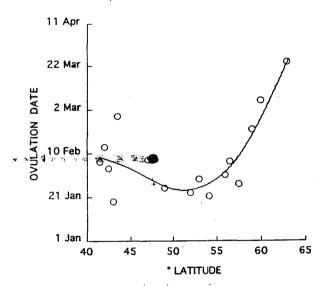


Fig. 2. Relationship between mean (or median, when the mean is unavailable) ovulation date in female red foxes and degrees of latitude North across different studies. The curve indicates distance weighed least squares smoothing (McLain, 1974).

Ovulation data was estimated for 93 female red foxes ($Vulpes\ vulpes$) collected in central Italy. Three methods were used: ageing embryos, examination of ovarian bodies, and of placental scars. Most females ovulated around 26 February (SD \pm 0.5 days). Estimates from different methods gave consistent results. Ovulation data was independent of physical condition and size, but was 5 days earlier in the north of the study area

than in the south. The testes of 154 males were also weighed. Male testicular mass decreased after the end of February. Results from this and other studies are consistent with the hypothesis that timing of reproduction in the red fox is constrained by a winter trophic bottleneck in the north, but can be more variable in the south. Small-scale variation in our study area was probably unrelated to food availability.

Z. Säugetierkunde 60: 337-342, 1995. 1 table, 2 figs., 31 refs. Authors' abstract.

Effect of mating with two different males on female mink reproduction

Ulla Lund Nielsen

Previous studies have shown that in mice and pigs it is possible to improve reproduction by producing a sort of allergic reaction in the uterus prior to fertilization. The allergic reaction can take place by treating the uterus to killed or live semen. Semen has antigenic properties and causes antibody production when injected into test animals.

The question is whether this is also the case in mink who, as opposed to mice and pigs, differ with respect to several reproductive conditions such as having induced ovulation. This study shows that even though the material was sparse, there was a significantly better breeding result when the female was mated by two different males instead of being mated by the same male twice. This method also increases the efficiency of male usage and eases the work load in the mating period.

However, the method also has some disadvantages, the greatest being that is it more unsure who the father of the kits is, which has a negative effect on the confidence of selection of breeding animals.

Technical Year Report 1996, pp. 15-17. In DANH. 3 tables, 6 refs. Author's summary.

Specific features of water balance and resistance to dehydration in some mustelids (Carnivore)

V.E. Sokolov, I.G. Meshcherskii, V.V. Rozhnov, S.V. Naidenko

Food consumption, total water intake, urine volume and concentration, and water content in feces were determined under the conditions of free access to drinking water and in its absence in some mustelids that inhabit territories with different water accessibility: *Mustela putorius*, *M. eversmanni*, *M. lutreola*, and *M. vison*. Specific features of water metabolism and resistance to dehydration in the studied species corresponded fairly well to differences in their ecology.

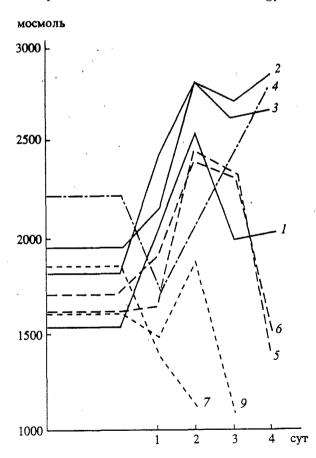


Рис. 4. Концентрация мочи животных при отсутствии питьевой воды. Обозначения см. на рис. 1.

Biology Bulletin, No. 2, pp. 163-171, 1995. In RUSS, Su. ENGL. 2 tables, 4 figs., 17 refs. Authors' summary.

The effect of dietary fiber level on nutrient digestibility, rate of chyme passage and activity of amylolytic enzymes in the digestive tract of nutria

Boguslaw Barabasz, Stanislaw Jarosz

The aim of this study was to investigate digestibility of nutrients, rate of chyme passage and activity of amylolytic enzymes in the digestive tract of nutria fed diets containing various levels of crude fiber (4.2-17.8%).

In our experiments were used a total of 40 nutria of Standard and Greenland varieties, coming from a nutria-farm at the University of Agriculture in Cracow.

Most nutrients (dry matter, organic substances, protein, carbohydrates) were best digested in diets with 6-11% of fiber whereas fat in diets with 11-15% of fiber.

It was found that an increase in dietary fiber level resulted in a faster rate of chyme passage through the digestive tract and a faster out-flow of indigested feed remnants. With an increased dietary fiber level an increase was noted in the activity of amylolytic enzymes in the digestive tract being the highest (2.5 times) in the small intestine.

Zootechnika, Vol. 31, pp. 65-72, 1996. In POLH, Su. ENGL. 4 tables, 9 refs. Authors' summary.

Physiological, reproductive and pathological effects of dietary bleached pulp mill effluent on mink (Mustela vison)

Judit E.G. Smits, Gary A. Wobeser, H. Bruno Schiefer

In this study mink (*Mustela vison*) were exposed to whole bleached-kraft mill effluent (BKME) through their diets. The investigation examined clinical, biochemical, induction of hepatic ethoxyresorufin-0-deethylase (EROD) activity, one-generational repro-

ductive, and pathological effects on mink exposed to BKME through water containing 25% effluent, and diet containing 75% (pilot and first subchronic study) fish caught downstream of the BKME discharge point of the pulp mill.

In a 6-week pilot study, no adverse effects were found on behavioural, gross pathological, histopathological, hematological, or biochemical variables tested. In an 8-month exposure study, no significant effects on these parameters, or on gestation, kit birth weight, kit survival, libido, estrus, sperm quality, or hormone levels were found.

This was followed by a second 8-month study using double the number of mink (30 BKME-exposed, 30 controls), and modified dietary formulations. Whole fish was decreased to 45%, and 15% softwood-run BKME was added to the food. In this study, the liver somatic index was greater in exposed males (p=0.068) than in control males. Hepatic EROD activity was 1.8 times greater in exposed female (p=0.0001) and 2.0 times greater in exposed male (p=0.0004) mink compared with controls.

Environmental Toxicology and Chemistry, Vol. 14, No. 12, pp. 2095-2105, 1995. 8 tables, 55 refs. Authors' summary.

Digestibility of nitrogen from feed with various proportions of cod for mink

D. Mertin, K. Süvegova, J. Rafay, B. Barabasz, Z. Ceresnakova

An experiment was conduced on the Experimental Farm of Fur-bearing Animals affiliated with the Research Institute of Animal Production in Nitra in August to September 1995. The animals were housed in special cages for metabolic trials. The experiments were conducted on five nonsib mink males at the age of four months. The animals were clinically healthy and examined for plasmacytosis. The experiment con-

sisted of three stages to determine the digestibility of nitrogen in the feed ration with various proportions of cod fillet administered to mink and nitrogen digestibility in the test feed (cod fillet). The animals received a basal feed ration in the first stage while in the second stage the content of test feed in the feed ration was 41.7% and in the third stage 62.5% of the original amount. Each experimental stage had two periods - a preparatory period and an experimental period. The preparatory period lasted three days, in which the animals adapted themselves to a new feed ration, and the experimental period lasted five days. Feed was administered twice a day (at 9 a.m. and 3 p.m.). The nutritive value of the feed rations was in agreement with the standard for the age category and physiologic stage concerned. Climatic conditions were observed throughout the experiments.

Average air temperature was 14.2±0.6°C in the first stage of experiments, 28.0±0.4°C in the second stage and 18.1±0.4°C in the third stage. Indicators necessary to calculate digestibility coefficients for feed rations and test feed were determined during the experiment: feed intake, remaining uneaten feed and amount of excrements. The indicators were recorded and samples were taken twice a day, always an hour before feeding at 7 to 8 o'clock. A direct method was used to calculate nitrogen digestibility in the feed rations with various proportions of cod fillet, and an indirect (differential) method was used to calculate digestibility in the test feed. The results of the calculations were processed by a mathematical-statistical method of one-factor analysis of variance with multivariate comparisons of differences in arithmetical means. No statistically significant intra-group differences were determined for the animals, which meant that the set of mink was homogeneous. Tests of the effects of replications in the particular groups revealed statistically significant differences at a significance level $p \le 0.5$ for the second stage of experiments at administration of feed ration with 41.7% portion of cod fillet. As the biological material is homogeneous, we believe that this fact was influenced by the environment factor (climatic conditions during the experiments). Coefficient of nitrogen digestibility in the basal feed ration was 67.661±1.180%, in the feed ration with 41.7% of cod fillet 72.327±0.613% and in the feed ration with 62.5% proportion of test feed 81.563±1.095%.

The obtained results of nitrogen digestibility in the test feed rations are statistically significant (P \leq 0.05). The coefficient of nitrogen digestibility in cod fillet used in the feed ration with 41.7% proportion of test feed (2nd stage) was 81.240 \pm 0.864% and in the feed ration with 62.5% of test feed amounted to 93.020 \pm 1.033% P \leq 0.05). It can be concluded from the results obtained that the coefficient of nitrogen digestibility in feed rations administered to mink increased in relation to the per cent proportion of test feed.

The nitrogen digestibility in the test feed was also related to the feed proportion in the feed rations (41.7%: 81.240±0.86% and 62.5%: 93.020±1.033%).

It is recommended to use cod fillet in feed rations for mink throughout the year as it is a dietetic feed with a high crude protein content (16.62%) and low fat content (0.60%).

Zivocisna Vyroba 41 (8), pp. 355-358, 1996. 6 tables, 10 refs. Authors' abstract.

Effects of feeding dry compound feed upon growth and nutrient utilisation in growing-furring sables

Zhang Keqin, Tong Yuren, Yuan Shoucheng, Qiu Xuejun, Wang Feng

In order to study the effects of feeding dry compound feed on the nutrient requirement for growth and nutrient utilisation in sables during the growing-furring stage, 4 feeding and digestion trials were conducted in which 100 sables of 60-70 days old were randomly divided into 4 experimental and one control group (12 male and 8 female sables in each group). 21% dry compound feed was added to the experimental diets and the animals in control group were fed a raw feed based diet. In the middle of the feeding trial period, 40 sables were selected to carry out a digestion and metabolic trial.

The results obtained from the feeding trials showed that there were no significant differences in weight gains, mortality, morbidity of biting and fur quality of the sables between the experimental groups and the control. The results collected from the digestion and metabolic trials indicated that there were significant differences in digestibility (P < 0.05) and metabolisability of gross energy (P < 0.01). Significant differences in digestibility (P < 0.01), but no significant difference in crude protein utilisation was found.

Acta Zoonutrienta Sinica (China), Vol. 7 (2), pp. 36-41, 1995. In CHIN, Su. + subtitles in ENGL. 3 tables, 4 refs. Authors' abstract.

Amino acid digestibility of feed mixtures and digestibility of crystalline amino acids fed to mink

Christian Friis Børsting

A series of experiments were performed over four years in collaboration with Research Farm Vest, Tvis, Denmark to elucidate the requirements of individual amino acids for mink. In the present paper data on the digestibility determined for feed mixtures with and without supplementation of crystalline amino acids are presented. The aim of this part of the study was to i) describe if deviations between the calculated and the measured content of digestible amino acids in the mixtures were due to var-

iation in the chemical content or rather in the digestibility of amino acids compared to the values taken from the Danish feedstuff table for mink and ii) to estimate the digestibility of the supplemented essential amino acids, when these comprised approx. 30-50 % of the total amount of these amino acids in the diet.

