Virology & Mycology

Farid ,Segervall, Virol mycol 2014, 3:3
DOI: 10.4172/2161-0517.1000137

Research Article Open access

A Comparison between ELISA and CIEP for Measuring Antibody Titres against Aleutian Mink Disease Virus

Farid AH[1]* **and Segervall J**[2]

[1]*Department of Plant and Animal Sciences, Dalhousie University Faculty of Agriculture, Truro,Nova Scotia, Canada*

[2]*Profur, PälsKraft. Kungsgårdsvägen Vaasa, Finland*

***Corresponding Author**: Farid AH, Department of Plant and Animal Sciences, Dalhousie University Faculty of Agriculture, Truro, Nova Scotia, B2N 5E3, Canada, Tel: 0019028936727; E-mail: ah.farid@dal.ca

Rec date: July 31, 2014**, Acc date:** October 8, 2014, **Pub date:** October 18, 2014

Abstract

Introduction: Enzyme-linked immunosorbent assay (ELISA) is used for the identification of mink with low antibody titre against the Aleutian mink disease virus (AMDV) who can tolerate the infection. There is no published information on the merit of ELISA for measuring antibody titre. An ELISA was compared with the counter immuno - electrophoresis (CIEP) for quantifying antibodies against AMDV.

Materials and methods: In experiment 1 (EX1), 41 black mink were inoculated with various amounts of a local strain of AMDV and were euthanized on day 218 post-inoculation (pi). In experiment 2 (EX2), 262 black mink were inoculated with a single doses of the same virus and euthanized 16 to 71 weeks pi. Antibodies to AMDV were quantified by CIEP using 10 two-fold serial dilutions of plasma, and serum samples were tested by the ELISA. Total serum proteins, albumin and globulins were measured in EX1.

Results and discussion: All mink were tested positive for AMDV infection by PCR in both experiments. There were moderate concordances between CIEP and ELISA results (Spearman's correlation of 0.77 and 0.71 in EX1 and EX2, respectively). Each ELISA class spanned a wide range of antibody titres in both experiments. Antibody titres of lower than 32 were not associated with serum proteins while greater titers were positively associated with total serum proteins and globulins ($P<0.01$) and negatively associated with albumin and albumin:globulin ratio ($P<0.01$). Regressions of serum protein measurements on ELISA classes were linear and in the same directions as, although weaker than, those on CIEP.

Conclusion: Although ELISA was not able to accurately measure anti-AMDV antibody titre, in the absence of any other practical method, it is a useful tool for ranking mink for anti-AMDV antibody titres.

Keywords: Aleutian mink disease virus; Antibody titre; Counter-immunoelectrophoresis; ELISA; Serum proteins; American mink

Introduction

The Aleutian mink disease virus (AMDV, *Carnivore amdoparvovirus* 1), is a member of the genus *Amdoparvovirus,* family *Parvoviridae*[1].The viruse causes a serious problem for the mink industry in many countries. The disease reduces reproduction and increases adult and kit mortality, has no cure and attempts aimed at developing a vaccine have failed or provided partial protection only [2-8]. For these reasons, the accepted control strategy around the globe has been testing mink with the counter-immunoelectrophoresis (CIEP) and eliminating sero-positive animals in combination with disinfection practices, depopulation and implementation of biosecurity measures. This strategy has been followed in Nova Scotia, Canada, for more than 30 years but has not been effective in permanently eliminating the virus from many ranches in this province [9]. Difficulties in virus eradication, the presence of mink that do not develop disease symptoms after exposure to AMDV [10] and evidence that response of mink to AMDV infection is genetically controlled, suggest that genetic selection for increased resistance to AMDV symptoms is a realistic strategy, although the genetic control of tolerance is complex [11-14].

In recent years, a number of mink ranchers in North America and Europe have embarked upon selecting their herds for increased tolerance to AMDV infection. Lack of a proven test for the identification of tolerant mink has forced ranchers to either select healthy animals on their infected ranches without a laboratory test or use the iodine agglutination test [15]. Recently, a number of ranchers in Canada, USA and The Netherlands have used ELISA to identify and keep animals with low anti-AMDV antibody titres. Although initial attempts to develop an ELISA for detecting antibodies against AMDV were not successful, more sensitive assays have recently been developed based on either the recombinant VP2 protein or AMDV-G antigen which were developed independently in Denmark and the USA (Scientilla Development Company, LLC, Bath, Pennsylvania) [7,16-18]. The published literature on these ELISA systems focused on estimating the sensitivity and specificity of the assays for AMDV eradication and no published information is available on their merits for measuring antibody titre [17-19]. Even though the VP2-based ELISA is being used by some mink ranchers for selecting tolerant mink, it has not been optimized for antibody quantification by the laboratory that developed the system [17]. The objective of this study

was to compare the VP2-based ELISA with CIEP for ranking mink for antibody titres against AMDV.

