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Response of American Mink to Selection for Tolerance to Aleutian Mink Disease Virus

A Hossain Farid*

Department of Animal Science and Aquaculture, Dalhousie University Faculty of Agriculture, Truro, Nova Scotia, Canada

***Corresponding Author:** A Hossain Farid, Department of Animal Science and Aquaculture, Dalhousie University Faculty of Agriculture, Truro, Nova Scotia, Canada. **E- mail:** ah.farid@dal.ca

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Abstract

Introduction and Aim: Aleutian mink disease virus (AMDV) causes a serious health problem for American mink (Neovison vison) worldwide. The disease has no vaccine or a remedy and virus eradication attempts have failed. For over 20 years, mink on a commercial farm which was chronically infected with AMDV have been selected based on health, high reproductive performance and low levels of serum gamma globulin assessed by the Iodine Agglutination Test (IAT). The objectives of this study were to document the consequences of long-term selection for tolerance on the prevalence of CIEP- and IAT-positive cases, as well as reproductive performance of mink.

Results: Frequencies of IAT-positive breeders during a five-year period were less than 5%. Anti-AMDV antibodies were measured by counter-immunoelectrophoresis (CIEP) in 969 adults (3659 records) and 1728 kits over five years. The incidences of CIEP-positive breeders ranged between 74.7% and 83.8% in the early years and showed a declining trend for those kept for breeding. The incidences of CIEP-positive kits in Sep and Nov tests were 45.3% and 55.2%, respectively, and the estimates of heritability of CIEP-positive kits in Sep and Nov were 0.239 and 0.218, respectively. Only two of 1057 females which were exposed to males did not breed, and the number of females whelped of those mated was 92.8%. The averages of number of live kits on day 3 after birth and at weaning were 5.51 and 5.11, respectively, and kit mortality until weaning was low (6.39%). Female fertility and kit mortality were not significantly affected by CIEP status of dams in Nov, breeding year or date of first mating, but multiple-sire mating significantly increased all measures of reproduction compared with single-sire mating. Female fertility was the lowest for mink which mated once (79.3%) and increased for those which mated twice (90.3%) or three times (96.1%).

Conclusion: The results suggested that continuous selection for healthy mink with high reproductive performance and negative IAT in this heavily infected environment resulted in the creation of a tolerant herd whose performances were comparable with those of non-infected herds.

Keywords: *American Mink; Selection for Tolerance; Aleutian Mink Disease Virus (AMDV); Iodine Agglutination Test; Counter -Immunoelectrophoresis (CIEP); PCR Polymerase Chain Reaction*

Abbreviations

AD: Aleutian Disease; AMDV: Aleutian Mink Disease Virus; CIEP: Counter-Immunoelectrophoresis; DNA: Deoxyribonucleic Acid; IAT: Iodine Agglutination Test; PCR: Polymerase Chain Reaction

Introduction

Infection with Aleutian mink disease virus (AMDV, Carnivore amdoparvovirus 1) causes the production of large amounts of non-neutralizing antibodies and immune complex formation [1], leading to a serious health problem for the mink industry worldwide. Aleutian disease (AD) has no cure nor an effective vaccine [2]. The control measure for this virus in mink producing countries has been regular testing of animals for antibodies against the virus by counter-immunoelectrophoresis (CIEP) [3] or recently by Enzyme-Linked Immunosorbent Assay [4,5] and elimination of infected mink. This strategy has not resulted in permanent elimination of the virus from mink farms in Nova Scotia, Canada [6] or other mink producing countries [7,8]. Selection for tolerance to AMDV was initiated by the majority of mink farmers in Nova Scotia after an AMDV outbreak in 2012 and 2013 (unpublished data) and has gained popularity in other countries as well [8]. This strategy is based on the presence of differences among mink in the development of clinical symptoms following infection; some infected individuals do not succumb to the disease, showing a non-progressive or inapparent infection [3,9-13] and the differences are genetically controlled [14].

AMDV-infected animals with low levels of serum gamma globulin often remain healthy [10,12], but there is no published information on the results of long-term selection of mink on AMDV infected farms with low levels of serum gamma globulin. There is a unique mink herd in Nova Scotia, Canada, where animals have been selected based on visually assessed health status, high reproductive performance and low levels of serum gamma globulin measured by Iodine Agglutination Test (IAT) for over 20 years. Severity of AD lesions on organs of mink collected from this farm between 2003 and 2010 were significantly lower than those for AMDV-infected mink from farms which practiced the test-and-cull strategy [15].

Objective of the Study

The objectives of this study were to document the consequences of long-term selection for tolerance on the prevalence of CIEP- and IAT-positive cases, as well as reproductive performance of mink.

Materials and Methods

Statement of animal care

Mink on the commercial farm which were used in this study were managed and pelted by the owner according the industry standards [16]. Samplings specific to this study were performed according to the standards of the Canadian Council for Animal Care (http://www.ccac.ca) after approval by the institutional Animal Care and Use Committee (https://www.dal.ca/faculty/agriculture/animal-science-aquaculture/about/animal-care.html).

The farm

The mink farm (MM) was established in 1944 on Cape Breton Island in eastern Nova Scotia, Canada. Black American mink (Neovison vison) have been kept on this farm since its establishment; some non-black mink that were raised for several years were discontinued in 1990. Since 1990, the herd consisted of approximately 700 to 800 breeder females and 180 males. Some black breeder mink, mostly males, were obtained from two mink breeders in Nova Scotia between 1990 and 1995, but the herd, which has been a mixture of Jet-black [17] and standard black, has been closed to outside stock since 1995.

Animal management

Animals were kept under conventional farm conditions in individual cages, which were equipped with wooden nest boxes for breeding and whelping. Aspen shavings were provided for nest building. Water was provided ad libitum via nipple drinkers. Animals were fed standard mink diets which were prepared on the farm, and their compositions changed based on the production cycles. The Iodine Agglutination Test (IAT) has been performed by the farmer, without interruption, in December each year since 1966. The iodine solution was prepared by the farmer by mixing 4 gr of potassium iodide and 2 gr iodine crystal in 28.35 mL of distilled water [18] and was stored

in dark bottles. The IAT results were scored as clear (0), weak positive as shown by a few precipitates after swirling of the mixture for up to a minute (1), moderate as shown by the presence of a cloudy mixture with many small precipitates (2), and positive as shown by immediate formation of dark clumpy precipitates (3). Animals that were positive on the IAT were discarded from the herd in pelting season. In addition to IAT results, selection of replacements was based on reproductive performance as well as fur quality traits, such as size and short naps. Juveniles which were born into large litters, and adult females that produced large litters were retained for breeding. Mink with exceptionally high fur quality were occasionally retained for breeding even if they were born into small litters or produced small litters. Low producing females were pelted in December and most breeder males were pelted in March after breeding. The CIEP test had not been used as a selection tool on this farm until 2006, after which some seronegative animals were kept for breeding in 2007 and subsequent years for experimental purposes.

