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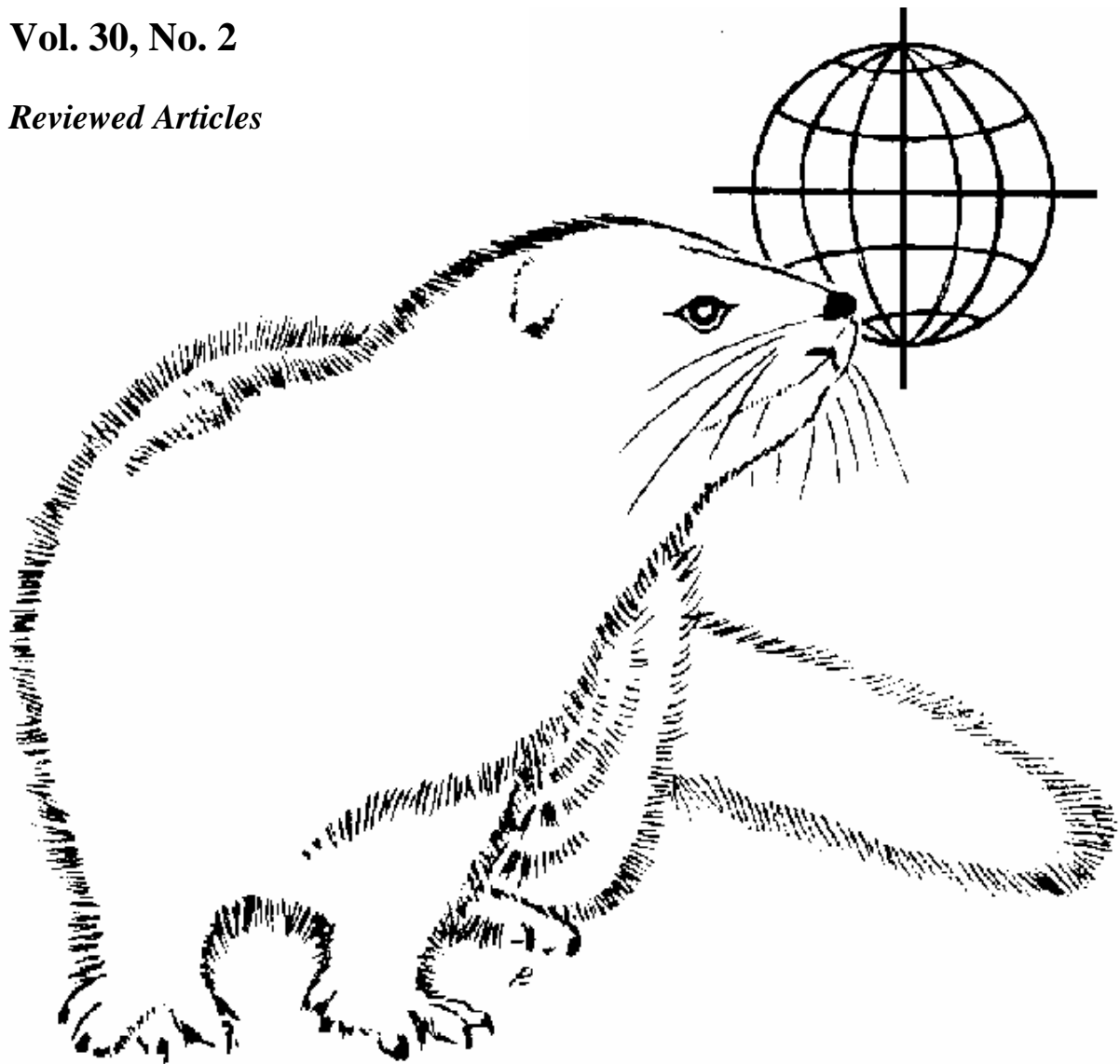
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Notes from the Group of Editors

This issue of Scientifur, Volume 30, No 2, contains two reviewed articles as well as a short communication.

More interesting articles are in the pipeline, however, the reviewing process takes time.

We kindly invite our readers to continue submitting proceedings, short communications, abstracts, letters and in particular articles for reviewing.

On behalf of the
Group of Editors

Birthe Damgaard

A national survey for Aleutian disease prevalence in ranch mink herds in Canada

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Abstract

Aleutian disease (AD) is a devastating viral disease of farmed mink that can significantly decrease ranch productivity through immunosuppression, concurrent disease, and reproductive losses. In order to determine the viability of a national AD eradication program, it was necessary to determine the ranch prevalence of the disease in Canada. Due to variability in the method of testing utilized and insufficient farmer participation in national testing through provincial diagnostic laboratories, this study was undertaken to estimate national AD prevalence and herd prevalence by province. In order to generate current, accurate, national results from accredited laboratory facilities, the CMBA Aleutian Disease task force requested each Canadian rancher to submit 200 blood samples from breeder animals for no-cost counter current immunoelectrophoresis testing at one of three designated laboratories in Canada. The submission rate was 47/238 (20 %). A survey collecting associated ranch information (ranch size, feed delivery, color phases raised, whelp average, and illness or reproductive problems) was returned at a rate of 61/238 (26 %). The mean prevalence of AD at the national level was approximately 5 %. The only variables with significant statistical association to AD (p<0.01) were ranchers' ability to detect signs of AD when it was present on a ranch, having had AD on the ranch within the previous three years and having high mortality rates.

Keywords: Aleutian disease, mink, prevalence, survey

Introduction

Aleutian disease is a chronic, progressive, non-treatable immune mediated disease of adult mink caused by a persistent parvovirus that particularly affects the kidneys, blood vessels, brain, eyes and lungs (Hunter, 1996). High mortality is produced secondary to immune suppression and terminal kidney failure (Johnson, 1995). Infected adult females may experience reduced fertility and increased kit mortality. When virus infects kits at less than 21 days of age, coughing is the principal sign of acute interstitial pneumonia (Alexandersen, 1994). Mink kits that survive early infection go on to develop classic signs of this immune mediated disease (Alexandersen, 1994).

In countries such as Denmark, where AD control programs are mandatory, there has been some success in controlling AD (Hansen, 1990). Originally, control was attempted by identification and removal of positive animals following counter current immunoelectrophoresis [CEP] tests performed at the time of pelting and again prior to the breeding season (Cho, 1978). Currently, if three

or more positive animals are detected on a ranch, eradication and repopulation is recommended. Similar mandatory programs could be instituted in North America to initiate control and eventual eradication of this disease.

The CEP test detects the presence of antibody to the AD virus in the mink's serum and is the gold standard test available for a test and slaughter eradication program (Wright, 1982; An, 1977). This test has a very high sensitivity (95 %) and specificity (95%) so that false positive and false negative results are few (Wright, 1982; Chriel, 2000). As a direct binding assay, false positive reactions are expected at a low rate (Aasted, 1986). The presence of false negatives in particular can compromise the success of a test and slaughter eradication program. The presence of false positives can initiate eradication procedures in ranches without the disease. The test is problematic when used to screen herds at low frequency of infection. A 100 percent sensitive confirmatory test is actually needed (but not currently available) to form the basis of an ideal national test and slaughter eradication program.

Control of AD is difficult for several reasons. The virus persists in the environment. Milder variants result in inapparent infections or carrier animals and in the case of aggressive variants result in explosive epidemics especially in certain coat color phases (An, 1978). Additionally, several types of AD virus may be present simultaneously during a ranch outbreak. Additionally, unlike many other viral diseases that can be prevented by appropriate vaccination, AD is actually worsened by the production of vaccine-associated antibody, since the main lesions are the result of immune complex deposition (Bloom, 1975). There has been some indication that development of antibodies might be protective for the acute pneumonic form seen in mink kits (Alexandersen, 1994). It is difficult to eliminate the antibody response entirely following vaccination with traditional vaccine products and hence this is the primary reason why vaccine development for this disease is a slow and costly endeavor. There is hope that new molecular technology will allow researchers to develop products that primarily stimulate the cell mediated immune response, thus preventing progression of the disease and providing adequate protection.

In the interim, development of control strategies and test and slaughter programs is paramount. In Denmark, an increase in farms from 2400 to 5100 and in mink breeders from 800 000 to 2 million over the period of 1976-1989 taxed the ability to keep AD at bay (Hansen, 1990). 80 % of farms took part in the test and slaughter program and increased mortality and infertility were the most common symptoms (Hansen, 1990; Chriel, 2000). It was difficult to maintain freedom from infection (Hansen, 1990). Currently Danish ranchers test over 3 million mink a year and positive animals have necropsy done to help confirm presence of AD lesions.

Cases of AD continue to occur in ranch mink in Canada. The herd prevalence of the disease nationally and by province is not known. As there is a mounting interest in developing control procedures for this disease at the provincial and national level, determination of AD prevalence by a national blood testing campaign is required. Epidemiological information on Aleutian disease in Canadian ranch mink could be accumulated concurrently by a questionnaire.