There were large differences between the apparent digestibility of individual amino acids within the same mixtures. The apparent digestibility of cystine was approx. 20-25 digestibility units lower compared to crude protein, whereas the apparent digestibility of threonine was 5-10 units lower than of protein. These low values were explained by the high content of these amino acids in the endogenous protein losses as has been found in other studies.

Tryptophan digestibilities were also lower than crude protein digestibilities, whereas for the remaining amino acids the apparent digestibility was at the same level or higher compared to protein. For mixtures with the same feedstuff composition there were only small differences from year to year in protein and amino acid digestibilities. Therefore, it was concluded that deviations in the content of digestible amino acids per MJ ME from calculated values were caused by variations in the chemical content of amino acids rather than by variations in their digestibility.

The estimated digestibilities of the supplemented essential amino acids showed the same pattern as found for the amino acids in the mixtures without supplementation, i.e. the digestibility of cystine was low (57-72 %) and digestibilities of threonine (73-97 %) and tryptophan (82-97 %) were rather low, whereas the digestibility of the other supplemented essential amino acids was close to 100 %. For all non-supplemented amino acids the digestibility was lower in the supplemented diets than in the equivalent mixtures without supplementation. These findings strongly indicate that supplement

tation of large amounts of amino acids increases endogenous losses of protein.

Technical Year Report 1996, pp. 57-65. In DANH. 4 tables, 7 refs. Author's summary.

Test of Imovet in mink

Ulla Lund Nielsen

Imovet is a vegetable product without chemical additives and contains the following: Indian gooseberry, ginger, basil, Indian ginseng, and Indian mango. In Denmark the product is handled by Natura Vet Int.

According to the company, the product has anti-stress/adaptogenic and immunostimulating effects as well as having anti-inflammatory, anti-pyretic, anti-tumoral, anti-rheumatic, anti-asthmatic and analgesic effects. In this study we have not been able to find any differences between giving mink Imovet or not in the latter pelation period.

Technical Year Report 1996, pp. 135-138. In DANH. 3 tables, 1 fig. Author's summary.

Different feed wire mesh sizes for mink

Ulla Lund Nielsen

As a result of external factors (limited amounts of industrial fish and, possibly, fish cuts) the present and future feed composition differs from in the past. The changes are expected to affect feed consistency. The feed will probably become more crumbly as industrial fish is substituted by dry protein sources. It can therefore be expected that there will be problems with getting the feed to hang onto the regular types of feed wire mesh properly.

It would be expected that feed wire mesh with smaller mesh size would ease the problem. It has previously been shown that

feed spillage is less when the mesh size is 3/4" x 3/4" instead of 1" x 1" (Nielsen, 1993). The problem with this type of wire mesh is that it is difficult for the mink to keep it clean and that it does not correspond to standard wire mesh measurements. The mesh type 1" x 3/4" would not be expected to give these problems. At the same time, a thicker wire (2,45 mm = no. 12.5), as opposed to 2,05 mm (= no. 14), would expect to result in less feed spillage and, thereby, cheaper feeding. The results showed that a change of feed wire mesh to 1" x 3/4" from 1" x 1" and slightly thicker wires in one of the mesh directions gave significantly less feed consumption per cage in the month of October. The amount saved was 5-7%. Part of the saving was probably due to reduced feed spillage. However, it was not possible to register the amount of feed spillage in this study.

There were no significant differences between the groups with regard to growth rates, but the control group tended to have the best growth rate. This could be due to a problem with adapting to the new wire mesh type. Judged visually, the control group was best at keeping the feed wire mesh clean while the group with the small wire mesh size was poorer in this regard.

Tecnical Year Report 1996, pp. 233-236. In DANH. 1 table, 1 ref. Author's summary.

The content of elastin and soluble collagen in dried skins from mink raised on fat of different quality

Bent Riis, Christian Friis Børsting

Introduction

Today mink skins are used for a variety of different purposes, and the skins are no longer used solely for classical fur coats. Because of this trend, the skin must fulfill additional quality demands as well as have good hair and skin quality and colour. One of the demands which seems to be of grow-

ing importance is a uniform ability for the skin to respond to stretching. A mink skin's ability to respond to stretching is determined by the molecular composition of the skin. This is a very complex mixture of many organic compounds with a large proportion of proteins carrying different post-translational modifications. Elastin and collagen are important for a skin's stretchability, and these tests were performed in order to measure the content of these two structural components in relation to the quality of fat fed to the animals.

Materials and methods

24 dried mink skins from pastel male minks were used for this investigation. The minks were fed fish oil as the sole fat source during the period of growth. Four different qualities of fat were used: Test group # 71 received fresh fish oil with an peroxide value of 10 meq/kg oil. Test group # 72 was fed an oil containing 30 meq/kg oil, test group # 73 was fed an oil containing a peroxide value of 70 meq/kg olie and test group 74 was fed oil containing 100 meq/kg oil. To all oils was added 300 ppm etoxyquin/kg.

The skins were treated according to the normal procedure of the experimental mink farm and kept at approx. 10°C and 70% relative humidity, until the samples were taken for analysis. Two times 0.8-1.2 g skin pieces were cut from the back of the skin and analyzed for insoluble elastin and for soluble collagen. Briefly, the analysis for elastin was performed by boiling the skin sample in 0.1M NaOH for 45 min followed by weighing the remaining insoluble elastin. The collagen analysis was performed by extracting the skin pieces with buffer A (50 mM Tris/HCl pH: 7.5, 1M NaCl, 1 mM PMSF, 2 mM EDTA) for 16 hours at 20oC.

Results

These experiments have measured the content of insoluble elastin and soluble collagen. This experimental approach found that

the content of elastin was correlated to the quality of the fat fed to the animals. Feeding the mink fat of lower quality resulted in less insoluble elastin in the dried mink (table 1). The lower content of elastin found in animals fed poor fat quality might reflect a lower synthesis of this structural protein or, alternatively, a larger degradation of elastin. The measured values are slightly lower than previously measured values from other dried mink skins. The measurements of soluble collagen did not show any significant correlation between the quality of the fat and the content of the protein in the dried skins (table 1). The reason for this is unknown at present, but it is known that the content of soluble collagen is not a good and consistent indicator for insoluble collagen. However, testing more animals per test group may show a correlation.

Table 1. Combined results of elastin and collagen extractions from dried mink skins. For explanation of feeding groups (Group #) please see text

Feeding group	n	elastin % (w/w)	S.D.	collagen % (w/w)	S.D.
71	6	5.75	2.02	0.49	0.23
72	6	5.56	1.79	0.45	0.21
73	6	4.56	2.04	0.33	0.19
74	6	4.09	2.41	0.43	0.26

Conclusion

The conclusion is that feeding the animals fat of lower quality affects the content of proteins responsible for the elasticity of the skin in a negative direction. The results of fat quality versus the content of collagen are inconclusive, but it is possible to influence the protein composition of the skin by changing the fat given to the animals during the growth period. Preliminarily, it seems as if it is possible to change the streatchability of the mink skins by manipulating the feed should this trait become important to the

customers. Whether it is practically possible to obtain similar results in a normal farm situation with a larger variance in the composition of the feed still has to be tested.

Technical Year Report 1996, pp. 169-173. In DANH, Su. ENGL. 2 figs., 5 refs. Authors' summary.

Phase feeding (Protein reduction in the growth period)

Carsten Hejlesen, Tove N. Clausen

The growth period for mink (Mustela Vison) is naturally divided in two. In the first part protein deposition is primary while fat deposition dominates the last part. Because of the distinct different depositions in the two periods it is reasonable to believe that the protein requirement in the last part is smaller than in the first part of the growth period.

In 1995 an investigation was conducted where the protein ration was reduced from 30 % to 25 % of ME at different dates. Fat was added to replace protein. The energy distribution before the protein reduction was 30:53:17 (protein : fat : carbohydrate) and 25:58:17 after. The sulphur containing amino acids methionine and cystine are believed to be important in hair formation. In order to ensure that the requirement for these amino acids was met methionine and cystine were added to the protein reduced diet to meet the level in the 30:53:17-diet. In one experimental group though, there was no addition of methionine nor cystine.

There were six experimental groups in the colour type wildmink and six groups in the colour type standard. Each group represented a date for protein reduction, which was 15 Aug., 1. Sept., 15. Sept., 1. Oct. and 15. Oct. with addition of methionine and cystine and one group where protein was reduced 15. Sept. without addition of these amino acids. Each group consisted of 76

wildtype males and 54 males and 54 females of the standard type. With respect to all measurements i.e. weight gain from the beginning of July until pelting, skin length and fur quality parameters the results were very conflicting and inconclusive. Therefore, it was impossible to conclude whether or not it is possible to reduce the protein level in the middle of the growth period without negative effects on skin size or fur quality.

Technical Year Report 1996, pp. 79-84. In DANH. 4 tables. Authors' summary.

Incidence of greasy kits with various feed levels in the first month of the lactation period in mink

Carsten Hejlesen

Greasy kits is a disease syndrome in suckling mink kits most often occurring at the age of 2-4 weeks. The incidence of greasy kits varies from farm to farm.

In 1995 and 1996 studies were carried out on nine Danish mink farms with low, normal and high feed levels from 25 April to 25 May in order to investigate if there is a correlation between feed levels in the first month of lactation and the incidence of greasy kits. "Normal" feed level was defined as being the feed level which was normal on each individual farm, while "low" and "high" feed levels were approximately 10% lower and higher, respectively, than the farms' normal levels.

It was not possible in any of the studies to demonstrate a correlation between feed levels and incidence of greasy kits.

Technical Year Report 1996, pp. 227-228. In DANH. 1 table. Author's summary.

Taste preference studies with pressed cake from sprat in mink

Carsten Hejlesen, Niels Therkildsen

In 1995 a study was performed to investigate if an 8% addition to mink feed of pressed cake made from sprat affected the taste of mink feed. The study with 18 standard type male mink was done so that the mink had a choice between the feeds. Pressed cake is derived from whole fish from which most of the water and fat have been removed. Pressed cake therefore has a low fat content and a more well-defined and consistent composition than the corresponding untreated whole fish. Pressed cake could be a potential feed component in mink feed especially in the first half of the year.

The conclusion of the study was that an 8% addition of pressed cake did not affect the taste of mink feed.

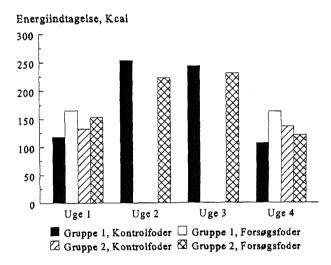


Fig. 1. Energy intake by the two groups of the two feed types.

Technical Year Report 1996, pp. 93-100. In DANH. 8 tables, 2 figs. Authors' summary.

Lactation feed preserved with acetic acid for mink - its effect on feed taste and kit growth

Carsten Hejlesen

Lactation feed for mink is traditionally composed of about 20% industrial fish which is preserved with 1% acetic acid. The full feed therefore contains about 0,20% acetic acid. In the beginning af May, when the ambient temperature increases, the full feed is additionally supplemented with up to 0,20% acetic acid in order to stabilize the feed.

In 1996 the taste of lactation feed containing 0,20%, 0,40% and 0,55% acetic acid, respectively, was investigated in 18 wild type mink females, each having 3 male and 3 female kits.

When the females could choose between feed containing 0,20% acetic acid and 0,55% acetic acid, 77,6% of their feed consumption was covered by the feed with the low level

of acetic acid (p<0,0001). If the females could choose between feed containg 0,20% acetic acid and 0,40% acetic acid, 63,7% of their feed consumption was covered by the feed with the low level of acetic acid (p<0,0002).