Breeding

Each female was exposed to a male between March 2 and 10 each year, to a different male 8 to 10 days later, and often to a third male one or two days after the second mating (multiple-sire mating). Approximately 100 females were bred to the same male up to three times in each of 2007 to 2010 (single-sire mating). The animals whelped between April 19 and May 14, and kits were weaned at the end of June (42 to 55 days of age) by removing the dam from the cage. The number of live kits was recorded on days 2 or 3 after birth and expressed as per female whelped (LS3) and recorded again at weaning. Kits were either separated and transferred to individual cages sometime after weaning or a male and a female sibling were caged together. Fostering was kept at a minimum in the single-sired group, and the number of weaned kits was corrected for the number of kits fostered in or out.

Sampling and laboratory procedures

Blood samples were collected by toenail clipping into heparinized capillary tubes for the CIEP test. On some sampling occasions, a second blood sample was collected for IAT. Sampling was performed between Nov 2005 and Aug 2010 for IAT (Table 1) and CIEP (Table 2). The CIEP test [3] was performed on plasma at the Animal Health Laboratory of the Nova Scotia Department of Agriculture in Truro, Nova Scotia, Canada, which is accredited for this test by the Standards Council of Canada. The test was performed using a cell-cultured antigen supplied by the United Vaccine, Inc., Madison, Wisconsin, USA. Spleen samples from 88 CIEP-positive and 10 CIEP-negative mink were collected aseptically after pelting in 2005, 2008 and 2009 and stored at –80°C until use. DNA was extracted from spleen samples, the presence of AMDV DNA was tested by the polymerase chain reaction (PCR) and the results were classified as positive, inconclusive or negative after multiple tests as previously described [15].

Data analysis

Data were analyzed using SAS, Version 9.4 for Windows (SAS Institute, Cary, NC, USA). There were a few inconclusive CIEP results which were considered as positive, and a few animals with IAT scores of 2 or 3 were combined with those with score 1 before analyses. A small number of 5- and-6-year old females were combined with the 4-year old group (4+) before analyses. Counts were compared using the likelihood ratio chi-square or Fisher's exact test. Two females which were exposed to males but did not breed were excluded from the analyses. The proportion of females which whelped of those mated and remained alive until weaning was analyzed using the GENMOD procedure with a binomial distribution and logit link function. The model included the fixed effects of system of mating (multiple- and single-sire), dam age, CIEP status of dams on the Nov test, number of matings and regressions of breeding year and date of first mating. The two-way interactions between the fixed effects were included in the model, but were not significant and were removed from the final analyses. The random effect of individual mink was used in the REPEATED statement to take care of the correlation among mink used in different years. Least squares means and their standard errors were obtained after conversion to the original scales by the ilink option, and multiple comparison of means was performed using Tukey's adjustment. The distribution of percent kit mortality of each litter from birth to weaning was positively skewed and was analyzed using the GENMOD procedure as explained above, with the exceptions of using Poisson distribution and log link function. The distributions of LS3 and the number of weaned kits were approximately normal and were

analyzed using a linear mixed effect model (PROC MIXED) with a variance component (VC) covariance structure. The model included the fixed effects of mating-system, age of dam, CIEP status of dam in Nov, number of mating 5, and covariances of breeding year and date of first mating, and random effect of individual mink.

CIEP test results of 17 males, 106 females and their 411 kits in Sep and Nov 2007 were used to estimate heritability of and genetic correlation between CIEP test results of kits at 5 and 7 months of age. Each male, with a few exceptions, was paired with 6 or 7 females in a single-sire mating scheme. The breeding pairs had different combinations of CIEP status. Fostering was kept at a minimum possible, and a few litters with kits fostered in or out were excluded from this analysis. Heritability was estimated using a multi-trait animal model which included the CIEP results of parents and kits in Sep and Nov, fixed effects of sex and age at the time of testing (month), the random animal additive genetic effect and the random residual effect. Variance components were estimated using restricted maximum likelihood (REML) as implemented in the program VCE-5 [19], despite the binary nature of the trait. Breeding values were obtained by the PEST software [20].

Results

IAT status of breeder mink

The frequencies of IAT-positive breeder males and females born between 2004 and 2008 and tested in Nov 2005, 2006 and 2008 were all less than 5%, but the estimates in Feb tests in 2008 and 2010 were variable and often greater than 7% (Table 1). Mink born in each year showed a decreasing trend for the incidence of IAT-positive cases over time, and most animals which were kept for breeding longer than one year tended to have negative IAT in Nov tests.

Test date	Birth year						All years
	2004	2005	2006	2007	2008	2009	
2005-Nov	3.1 (389)	4.8 (249)	.	.	.	.	3.8 (638)
2006-Nov	3.2 (313)	1.3 (160)	0.0 (34)	.	.	.	2.4 (507)
2008-Feb	7.1 (28)	0.0 (16)	16.7 (6)	12.8 (82)	.	.	9.8 (132)
2008-Nov	0.0 (9)	0.0 (9)	0.0 (3)	2.0 (49)	0.0 (64)	.	0.7 (134)
2010-Feb	0.0 (2)	.	100.0 (2)	0.0 (12)	0.0 (12)	13.9 (165)	12.9 (193)

Table 1: *Percentage of IAT-positive breeder mink by year of birth.*
Key: Number of animals tested are shown in brackets.

CIEP status of breeder mink

The incidence of CIEP-positive breeder males and females born in 2004 and 2005 ranged between 74.7% and 83.8% in the three tests conducted in 2005 and 2006, and those which were kept for breeding showed declining trends in subsequent tests (Table 2). Three of the six 2004-born mink which were retained for breeding until Feb 2009 (58 months of age) remained CIEP negative throughout their lives, whereas the other three showed variable CIEP status, of which one became CIEP positive in Aug. 2010 at 75 months of age. In contrast, one of the seven 2005-born mink which were retained for breeding until Nov 2009 (54 months of age) was CIEP negative throughout its life, two were CIEP-positive in all tests and the other four became CIEP-positive later in their lives. Some seronegative kits were deliberately selected for breeding in 2007, 2008 and 2009, resulting in low incidence of CIEP-positive cases in Sep of the year in which they were born (23.8%, 31.2%, and 0.6% in 2007, 2008 and 2009, respectively), but all gradually became seropositive over time. A few mink which were born in 2006, 2007 and 2008 and retained for breeding until Feb 2010 were all seronegative.