Materials and Methods

The statement of animal care

All protocols were performed according to the standards of the Canadian Council on Animal Care after approval by the institutional Animal Care and Use Committee. Animals were maintained according to the standard industry practices in 24"x12"x8" cages. Prior to inoculation or blood sampling, animals were anesthetized by intramuscular injection of ketamine hydrochloride (Ketalean, Bimeda-MTC Cambridge, ON, Canada) and xylazine hydrochloride (Rompun 2%, Bayer Health Care) at the rates of 10 mg and 2 mg per kg live weight, respectively. Euthanasia was performed by intracardial injection of sodium pentobarbital (Euthanyl, Bimedia-MTC) at the rate of 100 mg per kg body weight or by CO2 exposure.

Animal inoculation and sampling

Experiment 1 (EX1): Black American mink (*Neovison vison)* from an AMDV-free ranch were transferred to an enclosed facility (Aleutian Disease Research Centre) and were intranasally inoculated with 0 to 1,000,0000 dilutions of a spleen homogenate containing a local strain of AMDV. Forty-one mink which were tested positive for AMDV DNA by polymerase chain reaction (PCR) were euthanized on day 218 post-inoculation (pi) and blood was collected by heart puncture into heparinized tubes for plasma preparation for the CIEP test, in EDTA-coated tubes for viral detection by PCR, and in plain tubes for serum preparation. Samples were kept in a refrigerator overnight and centrifuged at 1397 g (Porta Spin C826 centrifuge, UNICCO, Dayton, NJ, USA) for 10 min. One fresh plasma sample was tested by CIEP and the remaining serum and plasma samples were stored at -80°C until use. Infectious materials were all handled in a biosafety Level 2 laboratory following approved Standard Operating Procedures.

Experiment 2 (EX2): A total of 262 male and female black mink were intranasally inoculated with 600 ID50 of the same spleen homogenate used in EX1. The first group was inoculated in October 2010 and euthanized 16 to 19 weeks later in February 2011 (74 mink) or 68 to 71 weeks later in February 2012 (95 mink). The second group was inoculated in September 2011 and euthanized approximately 20 weeks later in February 2012 (93 mink). Blood samples were collected and processed as explained above. All animals were tested positive for AMDV DNA by PCR.

Laboratory procedures

In addition to the fresh plasma samples that were tested by CIEP, frozen plasma samples were thawed, two-fold serially diluted 10 times (1/2 to 1/1024) and tested by the CIEP [20]. The CIEP test was performed by an experienced technician at the Animal Health Laboratory of the Nova Scotia Department of Agriculture in Truro, Nova Scotia, Canada, which is an accredited laboratory under the Standards Council of Canada. The test was performed on 75×50 mm glass slides coated with 10 mL of 0.8% standard low-Mr agarose (BioRad) in barbital buffer (VWR) using an antigen produced by the Research Foundation of the Danish Fur Breeders Association, Glostrup, Denmark. Plasma samples were placed in anodal wells and antigen in cathodal wells along with a positive control on each plate. The gels were electrophoresed for 50 min at 80 volts, soaked overnight in 2% sodium chloride and scored under a stereoscope. Formation of a visible greyish-white band in the agarose gel was the indication of anti-AMDV antibodies in the sample. In cases where bands were faint, the results were recorded as doubtful, which were the result of low antibody titres. The titre of anti-AMDV antibodies was recorded as the reciprocal of the highest dilution of plasma that produced a positive or doubtful result. A total of 130 of the samples in EX1 and EX2 were diluted in duplicate and tested with CIEP.