Materials and methods
All mink ranches in Canada were contacted for participation in the study and for provision of blood samples for AD testing. It was determined that approximately 200 (range of 167- 230) samples per farm would be statistically appropriate and financially achievable. This selection was based on the formula

$$n = (Z\text{-squared} \ x \ p(1-p)) \ / \ e\text{-squared}$$

using a 95% confidence interval (Z=1.96) about a prevalence rate of 0.50. A tolerable error rate(e) of 0.05 was used. Consequently, blood samples from 200 randomly selected breeder animals were requested from each ranch.

Questionnaires were developed to record farm name, province and size (based on number of mink raised and number of breeder animals), rancher suspicion of AD presence on ranch, litter size, genotypes raised, feed delivery methods, kit mortality, abortion rate, mortality rate, the presence or absence of concurrent diseases (such as nursing sickness, foot pad disease, distemper, kit pneumonia

and adult pneumonia) and a history of AD and/or previous eradication attempts on the ranch. These questionnaires were sent to all 238 ranches in Canada through the national association office (Canada Mink Breeders Association) with directions to return by mail or fax. In the same mailer package, ranchers were informed that three laboratories (one each in British Columbia, Ontario and Nova Scotia) would accept 200 blood capillary tube samples for standardized AD CEP testing from a randomly selected group of breeders. This free program would continue for a six month period from January to June 2004. Results from CEP tests utilizing Danish antigen were reported to ranchers and released to the CMBA following mandatory owner consent.

Farms participating in the study were categorized as either AD present or AD absent based on blood test results. The herd prevalence was estimated from the number of positive tests out of the total tested per farm (whilst almost always 200, there were occasional samples that had to be discarded or that were broken in transit, and rarely from farms with less than 200 breeders present).

Prevalence was determined nationally and by province. The prevalence for the nation was determined as a mean and standard deviation. Mean prevalence for provinces were calculated by first determining the ranch prevalence [ranch prevalence = # positive samples/total # submitted samples] and

secondly by dividing the sum of all ranch prevalences for a province by the total number of farms in the province. Additionally, the prevalence of positive farms out of the total farm number was calculated. Provincial differences in the interherd prevalence of AD were tested using chi square analysis. Chi square tests, using Fisher's exact method, were used to test for associations between the occurrence of AD and province as well as other categorical disease and management variables. Since AD prevalences were not normally distributed, appropriate nonparametric tests such as Kruskal Wallis and Mann Whitney were used to test for rate differences between provinces and between other possible influencing factors. In order to control the Type I error rate across multiple testings, a probability value of P= 0.01 was considered significant. All statistical analyses were performed using SPSS, version 11.5 (SPSS Inc, Chicago, IL).

The mean and standard deviation for total number of mink raised from the ranches that were positive for AD and those that were negative for AD were calculated.

Results
Self reported surveys were returned from about 26% of all ranches surveyed. Six provinces were represented: British Columbia (BC), Ontario, Nova Scotia (NS), Alberta, Manitoba, and Prince Edward Island (PEI) (see Table 1).

Table 1. Questionnaire and blood test result data

Province	BC	Ontario	Nova Scotia	Alberta	Manitoba	PEI	Total
Total # ranches	17	83	113	4	14	7	238
% of total ranches	7.14	34.87	47.48	1.68	5.88	2.94	100
# surveys returned	4	16	33	2	5	1	61
% returned by province	23.53	19.28	29.2	50	35.71	14.29	
% of all returned	6.56	26.23	54.10	3.28	8.20	1.64	100
# ranches submitting blood	2	13	27	2	2	1	47
% ranches submitting blood	11.76	15.66	23.89	50	14.29	14.29	
% of total ranches that submitted blood	1.32	27.66	57.45	4.26	4.26	2.13	100
# positive ranches	1	4	9	1	0	0	15
% of positive ranches by province	50	30.77	33.33	50	0	0	
% of all positive ranches across provinces	6.67	26.67	60	6.67	0	0	100
Mean prevalence rate	0.402	0.005	0.018	0.463	0	0	
Standard error	0.402	0.003	0.007	0.463	0	0	
Standard deviation	0.568	0.011	0.035	0.654	0	0	
Prevalence of AD (%)	25	25	27.3	50	-	-	-
Standard error	25	11.2	7.9	50	-	-	-
Standard deviation	50	44.7	45.2	70.7	-	-	-

Nova Scotia and Ontario together accounted for 82% of the documented Canadian mink ranches for provinces from which surveys were returned, but only had return rates of 19% and 29% respectively. Alberta, with only 4 ranches, represented less than 2% of the farm population but had a 50% survey return rate. Manitoba, at almost 36%, had the highest return rate for provinces with more than 10 ranches. BC with roughly 7% of the documented mink farms had a 24% return rate while PEI, with only 3% of known ranches, had only 1 returned survey (14 %). Blood samples were received from almost 20% of the ranches surveyed during the six month sampling period (see Table 1). Ontario (27.66 %) and Nova Scotia (57.45 %) ranches accounted for about 85 % of all submitted samples. Returned samples from BC, Alberta, Manitoba, and PEI each represented less than 5% of the total returned. Within these provinces, response rates ranged from 12% to 50%. While Alberta's response rate was the highest of these provinces, the 2 farms sampled represented only about 4% of the total blood tested. Nova Scotia (23.89%) and Ontario (15.66%) had the highest return rates for provinces with more than 10 farms. Manitoba, PEI, and BC each had return rates lower than 15%.

Positive AD reactors were identified at a total of fifteen farms (see Table 1). Nova Scotia produced 60% of all positive tests processed while close to 27% were from Ontario. BC and Alberta each had 1 farm with positive samples out of a total of only 2 farms sampled per province. Together, these two provinces had about 13% of all farms with positive tests.

No positive tests were obtained from Manitoba or PEI.

The mean prevalence rate of AD from the sampled population representing a national prevalence for Canada was about 5% (0.0473 + 0.175). Mean prevalence of AD by province is shown in Table 1. British Columbia and Alberta have the highest rates, both over 40%, however, only two farms returned blood samples in each of these provinces. Both Nova Scotia and Ontario had prevalence rates below the national average.

Differences between provinces, in the rate of AD disease, were tested using the Kruskal-Wallis test.

Results indicate that there were no statistically significant differences in the rate of the disease between provinces (chi-square=3.288, p = 0.678) (see Table 1).

Mean prevalence rate with standard error and standard deviation as well as percentage prevalence with standard error and standard deviation are listed for several tested variables in Table 2.

Additionally, there are significant differences in mean rank of percent positive animals between ranchers who self-reported an ability to detect signs of AD as opposed to those who did not (U=65.5, p <0.001). AD prevalence rates also differ significantly between ranches reporting a previous occurrence of AD within the past 3 years (U=144.5, p= 0.002) but not between ranches reporting any previous occurrence versus those having never had an outbreak (U=179.5, p= 0.015). Previous attempts to eradicate AD (disinfection and test and slaughter) also appear to have had no effect on the rate of AD in the ranches sampled (U=187.0, p=0.048).

Twelve mink color phases were reported by respondents. Due to few representative animals in several of the color phases tested, the mink were separated into light (sapphire, white, and violet mutations), medium (pastel and marble) and dark categories (black, mahogany, darks, brown, wilds, demi). Differences in the prevalence rate of AD between color phases and % prevalence of AD were determined (see Table 2). No significant differences were found between the three color categories (chi-square = 4.08, p= 0.253) but the lighter color group had numerically higher rates of disease, as would be expected due to the known association with the Aleutian genotype.

A positive blood test for AD was significantly associated (significant at alpha = 0.01) with a rancher's self-reported ability to detect signs of the disease (1.982, p=0.002) and a reported outbreak within the past 3 years (9.667m p=0.003). An acknowledged previous occurrence of AD at anytime, however, was not associated with a current incident (4.584, p=0.032) and neither were previous attempts at eradication (5.413, p=0.057).

Table 2. AD and management variables

	Mean prevalance rate	Standard error	Standard deviation	Prevalence of AD (%)	Standard error	Standard deviation
AD previously identified by blood tests						
Yes	.082	.044	.229	38.7	8.9	49.5
No	.003	.002	.009	10.0	5.6	30.5
AD identified in past 3 years						
Yes	.068	.042	.182	40.7	.096	.501
No	.036	.034	.178	12.5	.0059	.336
Previous eradication attempts						
Yes	.059	.040	.179	33.3	9.2	48.0
No	.038	.036	.181	15.1	6.3	36.4
AD signs identified by rancher						
Yes	.179	.093	.324	60.0	13.1	50.7
No	.004	.002	.011	14	.053	35.1
Color of mink						
Light	.449	.345	.500	66.7	33.3	57.7
Medium	.004	.004	.009	12.5	12.5	35.4
Dark	.053	.040	.193	20.0	7.4	40.7

Mixing of feed by ranchers as opposed to buying premixed feed was found to be independent of AD occurrence (0.035, p=0.851). Nursing status (2.671, p=0.475) and whelp average (2.264, p=0.384) were also not associated with an occurrence of AD. Additionally, no significant relationships were found between the incidence of AD and any disease vectors measured: kit mortality (2.932, p=0.463), kit pneumonia (2.190, p=0.127), adult pneumonia (4.845, p=0.084), footpad disease (4.55, p=0.146), 3 day enteritis (2.677, p=0.332), canine distemper (2.331, p=0.319), or spontaneous abortion (2.792, p=0.435). Increased mink mortality, however, was significantly associated with outbreaks of AD (8.207, p=0.007).