Test date	Birth year						All years
	2004	2005	2006	2007	2008	2009	
2005-Nov	79.2 (389)	74.7 (249)	.	.	.	.	77.4 (638)
2006-Jul	83.6 (318)	82.4 (182)	.	.	.	.	83.2 (500)
2006-Nov	80.8 (313)	83.8 (160)	38.2 (34)	.	.	.	78.9 (507)
2007-Feb	49.5 (97)	35.7 (42)	.	.	.	.	45.0 (139)
2007-Aug	54.5 (77)	36.8 (38)	25.0 (8)	.	.	.	47.2 (123)
2007-Sep	58.4 (77)	39.5 (38)	37.5 (8)	23.8 (84)	.	.	40.1 (207)
2007-Nov	61.6 (73)	50.0 (36)	.	34.5 (84)	.	.	47.7 (193)
2008-Feb	39.3 (28)	62.5 (16)	33.3 (6)	32.9 (82)	.	.	37.9 (132)
2008-Sep	30.8 (13)	61.5 (13)	33.3 (3)	59.1 (66)	31.2 (47)	.	47.2 (142)
2008-Nov	44.4 (9)	77.8 (9)	33.3 (3)	55.1 (49)	43.8 (64)	.	50.0 (134)
2009-Feb	16.7 (6)	71.4 (7)	0.0 (3)	48.8 (41)	50.0 (64)	.	47.9 (121)
2009-Sep	0.0 (4)	85.7 (7)	0.0 (3)	62.2 (37)	59.6 (52)	0.6 (166)	22.7 (269)
2009-Nov	0.0 (2)	85.7 (7)	0.0 (2)	58.1 (31)	62.9 (35)	2.4 (166)	20.6 (243)
2010-Feb	50.0 (2)	.	0.0 (2)	8.3 (12)	0.0 (12)	13.3 (165)	12.4 (193)
2010-Aug	0.0 (1)	.	0.0 (1)	0.0 (7)	0.0 (9)	13.1 (99)	11.1 (117)

Table 2: *Percentage of CIEP positive breeder mink in different tests by birth year.*

Key: Number of animals tested are shown in brackets.

Persistency of CIEP status of the same breeder mink in successive tests

The incidence of seronegative breeder mink which became seropositive in the consecutive test varied between 0.7 and 20.0% over various sampling occasions, with an overall mean of 6.4% (Table 3). The largest changes occurred from Feb to Sep in 2008 (20.0%) and 2009 (10.7%), and from Sep to Nov in 2007 (8.8%) and 2008 (10.3%). The change from seronegative to seropositive in the same periods in other years was rather low. The inconsistencies in the magnitude of changes for CIEP status between consecutive tests in the same period was particularly evident between Nov and Feb, which varied between 0.7% in 2006 and 11.9% in 2009. The incidences of CIEP-positive mink which turned negative in the next test were generally low, ranging between 0.4% and 4.1%, with an overall mean of 1.5%. Chi-square tests showed that differences in CIEP-status in consecutive tests were highly significant for all sampling occasions, except for those between Nov 2009 and Feb 2010.

Test dates	No. tested	Negative became positive	Positive became negative
Nov 2005 - July 2006	500	7.2	0.6
July 2006 - Nov 2006	380	2.4	1.8
Nov 2006 - Feb 2007	140	0.7	2.9
Feb 2007 - Aug 2007	115	4.4	0.9
Aug 2007 - Sep 2007	123	5.7	1.6
Sep 2007 - Nov 2007	193	8.8	1.6
Nov 2007 - Feb 2008	126	2.4	2.4
Feb 2008 - Sep 2008	95	20.0	3.2
Sep 2008 - Nov 2008	104	10.3	0.9
Nov 2008 - Feb 2009	121	5.0	4.1
Feb 2009 - Sep 2009	103	10.7	2.9
Sep 2009 - Nov 2009	243	3.7	0.4
Nov 2009 - Feb 2010	193	11.9	1.0
Feb 2010 - Aug. 2010	117	3.4	0.9
Overall	2535	6.4	1.5

Table 3: *Percentage change in CIEP status of the same breeder mink in consecutive tests.*

CIEP status of kits

Frequency of CIEP-positive kits in Sep tests ranged between 26.3% and 73.7%, with an overall mean of 45.3%, and the estimates for Nov tests ranged between 41.5% and 68.7%, with an overall mean of 55.2% (Table 4). The number of seronegative kits in Sep which became seropositive in Nov ranged between 6.2% and 18.5%, with an overall mean of 13.9%. No seropositive kit turned negative from Sept to Nov. Differences among years for the incidence of CIEP positive kits were significant in Sep ($\chi^2_{(3)}$ = 163, P < 0.001) and Nov ($\chi^2_{(3)}$ = 109, P < 0.001) as well as for changes from Sep to Nov ($\chi^2_{(3)}$ = 39.9, P < 0.001).

Birth year	September		November		% increase
	No	% Positive	No	% Positive	
2007	411	26.3	407	41.5	15.2
2008	247	73.7	99	68.7	17.2
2009	599	51.6	594	69.9	18.5
2010	471	39.3	470	45.5	6.2
Overall	1728	45.3	1570	55.2	13.9

Table 4: *Percent CIEP-positive kits in September and November, and changes in CIEP status of the same individuals.*

Joint distribution of CIEP and IAT results of breeder mink

The joint distribution of IAT and CIEP test results of breeder mink revealed that the majority of animals were CIEP positive and IAT negative in Nov tests in 2005, 2006 and 2008 (50.0% to 77.1%), followed by those which were CIEP and IAT negative (20.5% to 49.3%) (Table 5). The situation reversed in Feb tests in 2008 and 2010, i.e. the CIEP and IAT negative cases became the predominant group whereas CIEP-positive and IAT-negative cases became the second largest group. The incidence of CIEP and IAT positive (0.0 to 2.8%) and CIEP negative and IAT positive mink (0.6 to 0.9) in Nov were low, and both measurements were higher in Feb tests. The Chi-square tests revealed that the relative frequencies of CIEP and IAT were independent of each other at every testing occasion, but the difference between the joint distributions of CIEP and IAT in Nov and Feb was significant ($\chi^2_{(3)}$ = 301, P < 0.001).