Serum samples were thawed and 10 µL was transferred to a glass capillary tube, shipped at ambient temperature to the Fin Furlab, Vaasa, Finland, in 2011 and 2012 for testing by ELISA as previously described [17]. Reference sera (negative, low-positive, and positive) were run in each assay, the mean OD for two blank wells (containing all reagents except serum) was subtracted from each result, and the assay cut-off was set at the mean OD value of the CIEP-negative samples plus one standard deviation [17]. The results of EX1 were grouped into nine classes (0 to 8), and those of the EX2 were grouped into 7 classes (0 to 6) by the Fin Furlab. The relationship between OD values and classifications has not been released by the laboratory but it is stated to be non-linear.

DNA was extracted from 100 µL of plasma using Dynabeads Silane viral NA kit according to the manufacturer's protocol (Invitrogen, Burlington, ON, Canada), and eluted in 100 µL elution buffer. AMDV DNA was amplified by standard PCR using primers 60F/60R as previously described [21]. Three PCR tests were performed on each sample using 1.5, 2.5 and 3.5 µL of extracted DNA in 15 µL total PCR volumes. Three DNA volumes were used because the amount of viral DNA in the samples was not known. All tests included a reaction containing DNA from a known AMDV-infected animal (positive control), a reaction containing DNA from an AMDV-free mink and a blank reaction (negative controls). Sample preparation, PCR cocktail preparation, PCR amplification and PCR product testing were performed in four different laboratories with unidirectional sample movement to avoid cross-contamination. Sterile filtered-tips were used throughout the experiment. In addition, total serum proteins and albumin from animals in EX1 were measured using the Roche C501 chemistry analyser (Mississauga, Ontario, Canada), which uses the biuret and Bromcresol Green (BCG) methods for total serum proteins and albumin, respectively. The difference between total proteins and albumin was considered as total globulins.

Data Analysis

The relationship between ELISA and CIEP was tested by linear and quadratic regression and Spearman's rank correlation using SAS, Version 9.2 [22]. Antibody titres measured by CIEP were transformed to $\log_2(\text{CIEP})+1$ if CIEP>0 and 0 if CIEP=0 prior to regression analyses. Visual inspection of the distribution of serum parameters suggested minor changes at low antibody titres and a sharp change at higher antibody titres suggesting that a segmented regression would more accurately describe the associations between these parameters than a global regression. To determine the breakpoint, the nonparametric LOESS procedure of SAS was used. This procedure fits a local regression function to the data and provides a graphical diagnostic of trends in the data. The ODS GRAPHICS statement of PROC LOESS was used to overlay the Fit Plots on the scatterplots of the data. Visual inspection of the Fit Plots and Residual Plots suggested that CIEP class 5 (titre of 16) was the breaking point for all serum parameters, and linear and quadratic regression equations were fitted into each segment. The results were plotted using PROC GPLOT

of SAS. Likelihood Ratio Chi-Square of SAS was used to analyse contingency tables. Sensitivity of the ELISA relative to CIEP was calculated as the number of samples positive on both tests (true positives) divided by the total number of samples positive on the CIEP (true positives+false negatives on ELISA) [23]. The same procedure was used to compute sensitivity of CIEP relative to PCR results.

Results

Experiment 1

Descriptive statistics of the parameters measured in EX1 are shown in Table 1. Antibody titres measured by the CIEP ranged between 0 and 1024 and the ELISA classes spanned the entire scale of the test (0 to 8) and both showed large coefficients of variation revealing the great variation among mink for antibody titre. The coefficients of variation of total serum proteins and albumin were rather small, but total globulins and the albumin:globulin (A:G) ratio had large coefficients of variation reflective of variable response of mink to infection by the AMDV.

Measurement	No. observations	Mean ± SD[a]	% CV[b]	Range
CIEP[c]	41	241.0 ± 256.9	106.6	0-1024
CIEP, log_2 dilution [d]	41	6.73 ± 3.54	60.3	0-11
ELISA class	41	4.71 ± 2.95	62.6	0-8
Total serum protein, g/L	40	62.7 ± 7.8	12.4	50-83
Albumin, g/L	37	27.6 ± 3.4	12.3	19-33
Globulins, g/L	37	34.4 ± 8.9	25.9	20-56
Globulins as % of serum proteins	37	54.7 ± 8.0	14.6	40.0-70.2
Albumin: globulins ratio	37	0.86 ± 0.27	31.4	0.41-1.50

[a]Standard deviation, [b]Coefficient of variation, [c]The reciprocal of the highest dilution of plasma that resulted in a positive CIEP test, [d]Log_2(CIEP=0) was set at 0 and log_2(CIEP>0) was set as log_2(CIEP)+1

Table 1: Mean, standard deviation, coefficient of variation and range of values of the measurements in Experiment 1

The regression of ELISA readings on log_2 (CIEP) was linear and showed a close relationship between the two measurements (b=0.725 ± 0.037, R^2=0.904, P<0.001). The Spearman's rank correlation coefficient was 0.77 (P<0.01), indicating a moderate concordance. Eight mink were negative on both CIEP and ELISA tests, while two mink with titre of 2 on the CIEP were declared negative on the ELISA (Table 2).