When the kits were 24 days old the females were split into two groups. One group was only given feed containing 0,20% acetic acid while the other group was only given feed containing 0,40% acetic acid. Registration of the females' weight loss and the kits' weight gain up to weaning (at 42 days of age) showed no significant differences (p>0,58 and p>0,23, respectively).

The conclusion of this study is that extra addition of acetic acid to feed which contains 0,20% acetic acid from industrial fish, worsens the taste of the feed for mink. The kits' taste for the feed was not investigated, but if there is a difference it did not result in different weight gains.

Technical Year Report 1996, pp. 85-91. In DANH. 8 tables. Author's summary.



You can be sure.
This feed will prevent
metabolic disturbances.

Original Report

Distribution of lymphocytes in blood and tissues from mink infected with Aleutian disease parvovirus (ADV) - a pilot study

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Summary

A study on the distribution of lymphocytes in mink infected with Aleutian mink disease parvovirus (ADV) was performed. It showed that in peripheral blood the B cells and CD3⁺CD8⁻ cells (Th cells) did not change considerably during infection, while CD3⁺CD8⁺ cell counts increased dramatically after 90 days post infection (DPI). This cell population very likely represents the Tc lymphocyte. The ratio between CD3⁺CD8⁻: CD3⁺CD8⁺ cells decreased from 2 to 0.3 on 210 DPI. The distribution of lymphocytes in lymphoid tissues such as lymph node, spleen and thymus was also detected by flow cytometry and immunostaining. Generally, the lymphoid organs appeared normal at the early stage of the infection. However, on 210 DPI, some changes were apparent. The most interesting finding was in the thymus, where both the CD3 CD8 (CD4?) cells (immature double

negative thymocytes?) and CD3^{low}CD8*(CD4*?) cells (immature double positive thymocytes?) decreased and the CD3^{logh}CD8*(CD4?) cells (mature Tc cells) increased. Immunohistological studies on thymus showed that the cortex disappeared.

Introduction

Aleutian disease (AD) was first found in Aleutian genotype mink. These mink arose due to a color mutation in 1941. They are generally known to be susceptible to infectious diseases because they suffer from the Chediak Higashi type syndrome of immunodeficiency (Leader et al., 1963; Padgett et al., 1967; 1968). Today the disease has been shown to affect all color phases of mink (Porter and Larsen, 1964).

The pathogen of the disease is Aleutian disease virus (ADV), a non-defective parvovirus (*Bloom et al.*, 1980). The ADV single stranded

genome contains two large open reading frames, one coding for a non-structural protein (NS-1) and one for the virus capsid proteins VP1/2 (Alexandersen et al., 1988). ADV infection causes both acute and chronic disease in mink. Neonatal mink kits infected with ADV develop an acute interstitial pneumonia with clinical symptoms and pathological lesions (Alexandersen and Bloom, 1987; Viuff et al., 1994). In adult mink, a persistent infection is maintained until death and a classical Aleutian disease can be seen.

The classical AD is a slowly progressing lymphoproliferative disease and is characterized by plasmacytosis (*Porter et al., 1980; Lodmell and Portis, 1981; Aasted, 1985 and Bloom et al., 1994*). The animal often suffers from fatal immune complex disease (arteritis and glomerulonephritis). In this study, the classical form of AD was considered.

In classical infection, there is a profound host immune response with very high antibody titers against ADV (*Aasted and Bloom*, 1983) as well as self antigen such as DNA (*Hahn and Hahn*, 1983), with resulting immune complex formation and deposition. The anti-ADV antibodies are not able to neutralize the virus and are therefore unable to prevent disease *in vivo* nor infectivity in *in vitro* systems (*Porter et al.*, 1977). It has been indicated *in vitro* that the apparent lack of neutralizing antibodies in Aleutian disease is due to masking of antigenic sites by phospholipids (*Stolze and Kaaden*, 1987).

The study of T-lymphocyte reactivity indicated that mink infected with ADV maintained a T-lymphocyte reactivity against both ADV antigen as well as other antigens or mitogens during the progress of disease. Only in the terminal stage of the disease were some of these functions compromised (*Race et al.*, 1983).

An extensive study on the measurement of Band CD8⁺ lymphocytes (Tc cells) of ADV-infected and uninfected mink in peripheral blood was performed by Aasted (1989). The most pronounced finding was that the frequency of CD8⁺ lymphocytes, on average, increased two-fold during the development of AD, while the frequency of B-lymphocytes did not change significantly. The enhanced CD8 frequency was still apparent 6 months after initial ADV infection of mink.

Obviously, ADV interferes with the immune system and AD has therefore been characterized as a disease of the immune system. However, little is known about the cellular mechanisms of the disorder. This is partly due to the lack of lymphoid cell markers. After years of generation and collection of mAbs against mink leukocytes, a more thorough detection system of mink lymphoid cells was established in our lab (Chen et al., 1997). In the present study, we measured B-, Tc and CD3⁺CD8 (Th?) lymphocytes of ADV infected and uninfected mink. The study was designed to demonstrate the influence of ADV on the immune system, from the peripheral blood to lymphoid tissues.

Material and methods

Mink

Fifty-one 1 year-old female black mink (non-Aleutian genotype) were used in the experiment. Thirty-four of them were experimentally infected with ADV (10³ ID₅₀ given i.p.). Seventeen mink were included in the uninfected control group which were inoculated with non-ADV mink tissue antigen. All mink were shown to be negative for antibodies to ADV at the start of the experiment as measured by counter current immunoelectrophoresis.

Virus

The type IV ADV isolate (Gottschalck et al., 1994) was used for experimental infection. Virus was partly purified from an organ homogenate by a combination of freon extraction and ultracentrifugation as described by Cho and Ingram (1974). The virus concentration was high enough to be used as an antigen in the counter current immunoelectrophoresis.

Antibodies

Monoclonal antibodies (mAbs) against mink leukocytes antigen were produced and collected in our lab (Chen et al., 1997). Mouse mAb No.50 was used as a marker to separate mink monocytes, granulocytes and lymphocytes in peripheral blood. Mouse mAb No.165 was a mink CD3 marker, which was used as a pan T marker in blood, lymph node and spleen. In thymus it was used as a marker to separate immature thymocytes and mature T cells. Mouse mAb No.T8 (ATCC, cat. 8014) is a human CD8 marker which cross reacts with mink CD8 (Aasted, 1989). In our experiment, it was used as a Tc cell marker. Rabbit polycloantibodies against mink IgA/M/G (Aasted, 1989) was used as a B cell marker.

Blood Samples and Organ Samples

Peripheral blood samples collected in EDTA-coated tubes on 0, 10, 20, 30, 60, 90,150 and 210 DPI were used for the flow cytometric study. For each reaction 50 μ l of blood were used. Lymph node, spleen and thymus samples from 30 and 210 DPI were analyzed by flow cytometry. Cells were suspended in PBSA with 10 mM EDTA and 1 x 10 6 cells were used per reaction .

Lymph node, spleen and thymus tissues were also fixed in formalin and embedded in paraffin for immunohistology studies.

Serum Electrophoresis

Gammaglobulin quantitation was carried out by agarose electrophoresis (LSA agarose, Litex, Copenhagen), using the Tris-barbital buffer system (*Svensen et al., 1983*). The amido-black staining procedure was used and the protein fractions were quantitated by densitometry (LKB 2202 ultrascan densitometer).

Counter Current Immunoelectrophoresis

The normal counter current immunoelectrophoresis was performed according to Cho and Ingram (1974).

NS-1 ELISA

NS-1 protein were kindly provided by Dr. Jesper Christensen in our lab (Christensen et al., 1995). Briefly, the plate was coated with 50 µl 10 mM NS-1 protein in coating buffer (50 mM carbonate buffer, pH 9.6, 30 mM NaCl) per well. After blocking with blocking buffer (1% BSA, 300 mM NaCl in PBS) and washing with washing buffer (PBS, 300 mM NaCl, 0.1% Tween 20, pH 7.2), dilution of primary serum in blocking buffer was added. The incubation was 1 hr at room temperature (RT). After washing, 50 µl of Biotinylated rabbit antimink IgA/M/G (1:2000 in blocking buffer) was added per well followed by incubation for 1 hr at RT. After washing, 50 µl peroxidase-strepavidin (1:2000 in blocking buffer) (DAKO, Denmark, cat. P0397, Denmark) was added per well. After 0.5 hr incubation, the plate was washed and 100 µl OPD (DAKO, Denmark, cat. S2000) solution was added. The reaction was carried out in the dark for about 15 min and then stopped by adding 100 μl 2 M H₂SO₄ per well.

Flow Cytometry

Peripheral blood leukocytes and lymph node, spleen and thymus cell suspensions were used.

For each reaction 50 µl of blood or 106 cells from the cell suspension were applied. First, the red blood cells were lysed for 6 min at RT with the lysing solution (0.16M NH₄Cl, 0.10M KHCO₃, 0.10 mM EDTA) and the cells were washed with PBSA. The cells were then incubated with 1 μ l purified mAb or 50 μ l culture supernatant for 1 hour on ice. After washing with washing buffer (PBSA with 1% mink serum), the cells were incubated for 1 hour with a FITC-conjugated F(ab'), rabbit antibody to mouse Ig (DAKO, Denmark, cat. F313). The cells were then washed and fixed in 1% formaldehyde in sheat fluid and analysed on a FACScan flow cytometer (Becton Dickinson).

For double labelling, the red cells were lysed first. Then the cells were washed with PBSA and incubated for 1 hour on ice in the dark with FITC-conjugated No.165 and Biotinylated No.T8. After another wash, the cells were incubated for 1 hour on ice in the dark with R-phycoerythrin-conjugated strepavidin (DAKO, Denmark, cat. R438).

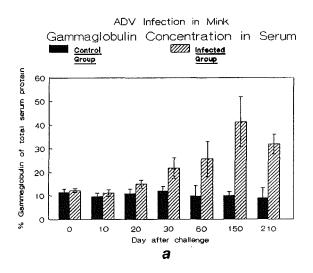
After a final wash, the cells were fixed and analysed on the FACScan flow cytometer.

Immunohistology

Formalin fixed (RT, overnight), paraffin embedded tissue sections were used for immunohistological studies. After deparaffinization and rehydration, the slides were transferred to 0.1M citrate buffer, pH 6.0 and boiled in a microwave oven for 15 min. The immunostaining protocol has been described as Alkaline Phosphatase Anti-Alkaline Phosphatase (APAAP) staining procedure (DAKO, Denmark).

Results

Serum gammaglobulin and anti-ADV NS-1 quantitation from ADV infected and uninfected mink. After challenge of 34 female black mink with ADV, a rise in serum gammaglobulin was observed with time as well as a rise of ADV specific Ab against the non-structural ADV protein NS-1 (Fig.1.).



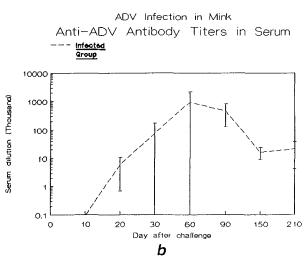


Fig. 1. Serum gammaglobulin and anti-ADV NS-1 quantitation from ADV infected and uninfected mink.

- (a) Gammaglobulin concentration was detected by Serum electrophoresis.
- (b) ADV specific Ab titers were detected by ADV protein NS-1 ELISA.