Test date	Number tested	IAT-negative		IAT-positive		$\varkappa^2_{(1)}$ (probability)
		CIEP-negative	CIEP-positive	CIEP-negative	CIEP-positive	
2005-Nov	638	21.6	74.6	0.9	2.8	0.08 (0.77)
2006-Nov	507	20.5	77.1	0.6	1.8	0.11 (0.74)
2008-Feb	132	56.1	34.1	6.1	3.8	0.01 (0.96)
2008-Nov	134	49.3	50.0	0.8	0.0	1.39 (0.23)
2010-Feb	193	77.7	9.3	9.8	3.1	2.98 (0.08)
Total-Nov	1279	24.1	73.0	0.8	2.1	0.09 (0.76)
Total-Feb	325	68.9	19.4	8.3	3.4	0.90 (0.34)

Table 5: *Joint distribution of CIEP and IAT results of breeder mink.*

Key: $\varkappa^2_{(1)}$ = Chi-square with one degree of freedom.

Heritability of seropositive kits

Estimates of additive genetic variance, residual variance and heritability for the CIEP-positive kits at five and seven months of age (Sep and Nov) are shown in table 6. Estimates of heritability were 0.239 and 0.218 at five and seven months of age, respectively, and the genetic correlation between the two measurements was 1.0.

Source	September	November	Covariance
σ^2_A	0.045705	0.051463	0.04850
σ^2_e	0.145740	0.18480	0.09999
h^2	0.239	0.218	1.000

Table 6: *Estimates of additive genetic variance (σ^2_A), residual variance (σ^2_e) and heritability (h^2) of seropositive kits in September and November.*
Key: Genetic covariance between additive genetic effect and residuals in Sep and Nov tests, and the genetic correlation between the two measurements.

Joint distribution of CIEP and PCR results

A high proportion of the 88 CIEP-positive mink were also PCR-positive (90.9%), whereas 40% of the 10 CIEP-negative mink were PCR-negative. The PCR results of CIEP-positive mink were inconclusive in 7.9% and negative in 1.1% of the mink, whereas the incidences of inconclusive and negative PCR results in CIEP-negative mink were 20% and 40%, respectively (Table 7).

Birth year	Sampling year	Number of samples	CIEP	PCR positive	PCR inconclusive	PCR negative
2002 - 2004	2005	43	Positive	43	0	0
2003 - 2007	2008	10	Positive	6	4	0
2005 - 2008	2009	7	Positive	5	2	0
2009	2009	28	Positive	26	1	1
2009	2009	10	Negative	4	2	4

Table 7: *Number of PCR positive, inconclusive and negative spleen samples by CIEP results in 98 mink sampled in December 2005, 2008 and 2009.*

Female fertility

Two of the 1057 females which were exposed to males did not breed and were excluded from further analyses. The number of females whelped of those mated (fertility) ranged between 79.6% and 97.0% in different years and mating systems, with an overall mean of 92.8% (Table 8). The GENMOD analysis showed that female fertility was not significantly affected by CIEP status in Nov, age of dam, breeding year or date of first mating (Table 9), but multiple-sire mating increased fertility by 10.0% ($P < 0.001$) over the single-sire mating scheme, and fertility of females which bred once was 11.0% and 16.8% lower than those bred twice or three times, respectively, and all differences were significant.

Litter size

The average of LS3 in different breeding years and mating schemes ranged between 4.24 and 6.06, and the average number of kits weaned varied between 3.86 and 5.77, with the overall means of 5.51 and 5.30, respectively (Table 8). Multiple-sire mating resulted in

Measurement	Multiple-sire mating			Single-sire mating				Overall
	2006	2007	2010	2007	2008	2009	2010	
Number mated	500	130	35	106	98	87	99	1055
Female fertility, %	97.0 ± 17.1	95.4 ± 21.1	88.6 ± 32.2	86.8 ± 34.0	79.6 ± 40.5	89.7 ± 30.6	91.9 ± 27.4	92.8 ± 25.8
Number whelped	485							
Live kits at 3 days		124	31	92	78	78	91	979
Per female whelped	6.02 ± 1.68	6.06 ± 1.76	4.77 ± 2.63	5.11 ± 2.36	4.24 ± 2.16	4.24 ± 2.38	4.90 ± 2.41	5.51 ± 2.11
Per female mated	5.84 ± 1.95	5.78 ± 2.14	4.23 ± 2.91	4.43 ± 2.80	3.38 ± 2.58	3.80 ± 2.61	4.51 ± 2.82	5.11 ± 2.48
Number weaned								
Per female whelped	5.73 ± 1.65	5.77 ± 1.76	4.76 ± 2.32	4.85 ± 2.37	3.86 ± 2.27	4.14 ± 1.87	4.76 ± 2.25	5.30 ± 1.98
Per female mated	5.56 ± 1.89	5.50 ± 2.11	4.21 ± 2.69	4.20 ± 2.75	3.06 ± 2.56	3.76 ± 2.27	4.35 ± 2.54	4.91 ± 2.36
Mortality, %	4.4 ± 10.5	4.6 ± 10.1	6.9 ± 19.3	9.0 ± 22.1	16.4 ± 30.0	7.4 ± 17.0	7.8 ± 15.3	6.4 ± 15.8

Table 8: *Means and standard deviations of female fertility, number of kits born alive and weaned and kit mortality from birth to weaning by mating system and breeding year.*

Key: Female fertility = % of females whelped of those bred. Two females which were exposed to males but did not breed were discarded from the analyses.

Live kits at 3 days = number of live kits recorded at 2 or 3 days after birth. Ranged between 0 and 11.

Number weaned = Number of kits weaned (42 to 55 days of age). Ranged between 0 and 10.

Mortality % = Mortality from day 3 until weaning of each litter. Ranged between 0% and 100%.

0.8 extra kit per litter at birth and weaning ($P < 0.001$) and 1.1 extra kit at birth and weaning per dams mated ($P < 0.001$) (Table 9). The average number of kits born and weaned were the highest and the lowest for 2-year old and 4-year old dams, respectively, and all differences were significant. Two-year old dams also had greater liter sizes at birth and weaning than juveniles, and differences were significant except for the number of live born kits per dams mated. There were linear, albeit non-significant, increases in litter size at birth and weaning as the number of matings increased, but differences in litter size at birth and weaning per dams mated were significantly smaller for dams bred once compared with those bred twice or three times (Table 9). The CIEP status of dams in Nov test, breeding year and date of first mating had negligible effects on the measures of litter size.

Kit mortality

Kit mortality from day 3 after birth to weaning ranged between 4.4% and 16.4% in different years and systems of mating, with an overall mean of 6.4% (Table 8). Kit mortality was almost twice as high for single-sire mating as for multiple-sire mating ($P < 0.05$), but the effects of CIEP status in Nov, age of dam, number of mating, breeding year and date of first mating were not significant.