The Mann-Whitney U (two independent groups) test was used to determine if ranches where no disease was detected and ranches with AD disease as determined by blood testing differed significantly by mean number of animals raised (U=199.5, p=0.370) and number of breeders raised (U=190.0, p=0.266). None of these variables were found to have significant between group differences, however, within group variability was pronounced.

Ranches where AD was not detected raised an average of 7,197.12 (+ 7,844.17) mink with an average of 1,366.99 (+1,630.10) breeders. Ranches where AD was detected by blood testing raised an average of 14,403.60 (+ 22,171.00) mink including an average of 3,141.20 (+ 4,902.09) breeders.

Discussion
Whilst analysis of pre-existent laboratory records from routine AD testing from the provincial laboratories across Canada could have been done, it was felt that a current independent evaluation of the prevalence of AD would be more beneficial. Several factors could bias large laboratory data and include ranchers utilizing their own on farm testing facilities, ranchers submitting duplicate samples to multiple labs, ranchers submitting only when there is a suspected problem, and testing only being requested from a small number of ranches. The purpose of this study was to expand the likelihood of obtaining data from a larger segment of the ranching population because of the free blood testing incentive. Additionally, there was also the chance to accumulate targeted questionnaire information at the same time.

Despite what was considered an adequate response rate for the submission of blood samples on this study, the disease prevalence was generally low on the small number of farms that had positive animals. There were two farms, one in BC (80%) and one in Alberta (93%), both provinces with few participating farms, where significant bias occurred because of the very high prevalence of AD in these

single establishments. Elevation of the national mean prevalence was seen because of the presence of these two farms with within herd prevalence greater than 80%. Approximately 70 % of the ranches that responded to the survey and submitted blood for testing were free of Aleutian disease. Thus the majority of ranches that tested were apparently unaffected and thus our affected sample size was small. This potentially created a significant healthy farm bias in our analysis making it difficult to extrapolate these results to the Canadian mink population at large (estimated at 1.6-1.7 million animals). There may have been rancher bias in the random selection of breeder animals for the study but it was unaccounted for and unintentional under the recommendations of sampling. To the author's knowledge, this survey represents the most recent and most extensive attempt to characterize the presence of this disease in Canada. Based on low numbers the survey may be deemed inadequate in its original intention to determine national and provincial herd AD prevalence.

Testing established that the prevalence of AD was independent of province (2.406, p=0.960). These results are heavily biased because of the disparity in number of farms by province, the unusually high prevalence of AD in two and the lack of AD in two of the 4 smallest mink raising provinces. Comparatively, during the 2002 year, 47 000 tests were performed in British Columbia, 30 000 tests were performed in Guelph, Ontario, and 545 000 tests were performed in Truro. While the total annual test numbers from the national laboratories (622 000) greatly exceeds that tested as part of this initiative (8,409 samples), we feel that our survey results still have merit. We attempted to eliminate a cost bias in testing and prevented any one establishment from being over-represented, as once an AD problem is confirmed, extensive sampling is performed at the provincial laboratory.

The likely reasons for minimal survey participation by ranchers despite no-cost blood testing over a six month response period may be that many ranchers fear that testing for the disease might actually uncover its presence on their ranch. Additionally, for those ranchers that knew they already had a problem, they may have chosen not to put additional time and labor into the testing. Despite the six month time interval for sampling, ranchers were busy with breeding and whelping during the first six months of the year and hence some may have been too occupied to oversee the additional testing required to participate.

The survey allowed us to accumulate disease data and management practice information from the participating ranches. Surprisingly, there were few statistically significant associations between the stated variables and AD prevalence rates. This did not mean that these variables were not important in disease development but in our survey population with its low prevalence, their significance may have been minimized and larger surveys to address this could be undertaken.

The only statistically significant variables associated with incidence of AD were: a rancher's suspicion/ability to detect the presence of AD on the ranch by recognizing signs and having had AD confirmed by blood testing on the ranch in the previous three years. This suggests both that ranchers are astute in monitoring and recognizing the signs of AD correctly and that AD is a persistent disease that may cause problems on ranches that have dealt with it before. Having had prior AD outbreaks beyond three years previously was not associated with a higher likelihood of a current problem. Ranches that had undertaken eradication procedures (defined as disinfection and test and slaughter) had no greater likelihood of having the disease and hence when performed aggressively, this form of control procedure may be effective.

Although AD may manifest in a variety of ways, it did not appear that concurrent disease processes due to immune suppression were commonly noted. Additionally, reproductive problems were not commonly associated with AD in this survey population. Instead, high mortality in the absence of specific clinical symptoms in adult animals was the most consistently associated variable to AD prevalence. This may be the feature that farmers detected that made them the most suspicious of AD on their premises.

Although it has been suspected that larger ranches may have more AD, this was not a proven statistically significant association in this study, but it was the trend. Unfortunately, total ranch population numbers were not requested and so the proportion of individuals selected for testing from a ranch was not determined. Whilst not statistically significant, a comparison of average mink breeders raised was higher in the ranches where AD was

detected (7,197 +/- 7844 vs. 14,403 +/- 22,171). While many factors could contribute it is worth considering whether the disease is more likely to occur in larger ranch facilities. If so, this may be because of the higher potential for spread of the virus between animals and because of inherent practices associated with more intensive management. Apparently, feeding formulation (mixed or delivered) was not associated with disease incidence. It has been previously postulated that spread of AD may be assisted by transportation vehicles used to deliver feed to multiple farms.

Additionally, there was a trend towards lighter color phases having a higher AD prevalence, as would be expected based on the predetermined association with the presence of Aleutian genotype. This association was not considered statistically significant and may reflect the arbitrary division of color phases into one of the three groups and the small numbers assessed overall.

Whilst reports of successful elimination of AD from ranches exist Cho, 1978, voluntary Danish eradication programs have been in effect since 1976 without complete elimination of this disease from that country Aasted, 1986. In its inception, 53 tested farms had AD infected mink and 65 % of the 30 000 tested were positive Hansen, 1986. Nine years later, 65 % of farms had joined the program and 30 % of farms were declared as free of AD for the past three test periods (1.5 years) Aasted, 1986; Hansen 1990. Fifty % of farms in 1985 were AD free based on single test data Aasted, 1986. Mandatory eradication programs were legislated in Denmark in 1999 Ostergaard, 2000. Since that time, 250 farms have depopulated, disinfected and repopulated with AD free mink as dictated by the program Ostergaard, 2000. Subsequent tests on these farms revealed that 90 % (225) had no AD test reactors Ostergaard, 2000.

There are a number of reasons why control of this particular viral disease is considered difficult and most control programs, including those utilized in Denmark, involve a test and slaughter approach. Before implementing any mandatory testing procedures in Canada, it was critical to determine the rate of prevalence of the disease and any clearly associated management practices that might predispose to it. The CMBA sponsored survey was the first attempt to assess the presence of this important disease in the Canadian market.

Unfortunately, small sample sizes and few participating ranches make extrapolation of this data difficult but still informative as to the epidemiology of the disease. Undoubtedly, it will lay the ground work for discussions about eradication procedures and for future surveys to monitor the changing herd prevalence of this disease in Canada.

References
Aasted, B., Alexandersen, S., Cohn, A. & Hansen, M., 1986. Counter current line absorption immunoelectrophoresis in an alternative diagnostic screening test to counter current immunoelectrophoresis in Aleutian disease (AD) eradication programs. Acta Vet. Scand. 27, 410-420.

Alexandersen, S., Larsen, S., Aasted, B., Uttenthal, A., Bloom, M.E. & Hansen, M., 1994. Acute interstitial pneumonia in mink kits inoculated with defined isolates of Aleutian mink disease parvovirus. Vet. Pathol. 31, 216-228.

An, S.H. & Ingram, D.G., 1977. Detection of inapparent Aleutian disease virus infection in mink. Am. J. Vet. Res. 38, 1619-1624.

An, S.H. & Ingram, D.G., 1978. Transmission of Aleutian disease from mink with inapparent infections. Am. J. Vet. Res. 39, 309-313.

Bloom, M.E., Race, R.E., Hadlow, W.J. & Chesebro, B., 1975. Aleutian disease of mink: the antibody response of sapphire and pastel mink to Aleutian disease virus. J. Immunol. 115, 1034-1037.

Bloom, M.E., Kanno, H., Mori, S. & Wolfinbarger, J.B., 1994. Aleutian mink disease: puzzles and paradigms. Infect. Agents Dis. 3, 279-301.

Chriel, M., 2000. Interpretation of test results in eradication programmes using multiple sampling. In: Proceedings of the 9th Symposium of the International Society for Veterinary Epidemiology and Economics, August 6-11, 2000, Colorado, USA.