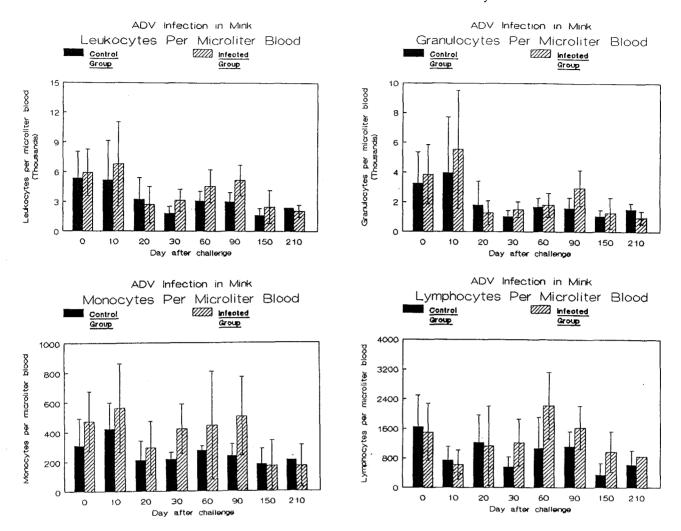


Fig. 2. Flow cytometric studies of peripheral blood leukocytes from ADV infected and uninfected mink.

Flow cytometric studies of peripheral blood leukocytes from ADV infected and uninfected mink.

Due to the large variation, no significant changes were observed in the number of leu-kocytes, granulocytes or monocytes per microliter of peripheral blood. A slight increase of lymphocyte number was observed in ADV infected mink after 30 DPI (Fig.2).

The total number of B cells did not change dramatically (although there was a slight increase around 30 DPI) (Fig.3.). The total num-

ber of T cells remained stable during the early stage of infection but increased after 60 DPI. The total number of CD3⁺CD8 (CD4⁺?) lymphocytes was found to be stable. However, the CD3⁺CD8⁺ lymphocytes(Tc cells) count increased dramatically. The ratio of CD3⁺CD8 : CD3⁺CD8⁺ cells decreased from 2 to 0.3 during the process of infection (Fig.4.). If the CD3⁺CD8 cells present the CD3⁺CD4⁺CD8 Th cells, this means the ratio of Th : Tc decreased dramatically in the peripheral blood of ADV infected mink.

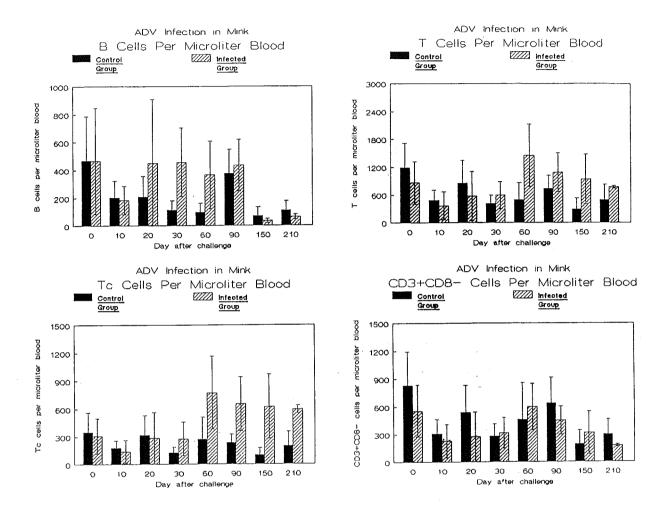


Fig. 3. Flow cytometric studies of peripheral blood lymphocytes from ADV infected and uninfected mink.

Flow cytometric studies of cells from lymphoid tissues from ADV infected and uninfected mink. No significant changes were observed in the early stage of infection. However, on 210 DPI, changes were apparent in the thymus. Both the CD3 CD8 and CD3 CD8 cells decreased dramatically. In the uninfected control mink (Fig.5a.), 36.76% cells were CD3 CD8 and 36.52% cells were CD3 CD8 and 36.52% cells were CD3 CD8 to the CD3 CD8 to

cell frequency for these two cell populations decreased 2-3 times (CD3 CD8: 16.82% and 17.52%; CD3^{low}CD8⁺: 12.28% and 21.16%). A marked difference was also seen in the CD3^{high}CD8⁺ cell population where uninfected mink had 9.92% of these cells while in the 210 DPI infected mink the percentage rose to 50.44% and 40.70%. No change was seen in the CD3^{high}CD8 cells between uninfected mink and 210 DPI infected mink. The ratio of

CD3^{high}CD8⁻: CD3^{high}CD8⁺ cells (Th :Tc) decreased from 1.8 to 0.4 in 210 DPI infected mink. We believe that the CD3 bow CD8 population very likely is the CD3^{low}CD4⁺CD8⁺ double positive thymocytes and that the CD3^{high}CD8 and CD3^{high}CD8⁺ population very likely is the CD3^{high}CD4⁺CD8⁻ and CD3^{high}CD4⁻ CD8⁺ single positive mature Th and Tc cells. At least a part of the CD3 CD8 cells should belong to the CD3 CD4 CD8 double negative thymocytes. If so, the immature thymocytes have decreased and mature Tc cells have increased in 210 DPI ADV infected mink. However, more data are required to confirm these findings. Similar studies have also been carried on lymph node and spleen but no apparent changes were observed between ADV infected and uninfected mink.

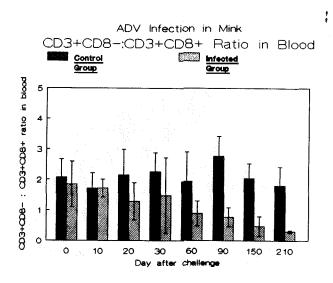


Fig. 4. Ratio of Th: Tc cells in peripheral blood from ADV infected and uninfected mink.

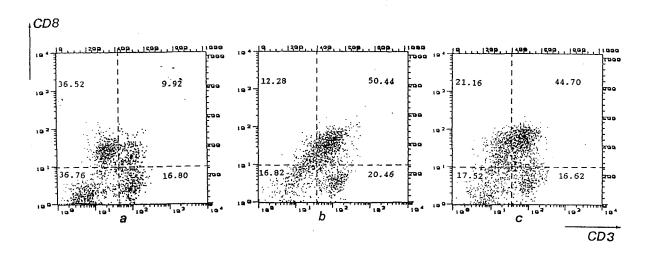


Fig. 5. Flow cytometric studies of cells from thymus from ADV infected and uninfected mink. (a) Thymocytes from non-infected control mink. (b) & (c) Thymocytes from Two 210 DPI ADV infected mink.

Immunohistological studies on thymus from ADV infected and uninfected mink.

In uninfected control mink (Fig.6a.), many CD3-positive cells could be seen in the medulla but only few in the cortex. In the 210

DPI ADV-infected mink (Fig.6b & 6c.), CD3-positive cells were scattered all over the thymus. It was difficult to recognize a distinction between cortex and medulla.

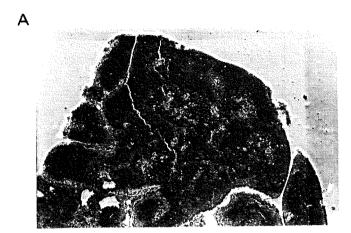




Fig. 6. Anti-CD3 Immunostaining on thymus (APAAP) from ADV infected and uninfected mink.

- (a) Thymus from non-infected control mink.
- (b) Thymus from 210 DPI ADV infected mink.

Discussion

In contrast to previous flow cytometric studies on blood from ADV infected mink (Aasted, 1989), we detected absolute numbers of leukocytes, granulocytes, monocytes, lymphocytes, T cells and B cells in peripheral blood. The number of CD3⁺CD8⁺ lymphocytes (Tc cells) increased dramatically. This correlated well with previous work of Aasted in

that the frequency of CD8⁺ lymphocytes doubled after ADV infection. At the same time, we also found that the ratio between CD3⁺CD8⁻: CD3⁺CD8⁺ cells (Th?: Tc) changed from 2 to 0.3 on 210 DPI. The number of CD3⁺CD8⁻ lymphocytes remained constant. Thus, it appears that in blood, instead of a decrease of the CD3⁺CD8⁻(CD4⁺?) cells (Th cells?), an increase of CD3⁺CD8⁺ lymphocytes (Tc cells) contributes more to the ratio change. The other interesting finding from this study was in the thymus. Thymus consists of two distinct regions, inner medulla and outer cortex. The cortex contains immature thymocytes and the medulla contains more mature T cells. During the development of thymocytes, the CD3 CD4 CD8 double negative and CD3^{low}CD4⁺CD8⁺ double positive immature thymocytes move from cortex into medulla and become either CD3high CD4 CD8+ CD3^{high}CD4⁺CD8⁻ single positive mature T cells (Janeway and Travers, 1996). In 210 DPI ADV infected mink, both the CD3 CD8 (CD4?) and the CD3^{low}CD8⁺(CD4⁺?) cells decreased dramatically and the CD3highCD8+(CD4?) cells increased (fig.5.). This correlated with the disappearance of the thymic cortex on the same mink on 210 DPI (Fig.6.). A possible explanation could be that ADV stimulates the CD3 CD4 CD8 (double negative thymocytes) and CD3^{low}CD4⁺CD8⁺ (double positive thymocytes) immature thymocytes into mature CD3^{high}CD8⁺ T cells (Tc cells). This is in accordance with ADV being present in thymus of naturally infected mink as detected by in situ hybridization (Haas et al., 1988). However, ADV replication was only detected in spleen and lymph node, no replicating ADV was found in thymus. Studies on the mechanism behind the stimulatory activity of ADV on thymocytes and on the specificities of mature Tc cells are of vital importance for understanding ADV pathogenesis. Such studies are ongoing.

Acknowledgments

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Original Report

Corynebacteria of group A: A frequent pathogen of breeding foxes in the perinatal period

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Summary

Studies were carried out on 14 different farms of breeding foxes located in three districts of Poland in which sterility of vixens, abortions and deaths of new-born animals were observed. Microbiological examinations showed that different pathogenic bacteria, such as Corynebacterium sp., Enterococcus faecalis 1, Enterococcus faecium 1, Escherichia coli, and Streptococcus equi subspecies equisimilis, were isolated from aborted fetuses, dead new-born foxes and from five vixens. The bacteria were identified on the basis of their morphological, cultured and biochemical characteristics using Coryne Api test (Bio-Merieux). It is concluded that, apart from other bacteria and viruses, under certain conditions Corynebacteria sp. may bring about severe infections in breeding foxes, especially during the perinatal period. These data indicate a change of the profile of bacteria pathogenic for breeding foxes; the microorganisms of Corynebacteria sp. should also be taken into consideration as potential causative agents of diseases on breeding farms.

Introduction

The clinical picture of diseases induced by infectious agents in foxes is highly differen-

tiated and depends not only on the species of the germ and its virulence, but also on the age of the animal, way of contracting the infection and environmental conditions. The large-scale introduction of prophylactic vaccinations against distemper and infectious inflammation of the brain and medulla has greatly diminished the occurrence of these prevalent infectious diseases in fox farms. However, at the same time, there emerged new and little known diseases, such as parvovirosis and dermatomycosis. For breeding foxes the perinatal period is particularly dangerous in which environmental-pathogenic agents frequently cause infections. In consequence of the high mortality of fetuses, still births or feeble pups, which usually die within several hours or days after birth, the economic losses in farms can be very high.

Our studies aimed at recording pathogenic factors which caused high losses during the prenatal period in the years 1994-1996 in breeding foxes in farms located in several Polish provinces which differed in respect to their size and sanitary-zoohygienic state.

Material and methods

Studies were carried out in 14 different farms of polar foxes in which abortions and

high mortality of young pups had been observed; the farms were located in the Lublin, Poznan and Zamosc provinces. The mortality index was from 10 to 50%, depending on the farm.