Variables		Live kits at 3 days of age		Number weaned		Pre-weaning mortality, %
	Female fertility, %	Per dam Whelped	Per dam mated	Per dam whelped	Per dam mated	
Mating system	**	**	**	**	**	*
Multiple-sire	94.4 ± 1.5a	5.52 ± 0.14a	5.08 ± 0.15a	5.26 ± 0.13a	4.84 ± 0.14a	5.35 ± 0.94a
Single-sire	84.4 ± 3.4b	4.71 ± 0.16b	3.94 ± 0.17b	4.44 ± 0.16b	3.70 ± 0.17b	10.85 ± 2.22b
CIEP, Nov	NS	NS	NS	NS	NS	NS
Negative	91.4 ± 2.0	5.15 ± 0.15	4.56 ± 0.16	4.86 ± 0.14	4.29 ± 0.15	8.23 ± 1.37
Positive	89.6 ± 1.7	5.08 ± 0.12	4.46 ± 0.13	4.85 ± 0.12	4.25 ± 0.12	7.07 ± 0.92
Age of dam	NS	**	**	**	**	NS
1	91.4 ± 1.7	5.12 ± 0.13ac	4.58 ± 0.14ab	4.81 ± 0.13ac	4.30 ± 0.14a	7.63 ± 1.15
2	92.5 ± 2.0	5.55 ± 0.14b	4.99 ± 0.16a	5.35 ± 0.14b	4.80 ± 0.15b	5.87 ± 1.03
3	88.1 ± 2.8	5.27 ± 0.16ab	4.58 ± 0.17ab	5.06 ± 0.15ab	4.39 ± 0.17ab	6.58 ± 1.27
4	89.5 ± 3.8	4.51 ± 0.25c	3.90 ± 0.27b	4.19 ± 0.24c	3.59 ± 0.26c	11.46 ± 3.14
No. of matings	**	NS	**	NS	**	NS
1	79.3 ± 4.9a	4.79 ± 0.26	3.84 ± 0.27a	4.51 ± 0.24	3.48 ± 0.25a	8.54 ± 2.58
2	90.3 ± 2.2b	5.18 ± 0.16	4.62 ± 0.17b	4.95 ± 0.15	4.36 ± 0.13b	7.30 ± 1.41
3	96.1 ± 1.0c	5.35 ± 0.12	5.09 ± 0.13b	5.10 ± 0.11	4.76 ± 0.16b	7.11 ± 1.07
Breeding year	NS	NS	NS	NS	NS	NS
b ± SE	0.060 ± 0.156	-0.123 ± 0.083	-0.091 ± 0.091	-0.035 ± 0.079	-0.022 ± 0.088	-.057 ± 0.106
First mating date	NS	NS	NS	NS	NS	NS
b ± SE	0.053 ± 0.083	-0.025 ± 0.046	0.001 ± 0.052	-.03 ± 0.044	-0.007 ± 0.050	0.046 ± 0.055

Table 9: *Least-squares means* ± **standard errors of** *mating system, November CIEP test, age of dam, number of matings, and covariances of breeding year and date of first mating for female fertility, kits born alive and weaned and mortality.*

Key: Means followed by different letters are different at P < 0.05 after Tukey's adjustment.

*** = Differences are significant at P < 0.01.*

** = Differences are significant at P < 0.05.*

NS = Differences are not significant.

Discussion

Selection for IAT-negative mink on the MM farm favored healthy animals regardless of the source of infection [18,21], including AMDV-infected mink with low levels of gamma globulin. The finding that the incidence of IAT-positive mink in Nov, when animals were tested prior to selection of the replacements, was less than 5% in all years is comparable with 5.3% reported previously on 773 mink from the same farm which were tested in Nov 2004 [22]. The finding was, however, at variance with the higher incidences of IAT-positive mink of an AMDV-infected herd of black mink in Nova Scotia which was under the test-and-cull strategy and measured over two years in Nov (27.3% and 16.0%) and Feb (35.0% and 21.0%) [23]. The large difference in the incidence of IAT positive cases between the two studies could be the result of selection for negative IAT on the MM farm, the difference in the overall health status of animals on these two farms, or because different operators dido not score the results exactly the same [21]. Breeder mink which were born in each year on the MM farm showed declining trends for the incidence of IAT-positive cases over time (Table 1), implying that animals which could maintain low levels of serum gamma globulin for an extended period of time had a higher chance of having a high reproductive performance and a better general health, and were thus retained for breeding.

Stability of serum gamma globulin level in infected mink over time is important when using IAT as a tool for evaluating the health status of the mink. Contrary to this requisite, serum gamma globulin levels and IAT results fluctuated in repeated tests [11,23-25]. Although all IAT-positive mink were culled in Nov, the selected animals often showed an increase in IAT-positive cases in Feb before the start of

breeding in the present (Table 1) and a previous study [23], which causes uncertainties on the reliability of IAT for assessing the health status of animals at breeding time in March. In another study, severe and reversible hypergammaglobulinemia was observed during pregnancy in the AMDV-infected violet and sapphire mink, and their kits had the lowest gamma globulin level in July, followed by either minor increases in some kits until Dec or sharp and irreversible increases in other kits [25], which shows the presence of natural changes in gamma globulin level, and confirms flaws in IAT as a tool for assessing health status of the mink over an extended period of time.

The high reproductive performance of mink in the current study, and the fact that mink on this farm had minor histopathological lesions of AD [15] are proof that the selection criteria used were effective for establishing a tolerant herd, but the relative contribution of each measurement to the final outcome cannot be assessed. It is tempting to speculate that visual assessment of health status and reproductive performance were the major forces for the establishment of tolerance, at least during the advanced phase of selection, and that the contribution of IAT results, which is an unstable attribute that was measured three months before breeding, was perhaps trivial. If this assumption is true, visual assessment of animal health on a regular basis, along with the measures of reproductive performance, offers a practical method for assessing the degree of tolerance of live mink on infected farms. Measuring the IAT status of animals in Feb, rather than during the pelting season (Nov - Dec), does not seem to be practical for economic reasons.

The high incidence of CIEP-positive breeder mink which were born in 2004 and 2005 and tested in Nov 2005 and 2006 (74.7% to 83.8%) is comparable with 84.4% in 773 mink from the same farm tested in Nov 2004 [22] and 84.7% in 59 mink tested in Nov 2000 (unpublished data), confirming that this farm has been persistently infected with AMDV. It is well documented that some mink with detectable levels of anti-AMDV antibodies remain healthy [3,11,12,14,23], which are possibly those with low antibody titers [9,10,13], suggesting that CIEP-positive mink on the MM farm likely had low antibody titers. The low antibody titer was also manifested as fluctuations in the CIEP status of individuals which were retained on the MM farm for 55 months and in a previous study [23]. In concordance with the observation on the MM farm, some mink on other chronically infected farms were CIEP-negative [3,15,26], which could be the result of low antibody titers falling below the detection threshold of CIEP. Alternatively, the presence of CIEP-negative mink which were also PCR-negative on MM farm (Table 7) and in previous studies [12,23] suggests that some seronegative mink could have either cleared the virus after infection, or had a non-functional virus receptor and were not infected, as shown for the parvovirus B19 in humans [27].