ELISA	Log_2 (CIEP)[a]									
	0	2	4	5	7	8	9	10	11	Total
0	8	2								10
1			1							1
4				1		1				2
5							1			1
6					2	3	4	4		13
7						2	4	3	2	11
8							2	1		3
Total	8	2	1	1	2	6	11	8	2	41

[a]The reciprocal of the highest dilution of plasma that resulted in a positive CIEP test. Log_2 (CIEP=0) was set at 0 and log_2(CIEP>0) was set as log_2(CIEP)+1

Table 2: Joint distribution of antibody titres measured by CIEP and ELISA in Experiment 1

Sensitivity of ELISA readings relative to CIEP was 0.94. CIEP values of 128, 256 and 512 each covered three to four ELISA classes and two mink with CIEP values of 1024 did not fall into the highest ELISA class. ELISA classes 6 and 7 each spanned 8-fold antibody titres (64 to 512 and 128 to 1024, respectively).

Serum parameters	CIEP 0 to 5			CIEP 6 to 11		
	b ± SE[a]	P[b]	R^2	b ± SE[a]	P[b]	R^2
Total protein, g/L	0.02432 ±0.5357	0.96	0.00	5.84273 ±1.19904	0.000	0.49
Albumin, g/L	0.27356 ±0.4249	0.54	0.05	-1.17062 ±0.64603	0.082	0.12
Globulins, g/L	-0.24924 ±0.44259	0.59	0.04	7.01335 ±1.18206	0.000	0.59
Globulin %[c]	-0.46713 ±0.60304	0.46	0.07	5.59162 ±1.07083	0.000	0.52
Albumin/ globulins	0.02100 ±0.02570	0.44	0.08	-0.18504 ±0.03414	0.000	0.54

[a]Regression coefficient ± Standard error, [b]Level of significance of regression coefficient, [c]Globulins as a percentage of total serum proteins, [d]The reciprocal of the highest dilution of plasma that resulted in a positive CIEP test. Log_2 (CIEP=0) was set at 0 and log_2(CIEP>0) was set as log_2(CIEP)+1

Table 3: Segmented regression of serum protein measurements on log_2(CIEP)[d] in Experiment 1.

Regression of serum parameters on each segment of CIEP values (0 to 5 and 6 to 11) was linear in all the cases (Table 3 and Figure 1). There was no association between serum parameters and CIEP classes of 0 to 5 (titres 0 to 16), but an increase in CIEP titre of greater than 16 was significantly associated with an increase in total serum proteins, globulins and globulins as a percentage of total serum proteins, and a significant decrease in albumin and A:G ratio. Regressions of serum proteins on the ELISA classes were linear and no segmentation was detected by PROC LOESS. Relationships between serum parameters and ELISA were in the same directions as for CIEP values, i.e. an increase in anti-AMDV antibody titre was significantly associated with an increase in total serum proteins, globulins and globulins as a percentage of total serum proteins, and a significant decrease in albumin and A:G ratio (Table 4, Figure 2). The R^2 of serum protein parameters on the CIEP values were almost 50% greater than those for ELISA readings. CIEP and ELISA readings more accurately predicted globulin measurements (amount, percentage and the A:G ratio) than did total serum proteins and albumin (Tables 3 and 4).