Chriel, M., 2000. Impact of outbreaks of acute aleutian disease in Danish mink farm. In: Proceedings of the VIIth International Scientific Congress in Fur Animal Production, September 13-15, 2000, Kastoria, Greece.

Cho, H.J. & Greenfield, J., 1978. Eradication of Aleutian disease of mink by eliminating positive counter-immunoelectrophoresis test reactors. J. Clin. Microbiol. 7, 18-22.

Gottschalck, E., Alexandersen, S., Cohn, A., Poulsen, L.A., Bloom, M.E. & Aasted, B., 1991. Nucleotide sequence analysis of Aleutian mink disease parvovirus shows that multiple virus types are present in infected mink. J. Virol. 65, 4378-4386.

Hansen, M., 1990. Procedure for, and efficacy of Aleutian disease eradication in Denmark. Proceedings of the 7 Arbeitstagung – uber – Haltung – und – Krankheiten-der-Kaninchen-Pelztiere – und – Heimtiere, May 31-June 1, Celle, Germany, 1990, 241-248.

Hunter, D.B., 1996. Aleutian disease. In: Mink… biology, health and disease. Ed. Hunter, D.B., Graphic and Print Services, Guelph, Ontario, Canada, pp. 1-29.

Johnson, M.I., Henson, J.B. & Gorham, J.R., 1975. The influence of genotype on the development of glomerular lesions in mink with Aleutian disease virus. Am. J. Pathol. 81, 321-336.

Ostergaard, J., Chriel, M. & Willadsen, C.M., 2000. Cleansing and disinfection procedures in connection with the Danish Aleutian disease eradication programme. Scientifur 24, 22-26.

Wright, P.F. & Wilkie, B.N., 1982. Detection of antibody in Aleutian disease of mink: comparison of enzyme-linked immunosorbent assay and counterimmunoelectrophoresis. Am. J. Vet. Res. 43, 865-868.

The use of vaginal electrical resistance (VER) as a diagnostic method for oestrus detection in the coypu (*Myocastor coypus Mol.*)

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Abstract

Myocastor coypus is native to South America and reproductive parameters in this species show large variations. These are probably due to differences in production systems in the region, which have not been studied in Uruguay. Seventy-one, 4, 5 and 6-mo-old females (*Myocastor coypus*, var. Silver) were studied in autumn and spring, and classified according to body condition (BC). Oestrus was identified by colpocytology and the vaginal electrical resistance (VER) was measured thereafter. Season, age, and BC affected oestrus frequency. Oestrus affected VER (P<0.001). During oestrus VER values were higher (P<0.001) than during non-oestrus (mean $\pm$ SEM: 334 $\pm$ 11.2 and 188 $\pm$ 1.3 Ω, respectively). The values of VER of the oestrous females ranged between 110 and 905 Ω, while those of the non-oestrous females ranged between 85 and 575 Ω. Our results suggest that measuring VER might be a useful method for oestrus detection in the coypu providing accuracy can be increased.

KEYWORDS: coypu, oestrus detection, colpocytology, vaginal electrical resistance

Introduction

Myocastor coypus is a native species of South America, and its habitat covers Argentina (East), Uruguay, south of Brazil, Paraguay and part of Bolivia. It belongs to the order Rodentia, sub-order Hystricognathi, family Myocastoidae (D'Elia, 1999). The coypu is a non-seasonal poly-oestrous species, although in wild life parturition generally occurs in winter and spring (Willner et al., 1979; Nes et al., 1988). The normal oestrous cycle has an average duration of 29 days, which can vary from 5 to 60 days (Iudica & Alberio, 1995; D'Elia, 1999; Felipe et al., 2001), with 1 to 5 days of oestrus (Baroch & Hafner, 2002). According to Kreatge (1937) ovulation is spontaneous. Ovarian hormone cycles are poorly understood, and the biological effects of nutrition on ovarian activity are only vaguely known (Sirotkin et al. 2000). This large variation is due, at least in part, to the regional differences in production systems and therefore, local studies should be carried out. To our knowledge, no such studies on reproductive behaviour of the South-American coypu have been done in Uruguay.

One of the key elements for reproductive success is the correct detection of oestrus. However, visual manifestation of oestrus is not observed in this species. Many studies have evaluated the oestrous cycle by cytological evaluation of vaginal smears (exfoliative colpocytology) (Jarosz et al., 1988; Iudica & Alberio, 1995; Felipe et al., 2001), as used in other species (guinea pig: Stockard & Papanicolau, 1917; dog: Bell et al., 1973 and Günzel et al., 1986; otter: Stenson, 1988). But this method implies additional manipulation of the animals and the need of a microscope for sample evaluation. The electrical resistance of the vaginal mucus (VER) has been used as a method for the detection of oestrus and the appropriate time of

mating in the coypu (Jarosz et al., 1988) as well as in other species (canines: Günzel et al., 1986; Boue et al., 2000; cattle: Pugh et al., 1999; swine: Stokhof et al., 1996). However, the value of VER varies among the different species. An increment in electrical resistance has been observed in the nutria (Jarosz et al., 1988) and the dog (Günzel et al., 1986) during pro-oestrus, attaining highest values during oestrus. On the other hand, Boue et al. (2000) working with vixens, observed that a majority of the females showed a rise in VER values on the day previous to oestrus, as well as a strong relation between VER values and the percentage of keratinised epithelial cells in the vaginal smears. The aim of this study was to evaluate the vaginal electrical resistance as a method for oestrus detection in the coypu (*Myocastor coypus*, var. Silver). Oestrus was confirmed by exfoliative colpocytology.

Materials and methods

Animals and working conditions
Seventy-one female nutrias (*Myocastor coypus*) Silver variety (34 in autumn and 37 in spring) were used. The study was carried out in Uruguay (34.5° S and 56.4° W), between March and December 2003. The experiment consisted of two 8-week periods, one in autumn and another in spring. In each period, 4, 5 and 6 months old females were grouped in 6 families (2 families for each age). Families were formed at weaning according to live weight at that time. Each family, consisting of 5 to 7 females, was kept in cages of 1 x 1.5 x 0.5 m in size (w x l x h), placed 0.8 m above the floor under a shed. The animals were fed a balanced mixture of maize grain, soy and sunflower expellers, bran, minerals and vitamins, with a minimum protein content of 18 %. Feed and water were available *ad libitum*. All of the animals were classified according to low and high BC at the beginning of each observation period. Live weight and body conformation (subcutaneous fat deposits inferred by palpation) were used to classify animals within age group into low BC (lean) and normal BC.

Collection and analysis of vaginal smear samples
The vaginal smear samples were obtained introducing a swab into the vagina at 3 to 6 cm. The samples were taken 3 times a week (25 samples per female), between 8 and 10 a.m.. The samples were placed onto a slide, dried at room temperature and, after 5 minutes fixation in ethanol, stained with a Giemsa solution. The slides were observed with a light microscope at a 450x magnification. For each slide the cells of different types present in 10 randomly chosen fields were counted (Iudica & Alberio, 1995). Females were classified in different stages of the oestrous cycle considering the cytological composition and relative proportion of each cellular type as described by Iudica & Alberio (1995) and Felipe et al. (2001).

Vaginal electrical resistance
The electrical resistance of the vaginal mucus was measured immediately after the collection of vaginal samples. The vaginal probe (Digital Heat Detector, Mod. IF-LI 3D, A/S LIMA, Sandnes, Norway) had a diameter of 10 mm and consisted of two metallic ring electrodes located at 8 and 16 mm from the tip of the probe. After each measurement the probe was cleaned and disinfected (Clorexidrina, 0.5% Aster S.A., Uruguay). The lips of the vulva were cleaned with paper tissues and water, prior to the introduction of the probe. The probe was inserted upwards at a 45° angle (relative to the spine) for 5 cm, and then placed parallel to the spine. Measurements were done holding the animals by the tail with their heads downward. The probe was gently moved forwards and backwards for 20-30 seconds to stimulate vaginal mucus secretion and to facilitate contact. The data were recorded when the conductivity reader stopped varying.

Calculations and statistical analysis
A statistical package (SAS 8.01, 1999-2000) was used to perform an analysis of variance of the data using the GENMOD procedure along with the Chi-square test. The variables analysed were the percentage of females presenting oestrus (as diagnosed by colpocytology) and percentage of females presenting one or more oestrus during the observation period. The effects of season (autumn or spring), age (4, 5 or 6 months) and BC (normal or low) at the beginning of the experiment, BC within each age, and family size (5, 6 or 7 females in the family) were evaluated. The electrical resistance was related with the presence of oestrus (by colpocytology), using the MIXED procedure along with the Tukey-Kramer test. Accuracy of the diagnostic value of VER was evaluated using the GENMOD procedure. The data are presented as mean values ± SEM. The level of significance was P<0.05.

Results

Exfoliative Colpocytology
In the two experiments 46 females (65% of all of the animals) presented oestrus at least once. Forty six percent (n=21) of the females showing oestrus presented only one oestrus during the 56-days observation period. Table 1 shows the frequency of females according to the number of oestrus observed during the experimental period. Complete oestrous cycles were registered for 25 females (showing two or more oestrus stages based on colpocytology) during the experiment, of which 14 had only one oestrous cycle, 8 had two cycles and only 3 had 4 cycles. In the 8-week experimental periods, cycle-length varied from 3 to 38 days, and the mean number of oestrus per female was 1.9 ± 0.12. For the females showing only one oestrus in the entire period, cycle length could not be estimated.