The declining trends in the incidence of seropositive cases on successive tests for mink which were born in 2004, 2005 and 2006 and were retained for breeding (Table 2), suggest that mink with low antibody titers, or those which cleared the virus, were more likely to remain healthy with high reproductive performance until old age. The decrease in antibody titer over time was previously reported [9,26] and the incidence of high antibody titers measured by ELISA was significantly higher for juveniles (73%) than for the older mink (14%) on the same farm in Sweden [8]. There was, however, an exception to the above statement for the 2005-born animals which were retained for breeding for almost four years and were mostly CIEP-positive. The rather low frequency of seropositive mink in Sep of the year in which they were born in 2007, 2008 and 2009 was the result of intentionally selecting some seronegative kits as replacements. The incidence of seropositive cases in these animals increased over time, but never exceeded 63% (Table 2), which is evidence that CIEP status is a heritable trait and could be gradually changed by selection.

The increases in the incidence of seropositive cases from Sep to Nov in the same breeder animals (Table 3) and kits (Table 4) agree with previous studies [14,23]. An increase in mean antibody titer in juvenile mink from Oct to the next Feb was observed, which resulted in 55.2% of seronegative mink becoming seropositive, with a poor Spearman's rank correlation coefficient between the two measurements [29]. The reasons for increases in the incidence of seropositive cases in chronically infected herds during the Fall and winter are not clear, and several factors could be involved, such as increase in antibody titer in previously infected mink as a result of stress caused by handling mink for pelt evaluation, cold weather or changes in the level of hormones which modulate molting of the summer fur and growth of winter pelage [30]. The observation that the magnitude of change in CIEP status in consecutive tests in the same period varied

among years implicates the contribution of other factors as well. Large increases in seropositive cases of breeder mink from Feb to Sep in 2008 (20.0%) and 2009 (10.7%) (Table 3) could be the result of stress caused by pregnancy, lactation or summer heat.

Persistency of antibody production is widely reported for experimentally inoculated mink [13,28,31], but the results of the current and previous studies [23,29] show that a small number of seropositive adult mink became seronegative, which could be those with marginal antibody titers. The observation that seropositive kits did not become seronegative from Sep to Nov agrees with a previous report [23] and could be the result of the short time between the two measurements. In a previous study, however, three of the12 seropositive kits at weaning (2 month of age) became seronegative four months later, and the presence of seropositive reaction in earlier times was attributed to the passively acquired maternal antibodies from the mother's milk [11].

The long-term selection for IAT-negative mink on the MM farm resulted in the greatest number of mink having low levels of serum gamma globulin, i.e. negative IAT, but with positive CIEP results (50.0% to 77.1%) in Nov test (Table 5). It was expected that IAT negative animals were CIEP-negative as well, because antibody titer is positively associated with serum gamma-globulin level [3,10,32,33], whereas these two measurements were statistically independent of each other in the current study (Table 5). The high frequency of IAT-negative and CIEP-positive mink could be the manifestation of the pattern of association between these two measurements; being weak at low antibody titers and positive and strong at high antibody titers [34]. Although not explicitly stated, a similar relationship can be inferred from the data presented before [10] as well. It is logical to hypothesize that selection for negative IAT in healthy animals on the MM farm resulted in reduced gamma globulin level and low antibody titer, causing a weak relationship between them. Antibody titer in some mink fell below the detection threshold of CIEP, resulting in animals with negative IAT and CIEP to be the second largest group in Nov test (21.6% to 49.3%). It is also possible that some of the mink which were negative on both tests were resistant to infection by AMDV, i.e. either cleared the virus [12,23], or did not become infected as a result of non-functional virus receptor.

A small number of mink in the current study (less than 3%) were positive on both IAT and CIEP in Nov, which were possibly unhealthy animals as a result of infection with AMDV. The smallest group of mink were negative on CIEP but positive on IAT, which were possibly those infected with pathogens other than AMDV, suggesting the presence of a low viral or bacterial burden on the MM farm. The joint distribution of CIEP and IAT results in Nov in the current study is comparable with the estimates on this farm in 2004, i.e. 79.4% CIEP-positive and IAT negative, 15.2% CIEP and IAT negative, 4.9% CIEP and IAT positive, and 0.4% CIEP-negative and IAT positive [22]. The higher incidence of IAT-negative and CIEP-positive group in Nov than in Feb by 53.6%, and the lower incidence of CIEP and IAT negative group in Nov than in Feb by 44.8%, were the manifestation of increases in IAT-positive (Table 1) and decreases in CIEP-positive cases (Table 2) from Nov to Feb.

The high incidence of PCR-positive in CIEP-positive mink (90.9%) is evidence for the persistence of viral infection in mink with detectable antibody titers. The incidence of PCR-negative in CIEP-positive mink (1.1%), agrees with a previous estimate (2.2%) in Canada [15] but is much lower than 9.2% and 34.7% in two Danish studies [35,36] and 17.3% in ELISA-positive and PCR-negative free-ranging mink in Sweden [37]. Multiple PCR tests performed on each sample is likely the reason for the low incidence of PCR-negative in CIEP-positive samples in the current study, which could be the result of low viral loads in the samples, differences between the sequences of the primers and their targets on the viral genome or false positive CIEP. The incidence of PCR-positive in the CIEP-negative mink on the MM farm (40%) was higher than previous estimates, namely 16.5% in Canada [15], 12.5% in China [38], 4.5% in Sweden [37] and 1% in Denmark [35]. These animals were infected but their antibody titers were below the detection threshold of the CIEP test, which was anticipated on the MM farm as the direct result of selecting healthy animals with low gamma globulin levels.