Serum parameters	b ± SE[a]	P[b]	R^2
Total protein, g/L	1.08418 ± 0.41655	0.014	0.16
Albumin, g/L	-0.45139 ± 0.18632	0.021	0.14
Globulins, g/L	1.53557 ± 0.45702	0.002	0.24
Globulin %[c]	1.48330 ± 0.39999	0.001	0.28
Albumin/globulins	-0.05068 ± 0.01337	0.001	0.29
[a]Regression coefficient ± Standard error, [b]Level of significance of regression coefficient, [c]Globulins as a percentage of total serum proteins			

Table 4: Regression of serum protein measurements on ELISA classes in Experiment 1

Experiment 2

Regression of ELISA readings on $\log_2$ (CIEP) in EX2 was linear and significant (b=0.621 ± 0.038, R^2=0.51, P<0.001), showing the same degree of association as the Spearman's rank correlation (0.71, P<0.001) and indicating a moderate concordance between the two measurements. The joint distribution of ELISA and CIEP values (Table 5) shows that 24 samples (9.16% of the observations) were positive on the CIEP, with titres of 1 to 64, but negative on ELISA. Conversely, one sample that was negative on the CIEP was positive on the ELISA. These figures indicate a relative sensitivity of 0.908 for ELISA. Each ELISA class covered a wide range of antibody titres, varying from 16 fold (ELISA class 6) to 512 fold (ELISA class 4).

Of the 130 duplicate plasma samples of the same animals that were tested by CIEP, none was positive and negative. Test results of two duplicate samples (1.5%) were positive and doubtful, and the results of nine duplicate samples (6.9%) were doubtful and negative.

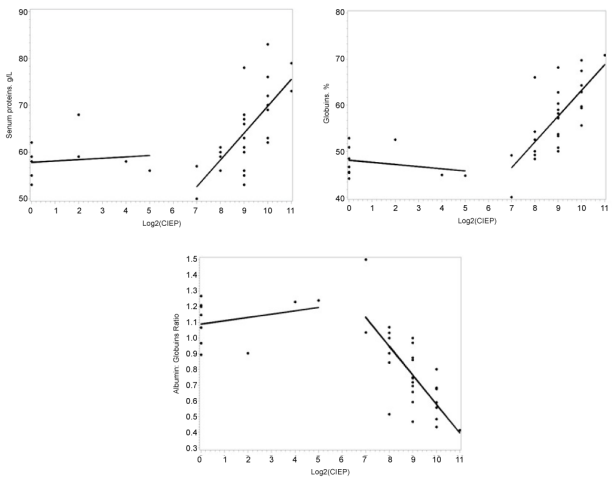

Figure 1 : Segmented regression lines of serum proteins (g/L) on $\log_2$(CIEP), Segmented regression lines of globulins as a percentage of total serum proteins on $\log_2$(CIEP), Segmented regression lines of albumin:globulins ratio on $\log_2$(CIEP)

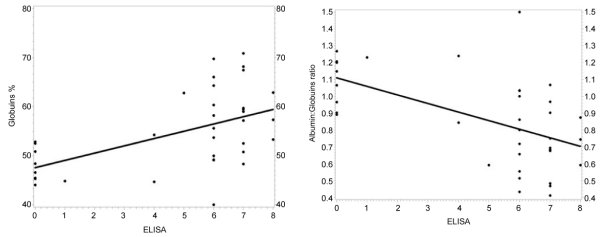

Figure 2 : Regression line of globulins as a percentage of total serum proteins on ELISA classes, Regression line of albumin: globulins ratio on ELISA classes

Discussion

A rather wide range of anti-AMDV antibody titres were generated using various doses of inoculum in EX1, and mink that were sampled 16 to 71 weeks post-inoculation in EX2. These data sets represented snapshots of animals on any ranch which is engaged in selection for tolerant mink, and thus provided reasonably good resources to investigate the relative accuracy of ELISA for ranking mink for their antibody titre. The CIEP test was employed as a reference because it is commonly used as a standard for evaluating ELISA systems [17-19]. While the CIEP has a high specificity and reasonably high sensitivity, it is too expensive for routine antibody quantification as it requires testing of multiple diluted serum or plasma samples [24-26]. Quantitative ELISA can potentially be a useful tool for ranking mink for anti-AMDV antibody titres.

Although all animals were inoculated with the same source of AMDV and were all confirmed infected by PCR, eight of 41 mink in EX1 (19.5%) and one of 262 mink in EX2 (0.4%) were CIEP negative, showing sensitivity of 0.75 and 0.91 for CIEP relative to PCR. The levels of antibodies in these animals were below the detection threshold of the CIEP, which occurs when animals are infected with low doses of the virus [27]. The maximum antibody titre of 1024 was observed in 2 and 8 animals in EX1 and EX2, respectively, all with doubtful test results, indicating that 1024 was the highest titre observed in these studies.