Table 1. Combined frequency (autumn and spring) of females according to the number of oestrus observed in a period of 56 days (total number of females: 71; and total number of females showing oestrus: 46)

Number of oestrus per female	n	% of Total number of females	% of females showing oestrus (n)
0	25/71	35%	- -
1	21/71	30%	46% (21/46)
2	14/71	20%	30% (14/46)
3	8/71	11%	17% (8/46)
4	3/71	4%	7% (3/46)

Season, age, BC and BC within the age of 6 month affected oestrus significantly, but not family size (Tables 2 and 3). Season affected oestrus (P=0.015) with more females showing oestrus during the autumn than during spring. Oestrus was more frequently observed in 4-mo-old females than in 5-mo-old (P=0.0279) and 6-mo-old (P=0.0483) females. More females with normal BC at the beginning of the experiment showed oestrus than females with low BC (P=0.004), but within age this difference was significant only for the 6-month-old females (P=0.022) (Table 3).

Table 2. Percentage of females showing oestrus by colpocytology according to season, age, body condition score and size of the family.

		Females showing oestrus	
		n	%
Season	Autumn	30/34	88[a]
	Spring	18/37	49[b]
Age	4 months	20/22	91[a]
	5 months	12/24	50[b]
	6 months	14/25	56[b]
Body condition	Normal	28/35	80[a]
	Low	18/36	50[b]
Family Size	5 females	11/20	55
	6 females	25/36	69
	7 females	10/15	66
TOTAL		46/71	65

Values with different superscript in column and within effect are different (P<0.05).

Table 3. Percentage of females showing oestrus by colpocytology according body condition within age

Body	4 months		5 months		6 months		Total	
condition	n	%	n	%	n	%	n	%
Normal	10/10	100	7/13	55	11/12	91[a]	28/35	80[a]
Low	10/12	85	5/11	47	3/13	30[b]	18/36	50[b]

Means with different superscript within column are different (P<0.05).

Furthermore, oestrus was classified according to its length in three types; short: when oestrus was observed on only one sampling occasion; medium: oestrus observed on two consecutive occasions; and long when oestrus was observed on three or more consecutive occasions. Table 4 displays the distribution of different types of oestrus.

Table 4. Distribution of the different types of oestrus (long, medium and short) according the number of oestrus showed by the females.

N° of oestrus	Number of females
One oestrus	21
Long Oestrus	8
Medium Oestrus	6
Short Oestrus	7
Two or more oestrus	25
Long Oestrus	2
Short Oestrus	8
Short and Medium Oestrus	6
Short and Long Oestrus	3
Medium and Long Oestrus	4
Short, Medium and Long Oestrus	2
TOTAL	46

Relationship between Vaginal Electrical Resistance and Colpocytology

A total of 1456 vaginal smears were evaluated by colpocytology, of which 155 were diagnosed as oestrus and the rest as non-oestrus. These vaginal smears were accompanied by VER measurements. The presence of oestrus had a significant effect on the value of the electrical resistance of the vaginal mucus (P<0.001). During oestrus the VER values were higher (P<0.001) than during non-oestrus (mean ± SEM: 334 ± 11.2 and 188 ± 1.3 Ω, respectively). The values of VER of the oestrous females ranged between 110 Ω and 905 Ω, while the values of the non-oestrous females ranged between 85 Ω and 575 Ω (Figure 1).
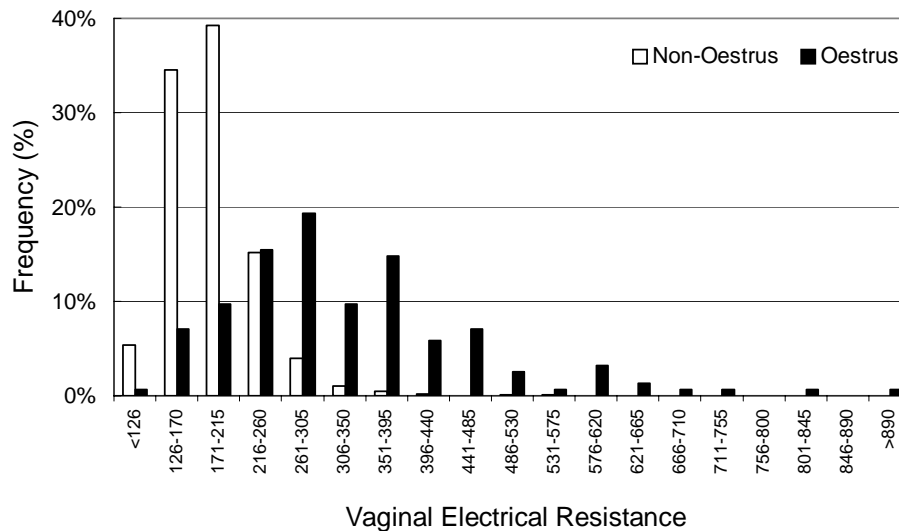
Figure 1. Frequency of VER for Oestrus or Non-oestrus. Classification of the females in Oestrus and Non-Oestrus was based upon the colpocytology results.

The cut-off limit for oestrus detection by VER was chosen arbitrarily as the arithmetic mean (261 Ω) of the mean VER values of females in oestrus (334 Ω) and non-oestrous females (188 Ω). Ninety four percent of the observations of non-oestrus (1226/1301) had a VER below 261 Ω and 67% of the observations of oestrus (104/155) had a value above 261 Ω. Age affected the accuracy of oestrus detection by VER ($P<0.05$), with the highest accuracy (93%) observed in the group of 5-mo-old females, followed by 88% in the 6-mo-old females, and the lowest accuracy (76%) in the 4-mo-old females.

The accuracy of diagnosis by VER varied with the type of oestrus. The majority of short oestrus had a VER value below 261 Ω and as the length of oestrus increased, fewer females had VER values below the cut-off limit. The type of oestrus affected the detection of oestrus by VER ($P<0.05$), since the detection rate was lower in the females with short oestrus (56%) than with medium or long oestrus (65% y 96%, respectively).

In 44% of the oestrus diagnosed by colpocytology the maximum values of VER occurred either on the sampling occasion previous to oestrus or on the first occasion of oestrus as detected by colpocytology. Analysing each female individually, accuracy of detection of non-oestrus was high, as 96% (24/25) of the females without oestrus had a VER value below 261 Ω.

Analysing each oestrus separately, 69% (59/85) were detected by VER. However, of the 46 females showing oestrus by colpocytology, VER detected each and every oestrus in only 27 (59%) of them.

Discussion

The most notorious finding in this study was the large variability of the oestrous cycle of the coypu. Oestrus was observed in the majority of the females (65%), and the females presented 1 to 4 oestrous stages during the period of observation. Iudica & Alberio (1995) reported similar findings in a study with 6 to 8 months old females. The rodent members of the sub-order Hystricomorpha, like the coypu, show large variation in reproductive parameters. Many studies done in some species of the genus *Cavia, Chinchilla, Dasyprocta*, and *Myoprocta*, show similar variation (Weir, 1971b, 1971c, 1974; Rood & Weir, 1970; Bland, 1980).

The higher number of females showing oestrus in autumn did not come as a surprise, since in wild life parturition occurs in winter and spring (average gestation length of 132 days) (Willner et al., 1979; Nes et al., 1989). Oestrus was affected by BC, however no reports on the effect of BC on oestrus in the coypus were found. This is a frequent observation in other species, such as cattle, were more females with normal BC presented oestrus than females with low BC (Rae et al., 1993). When discriminated by age, BC affected oestrus only in the older females (6 months). Nevertheless, the overall effect of age was not as one would expect, as the 4-month-old females presented more oestrus

than the rest of the females. The oestrous cycle of the females presenting only one cycle was longer than the oestrus cycles of females with 2 or 3 cycles. This observation on cycle length is similar to that observed by Iudica & Alberio (1995) and Felipe et al. (2001).

Studying nutrias, Jarosz et al. (1988), obtained overall VER values (90-960 Ω) similar to ours (85-905 Ω). Moreover, their mean values of VER during the other stages of the oestrous cycle (met-oestrus: 191 Ω; di-oestrus: 176 Ω; and pro-oestrus: 192 Ω) were similar to our non-oestrus VER value (188 Ω). Boue et al. (2000), working with foxes observed similar differences in VER between oestrous and non-oestrous females as we did. They established a basal VER value for non-oestrous vixens of 200 Ω, which was similar to our mean VER for non-oestrous females (188 Ω), although our results were not suitable for determining basal values.