Aborted fetuses and young foxes that died soon after birth constituted the initial material.

Bacteriological and viral analyses were carried out using the samples taken from the brain tissue, lungs, heart, liver, spleen, and kidneys. Cultures were poured simultaneously on a blood agar medium and on Wrzosek medium. The isolated microorganisms were evaluated under the microscope, followed by cultural and biochemical assessment using API-tests by Bio-Merieux.

Viral examinations were carried out on cellline CC81 (cat's xenotropic cells), multiplied in Eagle's liquid with an addition of 8% calf antibiotics (penicillin and units.m/ml, streptomycin 100 µg/ml, amphotericin 2 µg/ml, tyrosine-tartrate 2/100 ml). Extracts of homogenised organs of fetuses or newly-born pups, previously filtered through 0.6 and 0.2 µm millipore filters, were added to a suspension of the cells. Cultures were incubated at 37°C for 24 hours and then washed and again Eagle's fluid was supplemented. Analyses of possible cytopathic changes were carried out for 4-6 days and, when there were no changes, two blind passages were performed after a prior freezing of the culture at -20°C.

Results and discussion

Results of the studies are presented in Table 1. As the Table indicates, in the years 1994-96 the total of 132 animals were examined, including 85 fox pups aged 1-5 weeks, 42 aborted fetuses, and 5 mature vixens. The evaluated material was obtained from over a dozen farms highly differentiated in respect to the size of the basic peak of foxes and zoo-hygienic state.

The isolated germs were identified and their virulence examined on the basis of microbiological studies, by taking into consideration the evaluation of sections. The isolated pathogens were classified as: Corynebacterium of group A, Enterococcus faecalis 1, Enterococcus faecium 1, Escherichia coli, and Streptococcus equi subspecies equisimilis. The germs obtained from fetuses or newly-born fox pups from the same litter and having the same morphological, cultural and biochemical properties, were treated as strains belonging to the same species or genus. With the exception of *E. coli*, all the isolated microorganisms demonstrated pathogenicity for laboratory animals, which seemed to indicate their pathogenicity for fetuses and newly born farm animals as well. Depending on the species of isolated bacteria, the examined animals showed somewhat different pictures on autopsy. The bacteria classified as Corynebacteria were isolated from both aborted fetuses and newly born pups as well as from mature animals. Autopsy usually revealed degeneration of liver and swollen spleen.

Apart from congestion and oedema of liver and spleen, in fox pups infected with *E. faecalis* 1 there also sometimes occurred congestion of the brain and lungs.

Enterococcus faecium 1 was isolated from both aborted fetuses and dead fox pups. Dissection of the abdomen revealed extensive changes in the form of exudation and inflammatory changes of the gall-bladder. The liver was degenerated and often bilious, while the spleen was swollen. E. coli bacilli were isolated only from fox pups several weeks old and from one mature vixen. An autopsy examination revealed congestion and softening of the mucus of the alimentary canal, extravasation, liver degeneration and exudation in the abdominal cavity.

The studies mentioned above indicated that in the period 1994-96 the bacterial species,

Table 1. Results of the autopsy of bred foxes

Etiological factor	Year	Farm	Kind of	No. of	Autopsy	Biological assay and typica
		No.	material	animals	changes	anatomical-pathological changes
				examined		patriological chariges
	1994	7	pups	20		
		8	pups	5		
	İ	10	fetuses	6	_	
					Spleen:	White mice: death within 24-48 h
Corynebacterium sp.	1995	1	foxes	2	swelling and congestion	1011
		5	fetuses	6		Spleen: swelling, congestion
		11	fetuses	2	Liver: congested, fragile	T strained, configuration
		12	pups	3		Heart: congestion
	1996	2	fetuses	2		
		3	pups	3		
	1994	1	fetuses	4		
		2	fetuses	5		
		5	vixen	1		White mice and guinea pigs:
					Liver:	death within 24 h
Enterococcus faecalis 1	1995	2	pups	8	congested and swollen	
(beta-hemolytic		3	pups	5		Spleen congested and swollen
strains)		6	fetuses	6	Spleen:	
		10	pups	4	congested and swollen,	
					brain congestion (frequent)	
	1996	1	pups	2		
		4	pusp	4		
Enterococcus faecium 1	1994	4	fetuses	2		
		6	pups	4		
		.12	fetuses	5	Gall bladder: swollen	White mice and guinea pigs: death within 24 h
		8	pups	4	Liver: fragile; exudation in	death within 24 it
	1995	13	pups	4	abdominal cavity	Spleen: swelling, strong
						congestion
Escherichia coli	1994	13	pups	2		
		14	pups	2	Liver: fragile	Strains non-pathogenic for white mice
	1995	7	fox	1	Small intestine:	
		9	pups	2	extravasation in mucus	
		14	pups	2		
	İ				Abdominal cavity:	
					exudation	
	1994	3	fetuses	4		
		9	fox	1 ·	Spleen: congestion and	White mice: death within 24 h
Streptococcus equi		11	pups	3	swelling, sometimes	Spleen: strong swelling and
subspecies equisimilis			- *		exudation in abdominal	congestion
	1995	4	pups	8	cavity and brain congestion	

such as Salmonella choleraesuis (10), E. coli, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus pyogenes or Streptococcus viridans (9, 13), previously prevailing in fox farms in Poland, were no longer predominant. Nor were there observed cases of germs representing Proteus sp., Klebsiella sp.

(11), Listeria monocytogens (15) or Psedomonas aeruginosa (6, 7) discovered in the organs of dead pups from the 14 farms. Other European authors also reported infections in foxes caused relatively frequently by Pasteurella multocida, Listeria monocytogens, Brucella sp., Francisella tularensis, hemolytic

streptococci, Klebsiella sp. and Escherichia coli (1, 4, 8, 11, 12) as causes of diseases of foxes. The list of the germs mentioned above, which are pathogenic for foxes in the perinatal period, is supplemented by Nordstoga (11) with such pathogens as Campylobacter sp. and Mycobacterium sp. According to our studies this list should also include germs from the genus Corynebacterium which were isolated 9 times in three successive years from fox fetuses or dead newly born pups. Similarly to all the others, these germs were classified on the basis of their morphological,, cultural and biochemical properties. They were distinguished by a club shape, size of 1 x 3 µm, arranged singly or in pairs or else they formed typical palisades. Gram staining was unevenly distributed: the dye concentrated in the club-shaped, broadened poles. Colonies on blood solid medium had a diameter of 1.5 to 2.0 mm, and they were of cream colour or light yellow. The biochemical tests after Api-Coryne brought the following results: nitrate reduction +; pyrazinamidase -; pyrrolidonyl arylamidase +; alkaline phosphatase +; beta-glucuronidase; beta-galactosidase +/-; alpha-glucosidase -; n-acetyl-p glucosaminidase -; esculin +; urease +; gelatine hydrolysis +; glucose fermentation +; ribose fermentaion +; xylose fermentation +; mannitol fermentation +; maltose fermentation +; lactose fermentation +; saccharose fermentation +; glycogen fermentation -; catalase test +.

It should be emphasised that germs belonging to Corynebacterium constitute a group of heterogeneous microorganisms and therefore there have been several attempts at dividing them into some groups (2). In respect to their morphological, cultural and biochemical properties, the strains isolated by us corresponded to group A of Corynebacterium, (3) with the exception of different properties shown in respect to alpha-glucosidase and urease. These data confirm the opinion of other authors concerning the heterogeneous nature of germs classified as Corynebacterium sp. These are the bacteria which were isolated as primary pathogens

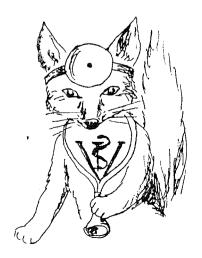
causing diseases of the upper respiratory tract in humans. As secondary germs, they were isolated from people with diphtheria, cases of paralysis, and from the mucous membranes of eye and ear (14). It is also supposed that, for instance, Corynebacterium ovis and Corynebacterium equi are the microorganisms largely adapted to a parasitic mode of life (2). It may be assumed that the isolated pathogens from the Corynebacterium group are precisely the kind of germs which, having penetrated the organisms of a pregnant vixen by infected feed aerogenously, found there favourable conditions for their own growth and then caused the death of fetuses or newly-born pups. This suggestion seems justified in so far as these diseases were observed only in small farms, with a low standard of hygiene, in which animals were fed on feeds acquired from various accidental sources of supply (16), which would confirm our suppositions concerning the alimentary way of infecting foxes. The negative results of viral studies, with special attention paid to parvo-viruses, would point to the isolated bacteria as the main causes of abortions or mortality of newly-born fox pups. Although one cannot exclude viral etiology, especially parvo-viral etiology, as an immunosuppressive factor favouring the multiplication of bacteria in the perinatal period, even of slightly pathogenic character, still their absence in the organs of fetuses or newly-born fox pups would rather indicate the isolated microorganisms as causes of their deaths.

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Original Report

Streptococcal infection of foxes during the perinatal period: Specific immunoprophylaxis

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Abstract

Studies were carried out on farms of breeding foxes located in the Lublin and Poznan provinces in the years 1994-96. Many late abortions, stillbirths or births of feeble, sick pups were noted. From the internal organs of dead foxes were isolated bacteria which, on the basis of morphology, cultural and biochemical properties, were classified as *Streptococcus equi subsp. Equisimilis*.

The vaccine prepared from the isolated strain of *Strep. Equisimilis* proved to be effective: the vaccine elicited a good immune response in the form of specific antibodies evaluated by a gel-precipitation test and a challenge trial. The vaccinated foxes gave birth to normal pups while in the control group (non-vaccinated animals) abortions were observed as before.

Introduction

Pyogenic streptococi producing hemolysins alpha or beta have been classified by Lancefield into groups A, B, C, D, E, G, P, R, S, U

or V (*Bergey*, 1994). Streptococci of group A are characterised by high pathogenicity mainly for man, while the others, especially those of group C, are mainly pathogenic for animals.

In humans pyogenic streptococci were isolated from the respiratory system, infected wounds, from cases of endocarditis, meningitis and from the urinary tract (Stamm and Cobbs, 1980). In animals these germs were isolated from: (a) horses with signs of catarrh of the respiratory tracts, abscesses of submundibular glands (distemper), inflammation of the uterus, abortions and sterility (Stableforth, 1959); (b) cattle with symptoms of septicaemia, pathologically changed udder gland (Bergey, 1986); (c) sheep with cases of joint inflammation (Bergey, 1986); (d) sick foxes with cases of septicaemia (Barrat, 1985); (e) the urinary system in dogs (Joubert, 1985). According to Bergey (1994), the pyogenic Streptococci of group C also include Streptococcus equi subspecies equisimilis, which is pathogenic for both humans and animals. In respect to animals the pathogen was isolated from cases of abortions in mares and pigs and uterus inflammation in cattle. Our own studies carried out in 1994/96 (Kostro, 1996) showed that both abortions in vixens as well as stillbirths or births of feeble pups may also be caused by Streptococcus equi subspecies equisimilis.

The present report has concentrated on the occurrence of disturbances in the reproduction of breeding foxes caused by *Streptococcus equi subsepecie sequisimilis* as well as a possibility of a prophylactic application of a vaccine.