The sensitivity and specificity of ELISA systems are not as important for selection purposes as they are for virus eradication programs because false positive cases, resulting from the low assay specificity, and false negative cases, resulting from low assay sensitivity, are likely those samples with low antibody titres. Animals in these cases will be grouped in negative or low ELISA classes, which will not impact ranking of animals for selection purposes. The central question is the accuracy of ELISA for ranking mink for antibody titre. It should be noted that the relationship between the VP2-based ELISA results and antibody titre is not linear, and this ELISA system has neither been optimized nor recommended by the Fin Furlab for antibody quantification. The non-linear relationship between ELISA and CIEP in the present experiments was not statistically significant because of a rather narrow range of antibody titres.

ELISA	$Log_2(CIEP)^a$												Total
	0	1	2	3	4	5	6	7	8	9	10	11	
0		4	1	3	8	4	3	1					24
1			2	1	5	6	7	6	2	3	1		33
2	1				1	3	9	4	5	1			24
3				1		2	3	8	8	8		1	31
4			1		1	2	5	9	19	8	6	1	52
5							3	5	13	24	7	4	56
6								2	7	8	23	2	42
Total	1	4	4	5	15	17	30	35	54	52	37	8	262

[a]The reciprocal of the highest dilution of plasma that resulted in a positive CIEP test. Log_2 (CIEP=0) was set at 0 and log_2(CIEP>0) was set as log_2(CIEP)+1

Table 5: Joint distribution of antibody titres measured by CIEP and ELISA in Experiment 2

The CIEP and ELISA results showed a moderate concordance based on Spearman's rank correlation of 0.77 and 0.71 in EX1 and EX2, respectively, suggesting that although ELISA did not accurately determine antibody titres, yet animals in the low ELISA classes had a higher chance of having lower antibody titres than those in the high ELISA classes. The observation that each of the ELISA classes spanned a wide range of antibody titres is, however, a matter of concern when ELISA is going to be used for selection of animals with low antibody titres. The wide range of antibody titres covered by each ELISA class would result in retaining some animals with high antibody titres and eliminating some with low titres. In EX2 for example, ELISA class 1 with 33 observations contained 4 mink (12.1%) with moderate to high CIEP values (256 and 512), and ELISA class 5 with 56 observations contained 8 mink (14.3%) with low CIEP values (32 and 64). The misclassification of animals for antibody titre in each ELISA class is thus not large but would negatively influence the rate of genetic progress for low antibody titre.

In addition to analytical inaccuracies in measuring antibody titres by each of the CIEP and ELISA methods, one reason for the wide range of antibody titres in each ELISA class may be the fact that antibodies are against both capsid (VP1, VP2) and non-structural (NS1) viral proteins and the antibody against NS1 proteins is associated with viral replication [10,28]. The VP2-based ELISA may only detect a portion of total antibodies against AMDV, which could vary amongst chronically infected individuals with different levels of viral replication. The reasons for the wide range of antibody titres within each ELISA class requires further investigation because failure of ELISA in detecting all types of anti-AMDV antibodies could potentially have a significant effect on the outcomes of selection for tolerance.

The presence of only one CIEP negative but ELISA positive sample in 303 tests (0.33%) is comparable with 0.33% of 306 mink in one report [19], but is smaller ($P<0.05$) than 2.84% of 211 mink in another report where VP2-based ELISA were used[17]. It should be noted that while both studies used the same antigen and both subtracted mean OD of two blank wells from each result, the assay cut-off was set at the mean OD value of the CIEP-negative samples plus one standard deviation in one study and three standard deviations in the other [17,19]. The numbers of samples that were positive on CIEP but negative on ELISA (26/303, 8.6%), were greater than those in the previous reports of 1/105 (0.95%) ($\chi^2_{(1)}=10.1$, $P<0.01$) and 1/58 (1.72%) ($\chi^2_{(1)}=4.5$, $P<0.05$) [17,19]. The high proportion of CIEP positive but ELISA negative cases in the current study may be due to the presence of antibodies against the non-structural viral proteins in chronically infected mink that were not detected by ELISA, as explained above, or anti-AMDV antibodies in mink infected with different AMDV strains showing divergent affinities for different antigens and the AMDV strain which was used in this study could have had a lower affinity for the VP2 antigen used in the ELISA system[29]. Furthermore, plasma samples were frozen, thawed and sent to Finland at ambient temperature, which could have caused some degradation of antibodies and an overall decrease in ELISA readings compared with fresh blood samples.