In contrast to Jarosz et al., (1988), we were unable to establish a VER value that distinguished the different stages of the oestrous cycle. This was due to the overlap of the range of VER values for the different stages of the oestrous cycle. We therefore restricted the evaluation to describing oestrous and non-oestrous females. No correlation was found between colpocytology and VER. Therefore; it was not possible to confirm the conclusions of Jarosz et al. (1988) regarding the use of VER as a method for oestrus detection. However, several differences between our study and theirs might explain the differences and should be taken into account: age of the females and the type of animal used (genetic type). In the present study we worked with 4 to 6 months old females as opposed to two years old in the study by Jarosz et al. (1988). The female nutria in our region in South America (from which it is native) reaches sexual maturity at 4-5 months of age.

In the fox, Boue et al (2000) detected heat accurately in 75-80% of the vixens, when using a daily VER evaluation. As we did not have a daily sampling schedule, our accuracy of detecting heat was somewhat lower (71%), but the accuracy of detecting non-oestrous females was similar (96% in our study vs 100% in Boue's study). Age also affected accuracy of heat detection by VER, as it was lower for the 4-mo-old females as compared to the older females (5 and 6-mo-old). The

reproductive tract was probably more developed in the older females accompanied by a more appropriate hormonal environment. Boue et al. (2000) found similar results in the fox.

Among the oestrous females, the duration of oestrus also affected the accuracy of detection by VER. Accuracy increased with increasing oestrus length. This was probably due to the sampling sequence in our study. Daily recording of VER might have improved the accuracy of detecting 'short' oestrus.

Conclusion

Our results suggest that measuring vaginal electric resistance might be a useful method for oestrus detection in the coypu, which shows no external cues. However, further research is needed to evaluate accuracy and to establish a sampling schedule that will give acceptable accuracy. Providing these standards are met, VER could be a good method to determine the time of mating. Furthermore, other aspects affecting oestrus, such as BC, season and age, should be included in those studies.

Acknowledgements

References

Bardoch, J. & Hajner, M., 2002. Biology and natural history of the nutria, with special reference to nutria in Louisiana. In: Nutria (*Myocastor coypus*) in Louisiana, Genesis Laboratories Inc. 155p.

Bell, E.T., Bailey J.B. & Christie, D.W., 1973. Studies on vaginal cytology during the canine oestrus cycle. Res. Vet. Sci., 14, 173-79.

Bland, K.F., 1980. Biphasic follicular growth in the guinea pig oestrous cycle. J. Reprod. Fert., 60, 73-76.

Boue, F., Delhomme, A. & Chaffaux, S., 2000. Reproductive management of silver foxes (*Vulpes vulpes*) in captivity. Theriogenology, 53, 1717-28.

D'Elia, G., 1999. "Myocastor coypus" (On-line), Animal Diversity Web. Accessed November 19, 2004   at http://animaldiversity.ummz.umich.edu/site/acco unts/information/Myocastor_coypus.html

Felipe, A., Cabodevila, J. & Callejas, S., 2001. Characterization of the estrous cycle of the Myocastor coypus (coypu) by mean of exfoliative colpocytology. Mastozoologia Neotropical / J. Neotrop. Mamad., 8 (2), 129-137.

Günzel, A.R., Koivisto, P. & Fougner, J.A., 1986. Electrical resistance of vaginal secretion in the bitch. Theriogenology, 25 (4), 559-570.

Kraetge, E., 1937. Uber die hitzigkeit und verpaarung von sumpfbibermetzer. DPZ, 12 (6), 117-120.

Iudica, C., Alberio, R., 1995. Preliminary study of the sexual cycle of the South American nutria (*Myocastor coypus*) by the method of exfoliative colpocytology. Scientifur, 19 (1), 33-42.

Jarosz, S., Szeleszczuk, O. & Jakubaica, I., 1988. Designation of an optimal term for mating in nutria (*Myocastor coypus*). In: Biology, Pathology and Genetics of Fur Bearing Animals (Eds. Murphy, B.D., Hunter, D.B.). Proceedings IVth International Congress in Fur Animal Production. 21-24 August 1988, Canada. pp. 1-3.

Nes, N., Einarsson, E.J., Lohi, O., 1988. *Myocastor coypus*. In: Beautiful fur animals and their colour genetics. Scientifur, 11 (3), 223-227.

Pugh, M.L., Pence, M., Caamano, J.N., Robbe, S., Timms, L.L., Thompson, J.U., Bredahl, R. & Youngs, C.R., 1999. The use of a vaginal conductivity probe to influence calf sex ratio via altered insemination time. www.iowabeefcenter.org/Pages/ansci/beefreprots/asi-1652.pdf.

Rae, D.O., Kunkle, W.E., Chenoweth, P.J.; Sand, R.S. & Tran, T., 1993. Relationship of parity and body condition score to pregnancy rates in Florida beef cattle. Theriogenology, 39 (5), 1143-1152.

Rood, J.P. & Weir, B.J., 1970. Reproduction in female wild guinea pig. J. Reprod. Fertil., 33, 393-409.

Sirotkin, A.V., Mertin, D., Súvergová, K., Makarevich, A.V., Genieser, H.G., Luck, M.R. & Osadchuk, V., 2000. Effect of restricted food intake on production, catabolism, and effects of IGF-I and cyclic nucleotides in cultured ovarian tissue of domestic nutria (*Myocastor coypus*). General and Comparative Endocrinology, 117, 207-217.

Stenson, G.B., 1988. Oestrus end the vaginal smear cycle of the river otter, *Lutra canadensis*. J. Repr. Fert., 83, 605-10.

Stockard, C.R. & Papanicolau, G.N., 1917. The existence of a typical oestrous cycle in the guinea pig with a study of its histological and physiological changes. Am. J. Anatomy, 22, 225-284.

Stokhof, S.J.H., Soede, N.M. & Kemp, B., 1996. Vaginal mucus conductivity as measured by the Walsmeta MKIV does not accurately predict the moment of ovulation or the optimum time for insemination in sows. Anim. Repro. Sci., 41 (3-4), 305-310.

Weir, B.J., 1971a. Some observations on reproduction in the female green acouchi. *Myoprocta pratti*. J. Reprod. Fertil., 24, 193-201.

Weir, B.J., 1971b. Some observations on reproduction in the female green acouchi. *Dasyprocta aguti*. J. Reprod. Fertil., 24, 203-211.

Weir, B.J., 1974. Reproductive characteristics of hystricomorph rodents. Symposium of the Zoological Society of London, 34, 265-301.

Willner, G.R., Chapman, J.A. & Pursley, D., 1979. Reproduction, physiological responses, food habits, and abundance of nutria on Maryland Marshes. Wildlife Monographs, 65 (2), 1-43.

Puerperal hepatic steatosis in mink (*Mustela vison*)

A case report

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Abstract

The authors report a case of puerperal steatosis affecting 75% of a farmed mink farm population. The syndrome was probably due to a diet partially lacking in essential amino acids and proteic factors, such as choline. The deficiency of both phospholipids and apoprotein precursors, such as choline, and/or amino acids, such as methionine and lysine, negatively affects the gluconeogenic metabolism, very active in the post-partum period. Hepatic steatosis could thus become irreversible following this histopathological lesion. A simulation model was built to evaluate the feasibility of vicary nursing of orphaned mink cubs; this practice appears to be successful only when daily incidence is lower than 50%.

Key words: fatty liver, pathogenesis, prognosis, simulation model

Introduction

Hepatic steatosis occurring during the puerperium of mink (*Mustela vison*) is a dysmetabolic syndrome reported by several authors - "hépato-néphrite par surcharge graisseuse" (Villemin, 1956); "sindrome del higado graso" (Martino & Villar, 1987); "Steatite" (Carboni & Lodetti, 1993), "fatty liver degeneration" (Hunter & Barker, 1996).

Villemin (1956) and Carboni & Lodetti, (1993) point out the causes both in a diet too high in fat, where the fat component is badly preserved (oxidated, rancid fats). On the other hand, Martino & Villar (1987) do not ascribe the disease to dietary factors, and describe the syndrome in farms where a high incidence of Aleutian disease and inadequate environmental conditions are the rule. Hunter & Barker (1996) review possible causes of fatty liver degeneration in mink: the disease appears to be particularly frequent during whelping time; hepatotoxic agents will produce fatty degeneration; sudden periods of fasting resulting in mobilization of large amount of fatty stores may result in fatty degeneration; epinephrine is known as a powerful lipids mobilizer, therefore, acute stress might cause sufficient fat mobilization to initiate fatty degeneration of the liver; some amino acids, such as choline and methionine, are required to properly metabolize certain forms of fat. A deficiency of these amino acids in the diet will therefore result in fat accumulation in the liver cells.

The present work reports a case of puerperal hepatic steatosis occurred in a mink farm located in the Isernia province (Central Italy), involving 75% of the dams; pathogenesis and prognosis are discussed. Prognosis is usually not an easy task; in this outbreak it was further complicated by the practice of giving orphaned newborn minks to other lactating females. This practice can indeed precipitate a pre-steatosic condition in the vicarious dams, due to the increased metabolic needs of the additional cubs.

A computer simulation model was built to identify the threshold within which vicarious nursing can be adopted without worsening the disease trend.

Material and methods

History, clinics, histopathology
The case farm consisted of about 300 females, belonging to the Standard Dark Mink genetic type; any females were housed individually in raised wire mesh cages, each one equipped with a covered nest-box; the cages were kept in three roofed, unwalled and unfloored sheds.