The negative CIEP and PCR test results implies that these mink were likely not infected by AMDV. The estimate (40%) is lower than 65.4% in a Canadian study [15] and 82.8% in a Danish study [35] for infected farms but is comparable with 37.6% ELISA and PCR negative free-ranging mink in Sweden [37]. The mink in this category were those which were exposed to the virus but were not infected because

they could prevent the establishment of infection when exposure to low viral doses, or they had a non-functional virus receptor. Alternatively, they were infected but cleared the virus, or their spleens contained low virus copy numbers which resulted in PCR failure. Regardless of the reason, it may be hypothesized that CIEP- and PCR-negative animals had a different genetic constitution that prevented them from persistent viral infection and needs to be treated separately from PCR-positive animals when selecting for tolerance. The presence of inconclusive PCR results, which was greater for the CIEP-negative (20%) than CIEP-positive cases (7.9%), was very likely the result of low virus copy number in the samples which caused weak and erratic PCR amplifications. The inconclusive results can only be estimated when multiple tests are performed on each sample [15,39,40]. Sample contamination was not the cause of low virus copy number because DNA extraction, PCR cocktail preparation, PCR amplification and evaluation of PCR products were performed in four different laboratories.

The estimates of heritability in the current study (Table 6) are smaller than those for seropositive kits of black mink at four (0.573) and seven (0.497) months of age (Aug and Nov) on a farm which implemented the test-and-cull strategy [23]. These estimates of heritability indicate that selection for CIEP negative kits will be effective in reducing seropositive kits, which would be the result of decreased antibody titer or delays in the time of infection beyond seven months of age. Long-term removal of seropositive animals on farms that followed the test-and-cull strategy is an unintended but direct selection for low antibody titer and thus healthier mink. This approach can possibly result in genetics of mink under the test-and-cull strategy to differ from mink on the MM farm where CIEP was not used as a selection tool and could explain the differences between the estimates of heritability in the current and previous study. Only a subset of data were used in computing heritability, because of difficulties in performing single-sire mating and avoiding cross-fostering on commercial farms. Tracing the pedigree of fostered kits is only possible when they can be mixed with a littermate of the opposite sex, which is often not practical. In the current study, heritability of binary data was calculated using a linear model, which resulted in the estimates being lower than those which would be obtained from a threshold model [42,43].

The observations that only two of the 1057 females which were exposed to males did not breed, and 92.8% of those mated whelped (Table 8) are comparable with the estimates on uninfected farms. For instance, number of females whelped of those exposed to males was 92.2% and 95.8% for CIEP-negative black mink in Nova Scotia in two years [23], 97.5% in Poland [44], 4.9%, 6.2% and 11.2% barren females under three mating systems in Sweden [45] and 8.7% barren females in Denmark [46]. The estimates of female fertility in the current study were greater than those on an AMDV-infected farm with 31% and 14% barren females in two studies in Sweden [8]. The results suggest that female fertility has noticeably been improved by selection on the MM farm.

The overall means of LS3 (5.51) is in the lower range of the reported values for the number of live-born kits on uninfected farms, such as 5.8 and 5.28 in two studies in Poland [44,47], 5.68, 7.17 and 7.46 live kits after 24h in seven mink color types on three AMD-free Danish farms [48], 6.75 and 5.0 kits within 48h after birth in brown and black mink, respectively, in Denmark [49], 5.67 in dark mink in Denmark [50], 5.0 (born dead or alive) in Denmark [46], 5.77 in 11 mink color types from 2002 to 2016 in Nova Scotia [51], and 5.6 in Sweden [45]. Numbers of live-born kits per female mated and per female whelped were 5.71 and 6.29, respectively, in wild-type CIEP-positive mink in Argentina [52]. The number of kits born alive was likely greater than 5.51 in the current study because it does not include mortality during the first two days of age.

The overall mean of the number of kits weaned per female whelped in the current study (5.30) is in the upper range of reported values for AMDV-free farms, including 4.67 at 43 days of age in a population of dark mink in Denmark [50], 4.48 and 4.9 in two Polish mink herds [44,47] and 5.12 in Nova Scotia [51]. Litter size at weaning per female mated in the present study (4.91, Table 8) was comparable with 4.5, 4.8 and 5.0 at three weeks of age under three mating systems in Sweden [45] and 4.71 and 5.29 for the number of kits weaned per CIEP-negative females exposed to males in a herd of black mink in Nova Scotia in two years [23]. Litter sizes per female whelped at three weeks of age on AMDV-infected farms in two studies in Sweden were 5.2 and 5.9 [8]. The above estimates and the results of the present study suggest minor negative effects of AMDV-infection on litter size at weaning.

The overall mortality rate of kits from day 3 after birth to weaning was low (6.4%), which is partly because it does not include mortality during the first two days after birth, a crucial period during which kit mortality is high. The preweaning mortality rate on an AMDV-free farm in Ontario (Canada) was 20%, of which 91% occurred within the first three days after birth [53] and kit mortality from birth to weaning at four weeks of age on two CIEP-positive farms in Argentina was 25.8%, of which 61.9% occurred during the first week after birth [52]. The estimates for kit mortality from birth to weaning at 50 days of age were 11.3%, 20.4% and 21.2% for three lines of mink in Denmark [46] and 27.4% and 21.0% from birth to weaning at 50 days of age in a herd of black mink in Nova Scotia in two years [23]. Survival rates of live-born kits until weaning at six weeks of age in a herd of mink in Nova Scotia was 78.4% [51] and that in a herd of dark mink in Denmark was 82% until 43 days of age [50], which are lower than 96.2% survival from day 3 to weaning in the present study. The exceptionally low rate of kit mortality in the present study was possibly one of the most important achievements of selection for tolerance on the MM farm.

There is broad consensus that AMDV infection has negative effects on reproductive success and kit survival [8,44,54-57]. For instance, the incidence of females which did not whelp of those expose to males were 11.8%, 12.4% and 2.5% in two AMDV-infected and one AMDV-free farms in Poland, respectively, and the corresponding values for the number of kits born alive were 3.4, 3.1 and 5.8, and for the number of kits weaned were 2.2, 2.1 and 4.9 [44], showing a considerable negative effect of infection on measures of reproduction. Fetuses and newborn kits of infected dams often carry the virus [54,55,57] and mortality rate of CIEP-positive kits (10.4%) was significantly greater than that of CIEP-negative kits (0.62%) in a previous study [23]. In the current study, however, the effects of CIEP-status of the dam in Nov were negligible on all measures of reproduction (Table 9), which is likely the manifestation of selection for tolerance. The absence of a negative effect of AMDV infection on measures of reproduction agrees with a previous field study showing that numbers of kits weaned and pelted were not different between naturally infected seropositive and seronegative dams [26]. In another study, mink with high antibody titer measured by ELISA had a higher risk of being barren, but AMDV infection status had a minor effect on litter size at three weeks of age [8]. Discrepancies among different studies could be the result of differences in virus strain and mink genotype [41].