Materials and methods

The studies covered polar fox farms localised in the Lublin and Poznan provinces; the foxes manifested the occurrence of fairly numerous late abortions (7-10 days before the regular date of birth) as well as stillbirths and births of feeble pups which died soon after birth (within 1-5 days). Depending on the farm, the disease affected about 10-50% of the fox herd. An inquiry indicated that the animals had been fed mainly raw meat from carcasses of farm animals (horses, cattle) that died for undetermined reasons and also raw poultry offal. In the period preparatory for reproduction (between November and December) foxes were prophylactically vaccinated against distemper and dermatomycosis. The sanitary and zootechnological conditions in the farms raised no objections.

The starting material for the studies were aborted fetuses, dead pups and, in one case, a vixen that died on the third week after abortion.

Microbiological examinations

The pathologically changed internal organs of dead fox pups served for the preparation of a 10% suspension in 0.85% NaCl. The suspension was then poured on a blood agar medium and on the Wrzosek medium. The cultures were incubated at 38°C for 24-48 hours. The morphological properties of the

microorganisms were evaluated after Gram staining. The cultural properties, i.e., the size of the colonies, extent of multiplication on liquid and solid media, and hemolytic properties on an agar medium with a 5% addition of sheep blood, were determined after 24-48 h incubation at 37°C. The biochemical properties of the isolated germs were assessed by employing kits API 20 Strep (BioMerieux, SA).

The sensitivity of the isolated strains to antibiotics was tested by the disc method on Muller-Hinton medium employing the kit produced by Bio-Merieux and Bio-Med. The pathogenicity of the strain was evaluated on white mice weighing about 18-20 g and on guinea pigs weighing 350-400 g which were injected intraperitoneally with 0.2 ml (mice) and 0.5 ml (guinea pigs), respectively, of a 24-hour broth culture.

Viral examinations were carried out on a single-layer culture of cat xenotropic cells (CC81). Homogenised material with antibiotics (crystalline penicillin 1000 units/ml, streptomycin 1000 mcg/ml) was filtered through filters Millipore 0.6 μ m and 0.2 μ m and used to inoculate cell cultures. The cultures were checked daily for five days, performing 3 blank passages. Blood samples were taken from vixens, which were aborting or giving birth to dead fetuses, in the fourth week in order to carry out serological examinations testing the presence of parvoviral antibodies in their sera.

Vaccine. The vaccine was made of a 24-hour culture on an isolated strain of Streptococcus equi subspecies equisimilis, multiplied on a soya-bean medium. A bacterial suspension of a density of about 2 x 10° cells per 1 ml (by McFarland standard) was inactivated with formalin (at a final concentration of 0.2%) for 24 hours at 37°C. This prepared vaccine was tested for sterility by inoculations on a blood agar medium and on the Wrzosek medium as well as for its harmlessness for guinea pigs and white mice. The preparation was administered to five mature primipa-

rous vixens twice intramuscularly at a dose of 1 ml at the interval of two weeks.

The remaining, non-vaccinated, vixens constituted the control group. Blood samples for serological assessment were taken from the saphenous vein three and six weeks after the administration of the vaccine.

Antibody level determination. The titres of antibodies in the sera of immunised foxes were determined by the precipitation reaction in a modified agar gel with an addition of PEG 6000 (Wawrzkiewicz et al., 1989). The antigen extract for the precipitation reaction was prepared by the Lancefield method (Pakula, 1958) and concentrated 30 times by means of filter Centriprep (Amicon Inc.). Results were read after a period of 48-hour of incubation at 37°C and after further 24 hours of storing at 4°C. Peripheral wells, 10 mm in diameter, were filed with the examined sera, while the central well with the antigen. The distance between the central well and the peripheral well was 5 mm.

The challenge test. The test was performed eight weeks after the beginning of immunisation, by a subcutaneous administration of 1 ml of a mixed culture of strains *Streptococcus equi subspecies equisimilis* isolated from various farms, at a density of 2 x 10° cells per 1 ml. The strains were pathogenic for white mice and guinea pigs, vaccinated intraperitoneally; the animals died within 24-48 hours.

Results and discussion

The inquiry indicated that, as a rule, no deaths of mature foxes were noted in the farms, although there occurred numerous cases of sterility in vixens (in spite of the presence of estrus and correct coition), as well as a high mortality index of newly born animals. The anatomo-pathological examinations of dead pups revealed swelling and

congestion of the spleen, degenerative changes of the liver and brain congestion.

Characterisation of the isolated germs

After 24 hours of incubation at 37°C the cultures of pathologically changed spleens of foxes and spleens of mice and guinea pigs (artificially infected with the examined material), the presence of small colonies, about 1 mm in diameter, with a clear zone of hemolysis of the beta type, was observed.

The preparations made showed Gram-positive cocci, about 1 μm in diameter, arranged in short or somewhat longer chains.

Biochemical properties, determined by the Api 20 Strep. test, proved that the isolated strains did not produce acetoin, did not hydrolyse sodium hippurate, did not produce β-glucosidase, pyrrolidonylarylamidase, αgalactosidase, β-galactosidase, nor arginine dihydrolase, and they produced β-glucuronidase, alkaline phosphatase, and leucine arylamidase. All the strains acidified ribose, lactose, threhalose, starch and glycogen; they failed to decompose 1-arabinose, mannitol, sorbitol, inulin, raffinose. The examined strains were characterised by the formation of a strongly demarcated zone of βhaemolysis on an agar medium with sheep blood.

The enumerated properties are regarded as characteristic of *Streptococcus equi subspecies equisimilis*. Biological tests performed on white mice and guinea pigs, infected with the isolated strians, proved their pathogenicity. The animals died within 24-48 hours after injection. Examinations of antibiotic-resistance in vitro showed that the examined strains were sensitive only to Baytryl (+++), augmentin (+++) and gentamicin (+++).

Viral tests brought negative results. No parvoviral antibodies were found in the sera of the examined vixens.

Post-vaccination immunity of foxes

All the sera samples taken from immunised foxes showed the presence of antibodies in titres of 80-160. The above concentrations of antibodies were maintained on the same level after both 3 and 6 weeks. The immunised animals were insensitive to artificial infection with the isolated bacteria (the challenge test). The positive titres in the group of 15 experimental foxes after the application of the inactivated vaccine and their insensitivity to infection in the challenge test proved the effectiveness of prophylactic vaccination. In the next production cycle the vaccinated group of vixen gave birth to healthy pups on the correct date and with numerous litters. On the other hand, in the control group there continued to occur cases of abortion or bearing feeble pups from which Streptococcus equi subspecies equisimilis was also isolated. These preliminary studies concerning the immunoprophylaxis against infections caused by Streptococcus equi subspecies equisimilis still require confirmation by tests carried out on a larger group of animals. According to the literature, hemolytic streptococci of group C constitute a frequent cause of diseases in carnivorous fur animals which often end with high mortality. These microorganisms are encountered in the breeding environment of the animals and, in a dried state, they remain alive for a long period of time. The source of streptococcal infections is often the meat of infected killed or dead animals. The disease may also be caused after feeding animals with milk from cows with signs of streptococcal mastitis. According to Steffenowa (1971), postslaughter meat and offal constitute a good medium for the multiplication of these microorganisms. Storing this kind of feed for foxes even for a period of a few hours in summer causes their quick multiplication which in effect leads to the infection of the animals after nourishment with it. It is assumed that silver foxes are more susceptible to streptococcosis, than polar foxes. Still, the studies showed that polar foxes are also characterised by high

susceptibility to streptococcal infections, especially during the perinatal period. The above results are in good agreement with the data obtained in Poland by Smielewska-Los and Lkimentowski (1996). It seems that active immunisation of breeding foxes by means of a inactivated vaccine in a farm contaminated with Streptococcus equi subspecies equisimilis is worthwhile because it considerably lowers the losses in the perinatal period of the foxes. This kind of prevention restricts to a minimum the possible necessity of applying antibiotics in cases of the occurrence of infections of this kind of etiology in fox farms.

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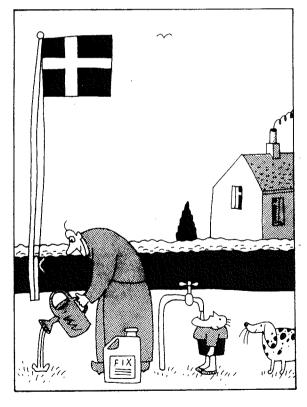
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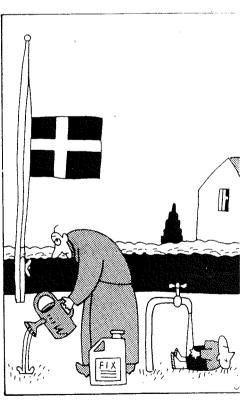
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Short Communication

Susceptibility of microorganisms recovered from dead mink kits (Mustela vison) to fourteen antimicrobial agents

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Summary

This investigation was undertaken to determine the antimicrobial susceptibility of 97 aerobic bacterial isolations (staphylococci, Proteus spp, coliforms, E. coli and Pseudomonas aeruginosa among others) and 5 fungal isolations from dead mink kits (n=102) against fourteen common antimocrobials. The total number of antimicrobial tests were 1079. Bacterial isolates had the greatest susceptibility to neomycin (80%), chloramphenicol (68%) and polymicin (65%). The rest of antibiotics showed intermediate or low efficacy. Natamycin was the most effective antifungal (80%).

Introduction

Postnatal mortality between birth and four weeks of age is a very important factor in the assessment of mink (*Mustela vison*) productivity. Surveys have shown 10 to 30% mortality in neonatal kits, with the highest mortality in the first week of life (*Einarsson*, 1980; *Martino & Villar*, 1990).

Mink are specially exposed to bacterial infections as their feed is to a great extent offal

from slaughter poultry and fish by-products. As slaughterhouse offal from mammals and poultry is mostly used in its raw state, a number of infections from other domestic animals may be contracted through the feed. A number of microbes, frequently transmitted from feed or drinking water, may give sepsis or localized inflammatory changes resulting in single or multiple deaths. These include staphylococci, hemolytic streptococci, E. coli and Pseudomonas aeruginosa among others (Nordstoga, 1992). Moreover, Argentinean farms tested have an unadvisable viable bacteria count per gram feed ranging from 106 to 107 (Martino, Marino & Villar, 1991).

In this survey young kits that had died between birth and 4 weeks of age due to septicemia or starvation were randomly collected from two commercial mink ranches. Primary cultures for aerobic bacteria and fungal culturing were made from the liver, brain and heart (Martino & Martino, 1995).