The farm was studied for three years. In the first year, when high mortality in the nursing mothers was first reported, the study focused on the identification of the affected animals, description of clinical signs and post mortem examination, diet composition and feeding protocols.

Liver, kidney and pancreas specimens were collected, fixed in 10 per cent buffered formalin and routinely processed for histopathology. 4-6 µm tissue sections were stained with haematoxylin and eosin and examined by light microscopy.

In the following two years, when the disease did not occur, the study focused specifically on diet composition at the start of the breeding season.

Feed composition and feeding management
At the time when the syndrome occurred for the first time (year 1), animals were fed with fresh meat, to which a compound fodder (40% of crude protein, 7.2% of crude fat), specific for breeding minks, was added. The fresh meat component derived from poultry scraps (wings, legs, heads), supplied weekly and successively grounded, portioned and frozen on farm. The final feed was prepared daily, mixing 4 parts of fresh meat and 1 part of compound fodder, adding water to obtain a semi solid mixture to be smeared on the top net of the cage (1 kg for 4 animals). Such ration was provided for about three months.

Producer's recommendations, as reported on the compound fodder's label (1.5 parts meat and 1 part fodder), were regularly adopted during previous production cycles; during the cycle when the syndrome occurred, these recommendations were disregarded due to financial reasons.

During the second and the third study year, producer's recommendations were observed again; moreover animals' diet was integrated with mineral and vitamin supplements and with amino acids.

Feed chemical analysis
Final feed and fresh meat stock were analyzed separately. Crude protein and crude fat were respectively analyzed with Kjeldahl (Dir. 72/199/EEC) and Soxhlet (Dir. 71/393/EEC) methods; nitrogen-free extract was calculated by difference from total dry matter (Dir. 71/393/EEC). Possible fat oxidative degeneration of the fresh meat component was assessed by determination of the peroxide value (by iodotitration) and by Kreiss reaction.

Vitamin E concentration (as alphatocoferols) was quantified by HPLC (Dir. 2000/45/EC). Methionine, choline and lysine daily intake (mg/animal/day) was estimated on the basis of what declared on feed and integrators labels.

Simulation model
A computer simulation model was built to assess the theoretical disease trend in the affected farm, using the following parameters:
- duration of the delivery season: 18 days
- number of females at the beginning of the delivery season: 300
- delivery trend: 5% from day 1 to day 5; 90% from day 6 to day 14; 5% from day 15 to day 18
- average number of newborns per dam: 3.5
- max number of newborns per nursing female: 7
- daily incidence of steatosis: 75%
- start of steatosis signs: 3 days *post-partum*

Results

Clinical signs, histopathology
Ailing subjects were very thin, showing and abnormal behaviour, ranging from simple listlessness to general carelessness towards the offspring and even refusal of sucklings, and prolonged stillness out of the nest until the insurgence of a pre-agonic dyspnoeic condition. These signs were observed within three days from delivery during the whole delivery period; they caused the culling of about 230 females and the subsequent loss of about 600 unweaned minks.

Post mortem examination revealed hepatic steatosis, characterised by a golden colour of the liver (see Figure 1); kidneys and pancreas were involved as well, although not as frequently and as severely as the liver.

Figure 1. Macroscopic aspect of the liver

Histologically, fatty degeneration was present in all the three organs. Fat was stored up in small globules in the cytoplasm, with a marked tendency to merge together (see Figure 2). The liver sinusoids were compressed and, at low magnification, resembled adipose tissue. Following globules' fusion, each cell usually contained one large globule, causing alteration of the contour of the cell and displacement of the nucleus. Fat infiltrated the interstitial connective tissue of the pancreas and the renal tubular epithelium.
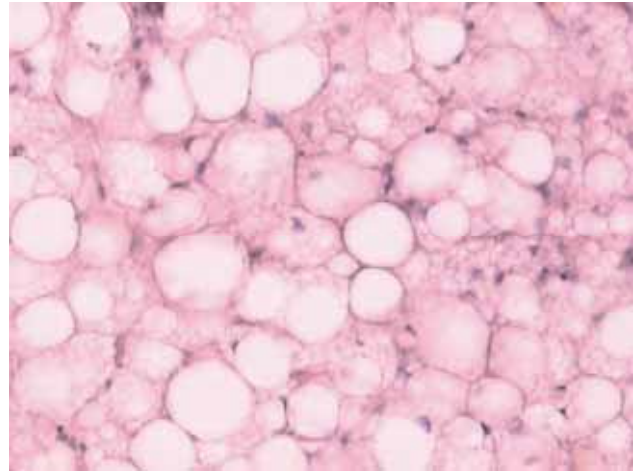
Figure 2. Fatty change of the liver. H&E 20X

Feed analysis results
Table 1 reports the percentages of crude protein, crude fat and nitrogen-free extract in the fresh meat and final feed both at the time of the puerperal syndrome and at the start of each pregnancy period in the following two years.

Fat oxidative degeneration of feed was not detected when the steatosic syndrome first occurred (negative Kreiss reaction; peroxide value = 7,4 meq O2/Kg fat, hexane extract).

Table 1. Feed main components during the study period

Components	Fresh Meat (*)			Final feed (*)		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Crude Protein	42.85	40.81	51.67	37.96	39.94	nd
Crude Fat	19.17	35.13	23.32	15.86	24.54	18.73
Nitrogen-free extract	29.48	11.69	5.72	37.51	24.94	nd
Kcal (kJ)		nd		435.2 (1820.6)	474.2 (1983.9)	nd

(*) % Dry Matter; nd = not determined

Table 2 reports the estimated quantities of some nutritional elements present in the feed, both at the time of the outbreak (year 1) and during the two following years. Methionine and lysine intake, during year 1 were estimated respectively as <0,7 and <4,7 mg/animal/day.

Table 2. Quantification of some nutritional elements in the feed during the study period

Nutritional elements	Year 1		Year 2		Year 3	
	mg/Kg	mg/animal/day	mg/Kg	mg/animal/day	mg/Kg	mg/animal/day
Vitamin E	29.3	7.3	58.4	14.6	43.3	10.8
Methionine	nd	nd	3.0	0.7	4.0	1.0
Choline	142.9	35.7	200.0	50.0	266.7	66.7
Lysine	nd	nd	19.0	4.7	25.3	6.3

nd = Not determinable

Nutritional elements supply was higher, altogether, of about 61% (year 2) and 51% (year 3), when compared with year 1.

Simulation model

Model results have reasonably simulated the actual disease trend, resulting in a final loss of 227 dams out of 303 and 483 cubs minks out of the 1061 theoretically expected at the beginning of the birth period (see table 3).

Table 3. Trend of simulated steatosis at a 75% daily incidence during the birth period

Day	Daily deliveries	Cumulative dams	Cumulative newborns	Daily affected dams	Cumulative theoretic weaned	Daily ratio affected/dam	Daily lactating	Daily newborns/ lactating	Cumulative survived newborns'
1	3	3	11		21	0	3	3.5	11
2	3	6	21		42	0	6	3.5	21
3	3	9	32		63	0	9	3.5	32
4	3	12	42	2	68	0.8	10	4.3	42
5	6	18	63	2	95	0.4	14	4.7	63
6	15	33	116	2	184	0.2	26	4.4	116
7	15	48	168	2	273	0.2	39	4.3	168
8	30	78	273	5	452	0.2	65	4.2	273
9	45	123	431	11	688	0.3	98	4.4	431
10	45	168	588	11	924	0.3	132	4.5	588
11	45	213	746	23	1082	0.5	155	4.8	746
12	30	243	851	34	1055	1.1	151	5.6	851
13	30	273	956	34	1029	1.1	147	6.5	956
14	15	288	1008	34	898	2.3	128	7.9	898
15	6	294	1029	23	782	3.8	112	9.2	782
16	3	297	1040	23	646	7.5	92	11.3	646
17	3	300	1050	11	588	3.8	84	12.5	588
18	3	303	1061	5	578	1.5	83	12.9	578
				2					