Multiple-sire mating is a standard procedure among mink farmers because its positive effects on fertility and kit survival have been well known for many years. The results of the current study clearly supported this practice, because multiple-sire mating improved female fertility by 10%, increased litter sizes at birth and weaning by almost one kit, and decreased kit mortality rate to half (Table 9). The reasons for the positive effects of multiple-sire mating cannot be pinpointed in this study, but it may be postulated that the negative effects of infertile or sub-fertile males on female fertility are compensated for by the other male(s). This is logical in mink which exhibits multiple waves of ovulation and delayed implantation. Blastulae which are implanted are possibly those with some genetic superiority inherited from one of the males, which help them survive until birth and up to weaning. In a previous study, females which mated once had a significantly higher incidence of infertility than those bred twice (apparently with two males), and the difference was more pronounced for yearlings than for older females, but the number of live kits at birth were not affected by the number of matings [45].

The observation that females which bred once had 11.0% and 16.8% lower fertility than those bred twice and three times, respectively, implies that females which refused to breed for the second time were those which had health or reproductive problems. In agreement with the results of the present study, the incidence of barrenness for mink in Sweden which mated once (11.2%) was significantly higher than those which mated twice (4.9% and 6.2%) [45], but difference in the incidence of barren females between those bred once or twice was not significant in a Danish study [46]. Although some females refused to breed for the third time, the reason for the majority of those which mated only two times was the lack of manpower on the MM farm. Three matings significantly increased female fertility by 5.8% over those mated twice, which agrees with a previous report where the incidence of barrenness decreased as the number of matings increased, although it was influenced by the intervals between repeated matings [58]. The incidence of barrenness in females which were mated on a comparable schedule with that in the current study, i.e. 1, 1+9, and 1+(>7)+1 were 32.6%, 15.2% and 6.8% [58], showing greater increases in female fertility by multiple mating than observed in the present study.

Non-significant increases in litter size at birth and weaning by increases in the number of matings in the present study agrees with previous reports [45,46], but is contrary to another report [59] showing that two matings resulted in significantly larger litters at birth and weaning compared with those mated 3, 4 or 5 times. Differences for litter size among mink mated 1, 2, or 3 times, as well as in the intervals between repeated matings was reported [58]. It was shown that litter size at birth and pre-weaning survival rate were not significantly affected by the number of matings (1, 2 or 3 matings), whereas this factor had a significant effect on the number of kits weaned, but no explanation was provided for such observation [51]. The significant increases in litter size at birth and at weaning when expressed as per dam mated in the current study (Table 9), was the manifestation of large increases for female fertility by increases in the number of matings.

Date of first mating had negligible effects on female fertility, litter size and kit mortality in this study. These findings contradict a previous report that late mating increased female fertility and improved litter size, attributed to increased ovulation and decreased pre-implantation mortality, particularly in older females [45]. The lack of a significant affects of date of first mating on fertility and litter size in the current study could be the result of a short duration of first mating (8 days).

The high percentage of mink whelped of those mated resulted in small and non-significant differences among age groups in the current study (Table 9), which is contrary to a previous study where the rates of barren females were significantly greater in old females (2 to 4 years) than in yearlings [45]. In Sweden, the incidence of barren females was significantly higher in juveniles (45%) compared to the 2 - 3 year old females (17%) and the risk of being barren was significantly higher among juveniles with high antibody titer compared to those with low antibody titer, but the estimates were comparable between high and low antibody titers in older mink [8], suggesting a complex relationship between antibody titer, age and previous selection history of females.

The finding that measures of litter size for 2-year old dams were greater than those for juveniles by almost 0.5 kit and the differences were mostly significant (Table 9) agrees with previous reports [45,48,49,58,60]. In previous studies, the 2-year old dams produced 0.31, 0.54 and 0.36 extra kits compared with juveniles on three Danish farms [48] and the mean number of kits per female whelped at 3 weeks of age was significantly smaller for juvenile mink (5.0) than that for the older females (5.2) [8], but litter size and kit survival were not different between juvenile and 2-year old dams in another study [50]. Data from nine farms in Finland showed that litter size at two weeks of age were 5.27, 5.77, 5.41 for parities one, two and three, respectively, and although 2-year old dams had 0.5 extra kits compared with juveniles, the differences were not significant [60]. The significantly higher litter size of 2-year old compared with juveniles in the current and previous studies is partly because of the high selection pressure applied against mink with small litters in the first parity.

The decrease in the measures of litter size in the third and subsequent parities compared with those in the second parity agrees with the previous reports [58,60]. The observation that the means of litter size in 4-year old dams were smaller than those in 2-year old dams by almost one kit (Table 9) and differences were significant, is in line with a previous study showing that compared with juveniles, litter size decreased by 0.53, 0.19 and 0.26 kits in the third and larger parities on three Danish farms [48]. Decreases in litter size in the third and larger parities, compared with the estimates for the second parity, were 0.19, 0.57, 1.27 and 1.69 [58]. Although 2-year old mink had the lowest (5.87%) and 4-year old females had the highest (11.46%) pre-weaning mortality rates, differences were not significant. The results suggest that keeping females older than two years of age is worthwhile only if they have exceptional size, fur quality or reproductive performance.

Breeding year had no significant effect on female fertility, litter size or kit mortality, which is contradictory to previous reports which covered more years than the present study [50,51,60]. The decreasing trend, albeit non-significant, for litter size by breeding year could be partly because some seronegative animals were kept in 2007 and subsequent years, which because of a rather small number of seronegative animals to choose from, were from smaller litters.

Conclusion

Success in selection programs for resistance or tolerance in any disease, including AMDV, is greatly influenced by accurately defining and measuring the phenotype, which is not always practical or inexpensive. This study demonstrated that selection for healthy mink based on visual assessment of animal health, high reproductive performance and low serum gamma globulin (negative IAT) on a farm chronically infected with AMDV was effective in the creation of a tolerant herd. The reproductive performances and kit survival of this herd were comparable with those for un-infected herds. IAT was used as a tool for the identification of healthy mink because it can be easily performed on farms at low cost, but fluctuations in the IAT status of individual mink over time indicated that the contribution of IAT to the final outcomes was possibly slight. Data on visual assessment of animals' health and reproductive performance, which are always recorded on mink farms, were likely very effective in the establishment of this tolerant herd. The moderate estimates of heritability of CIEP-positive kits at five and seven months of age suggest that selection for seronegative kits could be effective and would result in low antibody titer or delayed time of infection in kits. The presence of infected mink (PCR positive) which were CIEP-negative could have been one of the reasons for the failure of the test-and-cull strategy to permanently eradicate the virus from infected farms. Significant improvement in reproductive performance was observed when mink were mated more than once with different males, supporting the breeding methods which are commonly used by mink farmers.

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Conflict of Interest

The author declares no conflict of interest.

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