Antimicrobial susceptibility studies on the strains isolated were performed using disk-fusion methods (*Performance standard of antimicrobic disc susceptibility tests*, 1983). The testing was done on 97 aerobic bacterial iso-

Table. Antimicrobial susceptibility for the microorganisms isolated

Organisms	Total strains	PEN	AMP	OXA	AML	CAR	NEO	TET	CHL	POL	FUR	T/S
Bacteria												
E. coli Enterobact.	20	0	2	0	3	12	15	8	17	18	13	8
cloacae Alcaligen.	. 11	0	0	0	2	2	8	9	11	9	7	9
faecalis Proteus	5	0	1	0	1	0	5	4	2	5	2	1
vulgaris Proteus mor-	16	0	0	0	12	0	16	3	9	14	6	2
gagni Pseudomonas	12	0	1	0	8	0	12	4	6	12	5	2
aeruginosa Staphyl.	5	0	0	0	1	ND	1	0	0	5	1	3
aureus	14	7	14	14	12	8	13	10	12	3	10	2
Bacillus spp.	6	4	3	1	2	0	1	6	5	ND	ND	6
Citrobacter	4	1	0	0	1	1	3	2	2	4	3	1
Acinetobact.	4	0	0	0	1	0	4	3	2	4	2	2
Total (%)	97	12 12	21 22	15 15	43 44	23 24	78 80	35 36	66 68	63 65	49 51	36 37
Fungi		NAT	NYS	GRI								
Unidentified fungi	1	0	1	0				D I		EN.		
Mucor	2	2	1	1								
Alternaria	2	2	0	0						TH		
Total (%)	5	4 80	2 40	1 20				AASN	= ~/"	स ण		

PEN: penicillin, AMP: ampicillin, OXA: oxacillin, AML: amoxycillin, CAR: carbenicillin, NEO: neomycin, TET: tetracycline, CHL: chloramphenicol, POL: polymicin, FUR: furazolidone, T/S: trimethoprim/sulfamethoxazole, NAT: natamycin, NYS: nystatin, GRI: griseofulvin, ND: not determined.

lates for the following antimicrobials: penicillin, ampicillin, oxacillin, amoxycillin, carbenicillin, neomycin, tetracycline, chloramphenicol, polymicin, furazolidone and

trimethroprim-sulfamethoxazole. Diffusion methods were also used for testing antifungal agents on each of five fungal isolates. Commercial manufactured tablets (Neosensitabs, A/S Rosco, Denmark) that contained 50 g of natamycin, 50 g of nystatin and 25 g of griseofulvin were applied to buffered YMA agar (Yeast Morphology Agar, Difco, Detroit) that was inoculated with a single isolate and incubated at 30°C for 24 to 48 hours. Susceptibilities were determined by measuring zones of inhibition and applying interpretative criteria (*Casals*, 1979).

The Table shows the antimicrobial sensitivity of the microorganisms and fungi isolated (n=102) against the fourteen common antimicrobials. The total number of antimicrobial tests were 1079.

Mink breeders commonly treat animals with common broad spectrum antibiotics, penicillin or sulpha drugs, at the time of mating and whelping to guard against general infection. The procedure is expensive and dangerous. Knowledge of the most common bacteria isolated at given sites and their usual susceptibility to antimicrobials is central to rational therapy. The predominance of gram-negative with occasional gram-positive bacteria recovered from the sites investigated suggests that the use of neomycin, polymicin and a

broad spectrum antimicrobial such as chloramphenical would be advisable.

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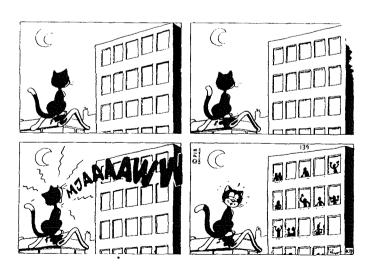
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On antiinfectional properties of cerumen in mammals

V.E. Sokolov, N.A. Ushakova, O.F. Chernova, A.V. Shubkina, L.M. Alimbarova, I.F. Barinskii

The cerumen (ear wax) of some mammals possesses antistaphylococcal, antimicrococcal and antiherpes activities. The cerumen of two thirds of individuals, irrespective of their species and sex, has antiviral properties. The mean chemotherapeutical index in the studied groups follows a significantly decreasing sequence: dogs, humans without signs of herpes infections, rabbits, and humans with clinically expressed herpes infection.

Cerumen of almost 25% of humans of the compared groups displays the immunostimulating activity. The cerumen of all studied individuals contains yeast-like fungi. A suggestion is put forward that the products of their metabolism stimulate local release of interferon-like substances by the lymphoid tissue in the cerumen.

Izvestiia Akademii Nauk SSSR, Serilia Biologicheskaia 5, pp. 579-585, 1995. In RUSS, Su. ENGL. 2 tables, 3 figs., 26 refs. Authors' summary.

Structure of the meatus acusticus externus skin and antiviral activity of the cerumen in carnivorous mammals (Mammalia, carnivora: Martes zibellina, Mustela vison, M. putorius)

V.E. Sokolov, N.A. Ushakova, O.F. Chernova, L.M. Alimbarova, I.F. Barinskii

The structure of the meatus acusticus externus skin was studied in the sable, mink, and European polecat with a parallel estimation of antiherpetic activity of the cerumen.

Morphologically, these species differ in the degree of fat cellulose development, vascularization, and association with the lymphoid tissue. Lymphocytes are present both in the tissue and along the skin gland ducts and hair follicles up to the external surface only in the sable. The cerumen samples have antiviral activity also in the sable alone. The relationship between physiological properties of the mammalian cerumen and morphogenetic features is discussed.

Izvestiia Akademii Nauk SSSR, Serilia Biologicheskaia 4, pp. 430-436, 1996. In RUSS, Su. ENGL. 2 tables, 5 photos, 7 refs. Authors' summary.

Determination of circulating immune complex in mink serum by measuring turbidity created by polyethylene glycol

Ji Yulin, Qu Weijiang, Zhao Yuankai

In this study, it was found that polyethylene glycol (PEG) precipitation turbidimetry was a reasonable method for detecting circulating immune complexes (CIC) in mink sera. It was carried out by mixing serum and 5% PEG (prepared with pH 8.4, 0.1 M borate buffer solution) for 1 h at 4°C and 0.5 h at 20°C.

Sera samples of 200 clinical healthy AD,CIEP negative mink and 600 positive mink with Aleutian disease were tested with this method. The OD value (X±SD) of healthy mink was 0.034±0.023 and that of AD mink was 0.241±0.152.

There was significant difference (p <0.01) between the OD value of healthy mink and that of AD mink by statistical comparison. The results suggest that the CIC are closely correlated to pathogenesis of AD.

PEG precipitation turbidimetry is rapid, simple, does not need special equipment and has good repetition. The development of this assay method is significant to research in pathogenesis and immunity of AD in mink.

Special Wild Economic Animal and Plant Research (China), No. 2, pp. 1-4, 1994. In CHIN, Su. + subtitles in ENGL. 6 tables, 5 refs. Authors' summary.

Immunization with an attenuated mink enteritis virus vaccine modified in calf testis cells

Quanfu Tao, Aiyu Dong, Jingang Zhang, Guojun Zhang, Zhen Yin, Kui Hu, Zhenfang Wu, Zhiwei Guo

A virulent mink enteritis virus (MV4) was isolated from naturally infected dead mink. When adapted to a co-culture of CRFK (a kind of feline kidney cell line) and calf testis (CT) cell, the cytopathic effect (CPE) did not appear in the CT cell until the 56th passage. The 57th-70th consecutive passages were made in CT cells only. An attenuated strain was obtained through end-point dilution. The vaccine was produced in CT cells. The protection rate was 100% when inoculated mink challenged with virulent strain of MEV on day 12, 60, 72, and 180 after inoculation, while the morbidity and mortality of controls were 100% and 40%, respectively. 58,940 mink were vaccinated by injection or oral administration in Liaoning, Hebei, Inner Monglia, and no side-effects or clinical signs appeared except in mink on two farms inoculated with CRFK-produced vaccine, which might have been contaminated with a virulent strain of MEV.

Chinese Journal of Veterinary Science 15, 2, pp. 130-134, 1995. In CHIN, Su. ENGL. 4 tables, 5 refs. Authors' summary.

Studies on the growth and decline of Aleutian disease antibody levels

Ji Yulin

The growth and decline law of AD antibody in mink was revealed by using AD-CIEP methods to examine the mink herd under normal breeding conditions from July to December annually for three years. The optimum time of general survey of AD mink once a year with AD CIEP was found to be suitable for the situation in China. And is was first found that there existed a phenomenon of long-term negative reaction to ADV antigen in a large number of mink with positive AD antibody, thus this perhaps contributed a scientific basis to immunize and prevent Aleutian disease.

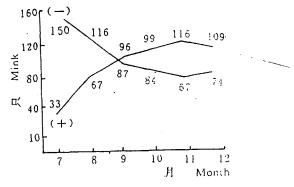


Fig. 2. Rule curve of AD antibody in 183 growth mink.

Acta Veterinaria et Zootechnica Sinica 26, 1, pp. 42-46, 1995. In CHIN, Su. + subtitles in ENGL. 2 tables, 2 figs., 8 refs. Author's summary.

Influence of circulating immune complex and antibody level in mink with Aleutian disease on litter size and surviving numbers of offspring

Ji Yulin, Qu Weijian, Zhao Yuankai, Xiaop Jiamei, Lu Yueying, Zheng Jun

The influence of circulating immune complex (CIC) and antibody level (Ab) in mink

with Aleutian disease (AD) on litter size and surviving numbers of offspring and the relationship between CIC and Ab was studied simultaneously in the female breeder mink before breeding and after weaning. The results of the experiment indicated that the female breeder mink with negative antibody reactions had the largest litter size and the best kits survival. However, when their antibody reaction converted from negative to positive, the litter size and surviving numbers of offspring were lower than those who revealed negative conversion. The relationship between litter size and surviving numbers of offspring and the antibody level was preliminarily established as follows: 6.05 kits for Ab (--), >5.40 kits for negative-converted, >4.33 kits for positive-converted, >3.11 kits for Ab (+). This study showed that female breeder mink with lower CIC • OD values had larger litter size and surviving numbers of offspring. The relationship between CIC • OD values and the litter size and surviving numbers of offspring based on the preliminary results was that female breeder mink with CIC • OD values ≤ 0.07 had the largest litter size and surviving numbers of 6.05 offspring and in those with CIC • OD value > 0.1 had 4.3 offspring. All of the female breeder mink with much higher CIC • OD values (such as 0.56, 0.65 etc.) had smaller litter size and surviving numbers of offspring or even failed to give birth. The results demonstrated that female breeder mink with lower CIC • OD values (<0.07) often showed lower antibody levels and AD (--) by CIEP, however, there was no direct correlation between CIC • OD values and antibody level. The results further indicated that female breeder mink with higher CIC • OD values and antibody level, or with lower antibody level but with higher CIC • OD values had much smaller litter size and surviving numbers of offspring or were even barren. On the contrary, female breeder mink with lower CIC • OD values and antibody level, or with higher antibody level but with lower CIC • OD values had larger litter

size and surviving numbers of offspring. Therefore, it is concluded that the content of CIC in female breeder mink might be a main factor directly affecting the litter size and surviving numbers of offspring, while antibody level may be not the only factor.

Chinese Journal of Veterinary Science 15 (3), pp. 224-227, 1995. In CHIN, Su. ENGL. 4 tables, 3 refs. Authors' summary.

Investigation of the pathogenesis of transplacental transmission of Aleutian mink disease parvovirus in experimentally infected mink

Susanne Broll, Søren Alexandersen

The transplacental transmission of Aleutian mink disease parvovirus (ADV) was studied in experimental infection of 1-year-old female non-Aleutian mink. The ADV-seronegative female mink were inoculated with ADV prior to mating or after the expected implantation of the embryos during pregnancy. A group of uninfected females served as a control group. Animals from each group were killed prior to or shortly after parturition. The in situ hybridization technique with radiolabeled strand-specific RNA probes was used to determine target cells of virus infection and virus replication. In both infected groups, ADV crossed the endotheliochorial placental barrier, although animals infected before mating already had high antibody titers against ADV at the time of implantation. The percentage of dead and resorbed fetuses was much higher in dams infected before mating. In the placentae of these mink, virus DNA and viral mRNA were detected in cells in the mesenchymal stroma of the placental labyrinth and hematoma but only occasionally in the cytotrophoblast of the placental hematoma. Placentae of animals infected during pregnancy showed in addition very high levels of virus and also viral replication in a large number of cytotrophoblast cells in the placental hematoma, which exhibited distinct inclusion

bodies. In both groups, neither virus nor virus replication could be detected in maternal endothelial cells or fetal syncytiotrophoblast of the placental labyrinth. Fetuses were positive for virus and viral replication at high levels in a wide range of tissues. Possible routes of transplacental transmission of ADV and the role of trophoblast cells as targets for viral replication are discussed.