The trend change in the number of dams and cubs during the delivery period shows the ineffectiveness of nursing the orphaned newborns when steatosis daily incidence amounts to 75%. As a matter of fact, in the simulation model, the number of survived cubs at the end of the birth period is roughly similar to the one of the minks theoretically weaned (578/578). This is due to the ratio between the affected dams and the dams starting lactation,

already exceeding 1 on day 12 of the delivery period, as well as to the ratio between newborns and lactating dams, already exceeding 7 on day 14. The model has been run against a disease daily incidence of 50%; in this case the ratio between suckling cubs and nursing dams remains below 7 (see Figure 3).
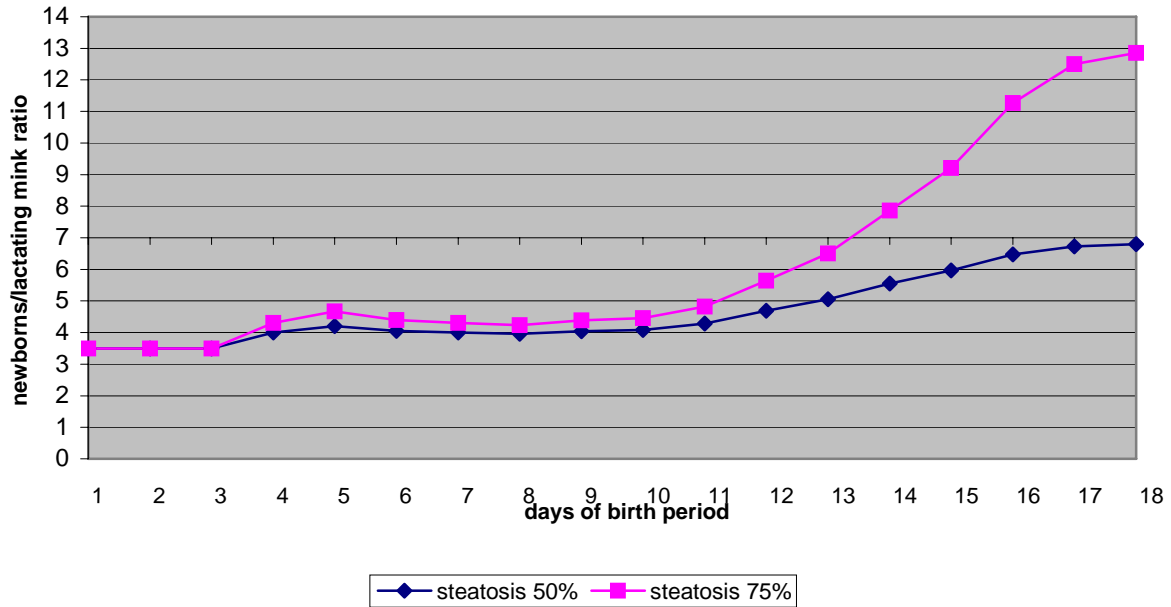
Figure 3. Trend of ratio between suckling cubs and dams mink at different daily incidence of steatosis

Discussion

Feeding

Mink feed is of critical importance to prevent the occurrence of puerperal steatosis. Both the type and the preservation characteristics of fresh meat (or better its fat content and oxidation level) are risk factors for steatosis (Villemin, 1956). In the present case, although vitamin E intake was lower than the recommended daily allowance (10-20 mg/Kg DM- Carboni & Lodetti, 1993), fat oxidative degeneration was not observed; crude fat content, particularly, was higher during the years following the one in which the syndrome occurred; anyhow crude fat content remains always within the standard range recommended for mink feed (20 to 30% - Carboni & Lodetti, 1993).

On the contrary, the reduced intake of compound fodder during the year in which the syndrome occurred, brought to a parallel decrease in the intake of essential amino acids, (methionine and lysine), and of other proteic factors, such as choline. The importance of a good quality diet during pregnancy and lactation has been emphasized by many studies investigating mortality causes in these particular stages of mink farming (Ingo et al., 1992; Rouvinen-Watt, 2001).

Pathogenesis

Classical steatosis is a degenerating disease characterized by intracytoplasmatic accumulation of fatty acids in organs that normally are able to metabolize them; cellular lesions can become irreversible, due to the severe compression of the nucleus and other vital cell structures (Marcato, 1997).

Clinical signs, such as food refusal and progressive apathy, followed by high mortality of the newborn minks, are typical also of "nursing sickness"; this condition, however, occurs in the last stages of the lactation period, associated with high energy diets and sodium deficiency (Clausen et al., 1992; Clausen et al., 1996).

Environmental stressors, especially if chronically present, may cause the prolonged release of corticosteroids, that are able to induce vacuolar degeneration of the liver in carnivores; here, the hepatic lesion is due to the accumulation of glycogen (Marcato, 2002). On the other hand, acute stress, as in the days following delivery, may be a risk factor for steatosic disease.

The pathogenesis of steatosis, especially when it occurs at the start of lactation, obeys to a more complex mechanism, not yet fully explained.

Lactose biosynthesis, which is the biochemical basis of lactation physiology, directly affects the hepato-pancreatic function involved in glycemia regulation (hence, the biological availability of glucose, precursor of lactose); to support the increased demand of glucose during lactation, the hepato-pancreatic function switches therefore from glycogenolysis to gluconeogenesis.

Glucose homeostasis in mink and carnivores is effectively maintained by gluconeogenesis (Børsting & Gade, 2000). Gluconeogenesis sustained by lipolysis, is supported by the hepatic synthesis of soluble lipoproteins able to transport fatty acids in the blood. This brings to hyperlipemia due to the increased bioavailability of triglicerids to be used in the glucose synthesis and to the concomitant release of free fatty acids from liver cells (Viviani, 1984).

According to molecular density, lipoproteins are distinguished in Very Low-, Low- and High Density Lipoproteins (respectively VLDL, LDL and HDL). They consist of a proteic component called apoprotein and a lipidic one made by phospholipids, cholesterol and triglycerides in various ratios. In particular apoprotein B, having a lower molecular weight, is the basic element of the low density lipoproteins (LDL), usually transporting fat from the liver (Viviani, 1984).

In the dairy cow, hepatic steatosis has been correlated with a decrease in LDL blood level (Rayssiguier et al., 1988). Therefore, the absolute deficiency of the proteic precursors of phospholipids and apoproteins (as choline, lysine and methionine) or a relative deficiency due to higher demands, that are the rule during lactation, causes a pathologic increase of triglycerides in the hepatic tissue (Buonaccorsi et al., 1997). Moreover, steatosis is characterized by some severe cytoplasmatic lesions affecting the endoplasmic reticulum (where the synthesis of apoproteins occurs) and this speeds up the pathogenic mechanism, making it irreversible in the end (Viviani, 1984).

Prognosis
The chronic events responsible for steatogenic conditions in farmed mink do not allow a favourable prognosis, however prompt the nutritional balancing intervention might be. However, on the grounds of our simulation model results, steatosis daily incidence appears as an important prognostic tool, allowing both to choose whether to wean or not

orphaned newborn minks and also the extent to which this practice can be adopted.

References
Børsting, C.F. & Gade, A., 2000. Glucose homeostasis in mink (*Mustela vison*). A review based on interspecies comparisons. Scientifur 24, 9-18.

Buonaccorsi, A., Guidi, G., Melosi, M. & Baroncelli, O., 1997. La lipidosi epatica puerperale della lattifera: momenti eziopatogenetici. Large Animals Review 3, 19-25.

Carboni, A. & Lodetti, E., 1993. Allevamento e malattie del visone. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia.

Clausen, T.N., Olesen, C.R. & Wamberg, S., 1992. Nursing sickness in lactating mink (*Mustela vison*). I. Epidemiological and pathological observations. Can. J. Vet. Res. 56, 89-94.

Clausen, T.N., Wamberg, S. & Hansen, O., 1996. Incidence of nursing sickness and biochemical observations in lactating mink with or without dietary salt supplementation. Can. J. Vet. Res. 60, 271-276.

Directive 71/393/EEC of 18 November 1971 establishing Community methods of analysis for the official control of feedingstuffs. Official Journal L 279, 20/12/1971, 7-18.

Directive 72/199/EEC of 27 April 1972 establishing Community methods of analysis for the official control of feedingstuffs. Official Journal L 123, 29/05/1972, 6-34.

Directive 2000/45/EC of 6 July 2000 establishing Community methods of analysis for the determination of vitamin A, vitamin E and tryptophan in feedingstuffs. Official Journal L 174, 13/07/2000, 32-50.

Hunter, D.B. & Barker, I.K., 1996. Digestive System of Mink In : Hunter D.B. & Lemieux N. (Eds.). Mink … biology, health and disease. Graphic and Print Services, University of Guelph, Guelph, pp 16-17.

Ingo, R., Luoma, M. & Virtanen, I., 1992. Some factors causing mortality among female minks during the puerperium and lactation periods. Acta Vet. Scand. 33, 59-69.

Marcato, P.S., 1997. Anatomia e Istologia Patologica Generale Veterinaria. 3rd Ed., Società Editrice Esculapio, Bologna.

Marcato, P.S., 2002. Patologia Sistematica Veterinaria". 1st Ed., Edagricole, Bologna.

Martino, P.E. & Villar, J.A., 1987. El sindrome del higado graso en visonas en gestacion y lactancia. Veterinaria Argentina 39, 810-812.

Rayssiguier, Y., Mazur, A., Gueux, E., Reid, I.M. & Roberts, C.J., 1988. Plasma lipoptroteins and fatty liver in dairy cows. Research in Veterinary Science 45, 389-393.

Rouvinen-Watt, K., 2001. An overview of protein and amino acid nutrition of the mink - a true carnivore. Animal Nutrition Association of Canada 37th Eastern Nutrition Conference. May 15-16, 2001, Halifax/Dartmouth, Nova Scotia.

Villemin, M., 1956. Le Vison. Biologie, Elevage, Pathologie. Vigot Frères Editeurs, Paris.

Viviani, R., 1984. Elementi di Biochimica. UTET, Torino.

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