Journal of Virology, pp. 1455-1466, 1996. 5 tables, 5 figs., 42 refs. Authors' summary.

Studies on *in vitro* neutralization of mink Aleutian disease parvovirus

Wu Wei, Nie Jinzhen, M.E. Bloom

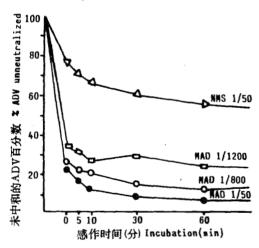


Fig. 1. Kinetics of neutralization of ADV-G.

This paper first report that ADV-G treated with ether can be neutralized by anti-ADV antibodies *in vitro*. The experiments indicated that after treatment with ether, ADV-G antigen epitopes were fully exposed and the virus can be effectively, rapidly neutralized by specific anti-ADV antibody. Mink produced neutralization antibodies 10 days post infection, and the antibody titer rose to 1: 128, 800 60 days post infection. ADV-G treated with ether could be neutralized by

rabbit anti-ADV structural protein antibodies *in vitro* indicating that ADV antigenic determinants responsible for neutralization are located (on) ADV structural proteins. ADV-G treated with ether could also be neutralized by purified anti-ADV Fab fragment. Compared with CIEP, immunohistochemistry and gammaglobulin assay, the *in vitro* neutralization test of Aleutian disease virus is the most sensitive method.

Chinese Journal of Virology, 10 (4), pp. 334-338, 1994. In CHIN, Su.+ subtitles in ENGL. 2 tables, 1 fig., 10 refs. Authors' summary.

Prevalence of microorganisms in dead mink kits from Aleutian-disease-infected and non-infected farms

P.E. Martino, J.J. Martino

Bacteria and fungi were isolated from different tissues (brain, liver, heart) taken from 81 dead newborn mink originating from Aleutian disease (AD) infected and AD-non-infected farms. Of the 123 isolates obtained, 96% were bacterial isolates (predominantly Gram-negative) and 4% were fungi. The prevalence of microorganisms appeared less common in kits from AD-non-infected farms (55%) than from AD-infected farms (73%), although the difference was not significant. The liver was the most highly infected site in both groups and generally was only infected by one microorganism species. Proteus spp (23%), Escherichia coli (16%), Staphylococcus aureus (11%) and Enterobacter cloacae (9%) were the most frequently isolated germs. These findings are similar to those of other studies but the role of these microorganisms as specific pathogens or secondary invaders remain controversial.

Vet Res 27, pp. 607-612, 1996. 3 tables, 20 refs. Authors' summary.

Breeding, acclimatization and identification of a mink virus enteritis strain attenuated through successive culture in cells of cattle testes

Tao Quanfu, Dong Aiyu, Zhang Jingang

A virulent strain of mink virus enteritis was isolated from naturally infected dead mink. This virus strain was cultured in a mixture of cattle testes cellc (CT) and CRFK and passed serially. The cytopathic effect (CPE) of CT cells did not appear until the 56th passage.

After the 56th passage, the virus strain was cultured only in CT cells and identified to be a typical parvovirus by EM, HA, and HI test at the 70th passage. An avirulent strain was gained by end-point dilution and proved by inoculation of normal mink. The HI dilution in the serum from inoculated mink was increased greatly.

Chinese Journal of Animal and Poultry Infectious Diseases, No. 5, pp. 21-25, 1995. In CHIN, Su. ENGL. 5 tables, 18 refs. Authors' summary.

Clinical picture and antibody response to experimental *Sarcoptes scabiei var vulpes* infection in red foxes (*Vulpes vulpes*)

S. Bornstein, G. Zakrisson, P. Thebo

Sarcoptic mange is a common skin disease of mammals. It is caused by the burrowing mite *Sarcopts sabiei*, which can infect over 40 mammal species, domestic as well as wild animals and humans. In the early 1970ies an epizootic of *S scabiei* broke out amongst Swedish wild red foxes (*Vulpes vulpes*). The red fox in Sweden were previously naive to the infection.

Within 8 years the infection had spread throughout the country and 50 to 80 per cent of the wild red fox population had died from the infection.

In order to describe the clinical appearance of the infection in the animals and the serum antibody response to the mites an experimental study was made.

Three red foxes were experimentally infected with Sarcoptes scabiei isolated from a naturally infected wild red fox. A fourth red fox served as a control. The first signs of sarcoptic mange became evident on the 31st day post infection (dpi). The signs gradually increased thereafter and between dpi 49 and 77 characteristic lesions of hyperkeratosis developed. Two of the infected foxes developed severe sarcoptic mange, and one of these animals died on dpi 121. The third fox developed a localized chronic hyperkeratotic lesion on its back, at the site where the mites had been applied. This fox did not show any systemic involvement and if it had been free in the wild it would most certainly have acted as a carrier animal.

Many sarcoptic mites were found in skin skrapings from the chronic lesion. On dpi 127 the surviving foxes were treated systemically with ivermectin and within 4 weeks the skin lesions had healed except on the pinnae of one animal.

Antibodies to *S scabiei var vulpes* were demonstrated in the infected foxes by an ELISA with which seroconversion was seen around 4 weeks post infection (wpi). Western blot analysis of sequential sera of the infected animals demonstrated antibody activity consistently after the 2nd wpi.

The fourth, non-infected, fox did not show any skin lesions throughout the experimental period nor any specific antibodies to *S* scabiei var vulpes.

Acta vet. Scand. 36, pp. 509-519, 1995. 2 tables, 6 figs. Authors' abstract.

Superficial spreading pyoderma and ulcerative dermatitis in a ferret

W.W. King, S.L. Lemarié, R.S. Veazey, E.C. Hodgin

A case report of an 8-month-old ferret with severe ulcerative dermatitis of the ventral abdomen and medial thigh regions is presented. Cutaneous biopsies of the periphery of the ulcerative lesions revealed large, confluent, superficial epidermal pustules containing inflammatory cellular debris and Gram-positive cocci, as well as perifollicular dermal necrosis.

These histological findings are consistent with superficial spreading pyoderma in conjunction with dermal coagulative necorisis.

Veterinary Dermatology 7, pp. 43-47, 1996. 3 figs., 14 refs. Authors' abstract.

Cutaneous epitheliotropic lymphoma in a ferret

Michele R. Rosenbaum, Verena K. Affolter, Amy L. Usborne. Neal L. Beeber

Treatment included isotretinoin and amoxicillin trihydrate plus clavulanate potassium administered orally and oatmeal-based shampoos. Isotretinoin was tolerated well and cutaneous lesions resolved after 60 days of treatment, but pretreatment azotemia worsened and the ferret was euthanatized.

Necropsy revealed cutaneous epitheliotropic lymphoma, pyelonephritis, and interstitial nephritis. Renal disease most likely was caused by immunosuppresion secondary to chronic treatment with corticosteroids and aging. Isotretinoin, although not curative, may be useful for the palliative treatment of

cutaneous epitheliotropic lymphoma in ferrets.

Journal of the American Veterinary Medical Association, Vol. 209, No. 8, pp. 1441-1444, 1996. 2 figs., 25 refs. CAB-abstract.

Clinical and pathologic findings in ferrets with lymphoma: 60 cases (1982-1994)

Susan E. Erdman, Susan A. Brown, Thomas A. Kawasaki, Frances M. Moore, Xiantang Li, James G.Fox

Objective – To examine clinical and pathologic findings in 60 ferrets with lymphoma.

Design – Retrospective case series.

Animals – 60 ferrets in which the diagnosis of lymphoma had been confirmed by means of histologic examination of biopsy or necropsy specimens.

Procedure – Information including age, sex, coat colour, history, clinical signs, clinicopathologic abnormalities, treatment, outcome, and results of histologic examination of biopsy and necropsy specimens were retrieved from medical records of ferrets with spontaneous lymphoma examined between 1982 and 1994 at the Massachusetts Institute of Technology or private veterinary practices in 10 states. Classification of lymphoma was assigned according to the National Cancer Institute's working formulation for non-Hodgkin's lymphomas. χ^2 Trend analysis was used to determine whether age was associated with history, clinical signs, hematologic abnormalities, stage, histologic grade, or outcome.

Results – Acute onset, mediastinal mass, lymphocytosis, and multicentric distribution were linked with younger ferrets, and lym-

phopenia and survival longer than 2 months after diagnosis was associated with older ferrets. Twenty percent of ferrets in this study had cohabitated with another ferret with lymphoma. Chemotherapeutic efficacy was evaluated.

Clinical implications – Clinical and pathologic features linked with age should be considered when evaluating diagnostic and therapeutic options for ferrets with lymphoma.

JAVMA, Vol. 208, No. 8, pp. 1285-1289, 1996. 1 table, 3 figs., 25 refs. Authors' summary.

The health condition of farm fitches on some Polish farms

Olga Szeleszczuk, Rafal Przybyla, Piotr Niedbala

The aim of this study was to estimate the health condition of farm fitches with particular reference to Aleutian disease – plasmacytosis – and its diagnosis by the use of non-specific Iodine Agglutination Test (IAT), as specific test – Counter Current Immunoelectrophoresis (CIEP) and anatomopathological examination.

The research was done on 275 young fitches on three farms situated in the district Rzeszow (Farm A), Czestochowa (Farm B) and Suwalki (Farm C).

The evaluation of salubrity of farm fitches on the farms under study showed that 64.8% of the animals were ill, including 2.3% with Aleutian disease. Plasmacytosis in farm fitches is of a chronic nature, its course being mild without the characteristic anatomopathological lesions such as are observed in mink.

Zeszyty Naukowe 15, pp. 193-199. In POLH, Su. ENGL. 3 tables, 18 refs. Authors' summary.

A helminthological survey of wild red foxes (*Vulpes vulpes*) from the metropolitan area of Copenhagen

A.L. Willingham, N.W. Ockens, C.M.O. Kapel, J. Monrad

Sixty-eight red foxes were collected from the metropolitan area of Copenhagen and examined for helminth infections. Standard fecal flotations for intestinal parasites gave the following results: Strongyle (75.0%), Capillaria eggs (36.8%), Toxocara eggs (23.5%), Taenia eggs (1.5%), and coccidia oocysts (2.9%). Gastrointestinal helminths were collected from 21 of the 68 foxes with the following specimens found: Uncinaria stenocephala (85.7%), Toxocara canis (81.0%), Taenis spp. (38.1%), Mesocestoides lineatus (23.8%) and Polymorphus spp. (9.5%). Feces of 39 foxes were examined by the Baermann method for larvae of cardiopulmonary worms with 20 foxes (51.3%) being infected. Fourteen foxes (35.9%) were infected with Angiostrongylus vasorum, 11 (28.2%) were infected with Crenosoma vulpis, and 5 foxes (12.8%) were infected with both species. Muscle digestion of diaphragms from the 68 foxes indicated that none harboured larvae of Trichinella spiralis.

Journal of Helminthology 70, pp. 259-263, 1996. 3 tables, 31 refs. Authors' abstract.

Diseases of chinchillas

H. Kraft

Among chinchillas kept in Germany as furbearing animals or pets, infectious diseases and parasitoses were rare. Most diseases arose from errors of management, particularly inappropriate feeding, and this is reflected in the contents of this fifth edition.

Krankheiten der chinchillas; Ed. 5; 74 pp, 37 figs., 83 refs., 1994. Paperback; DM48. Only abstract received. CAB-abstract.

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