Infection with AMDV results in a significant increase in globulins and a significant decrease in serum albumin [30-33]. The higher rate of increase in serum gamma globulin relative to the decrease in albumin causes an increase in total serum proteins and a decrease in A:G ratio [30,32]. In the present study, total globulins were the non-albumin segment of the serum proteins, of which the largest fraction was gamma globulin [30]. The higher rate of increase in serum globulins than decrease in albumin explains the observation that both CIEP and ELISA readings were more strongly associated with globulin measurements than total serum proteins or albumin (Tables 3 and 4). The moderate and positive associations between CIEP and ELISA with globulins could be interpreted as an evidence for the usefulness of antibody measurement in selecting tolerant mink because high levels of gamma globulin are associated with the development of AD symptoms, although genotype of the mink and strain of the virus play crucial roles as well [27,31,33].

The segmented regression procedure more clearly showed the relationships between serum parameters and antibody titre compared with a single-line regression, and revealed an interesting phenomenon of considerable importance when selecting mink for tolerance to AMDV. The absence of a significant change in serum proteins at low antibody titres supports the idea that selection for low antibody titres could potentially result in the creation of tolerant mink herds.

Although not explicitly stated, a similar trend can be deduced from the data presented in another study [33]. One drawback of the segmented regression analysis is that each segment of the regression line, particularly at low CIEP values, was based on a small number of observations and further research is needed to confirm these findings. The closer associations between the CIEP than the ELISA readings with serum proteins, particularly globulins, could be considered an indirect indication that ELISA is not capable of accurately identifying animals with high serum gamma globulins.

The degrees of association between the CIEP classes greater than 6 (titre of 32) and amounts and percent globulins in this study (R^2=0.59 and 0.52, Table 3), are comparable with the correlation coefficients of 0.81 and 0.609 between antibody titres and serum gamma globulin in naturally infected pastel mink, and 0.75 in experimentally infected sapphire mink [27,33,34]. The moderate associations between CIEP readings and globulin levels could be due to the fact that only a portion of gamma globulin is specific anti-AMDV antibodies [27,29,35]. In addition, the sustained production of serum immunoglobulins in AMDV-infected mink is the outcome of both specific antiviral antibodies and an autoimmune response resulting from the presence of anti-DNA antibodies and the latter is more strongly associated with gamma globulin levels than anti-AMDV antibodies [34,36]. Anti-DNA antibody production could be one of the reasons that, at least in some mink, anti-AMDV antibodies reach a plateau, or even decrease, while gamma globulin continues to increase [10,27]. The patterns of changes in the amounts of anti-DNA and anti-viral (VP1, VP2, NS1) antibodies over time, and the accuracy of detection of these components by the CIEP and the VP2-based ELISA is not clearly known and more information is needed before recommending large scale selection programs based on anti-AMDV antibody tests, such as ELISA.

Conclusions

Results of two experiments on 303 AMDV-inoculated black mink with a wide range of infection histories showed a moderate concordance between the VP2-based ELISA classes and anti-AMDV antibody titres measured by CIEP. Each ELISA class spanned a wide range of antibody titres, suggesting that high and low ELISA classes contained some animals with low and high antibody titres, respectively. Although the VP2-based ELISA has not been validated for antibody quantification, in the absence of any other practical method, this ELISA system is a useful tool for ranking mink for anti-AMDV antibody titres. Antibody titres of lower than 32 were not associated with changes in serum proteins, suggesting that selection for low antibody titres may be useful in establishing mink herds that can tolerate AMDV infection. More information on the relationships between anti-AMDV antibody titres and degree of tolerance of mink to AMDV is needed before recommending the use of CIEP or ELISA as selection tools.

Acknowledgments

We would like to acknowledge Priyanka Rupasinghe and Irin Arju for their technical support, the staff of the Pathology Laboratory, Nova Scotia Department of Agriculture for performing the CIEP tests, and the Fin Furlab for performing the ELISA tests. Financial support from mink industry organizations (Canada Mink Breeders Association, Nova Scotia Mink Breeders Association, Fur Farmers of Canada Marketing Association, American Legend Cooperatives, North American Fur Auctions, American Mink Council, American Mink Farmers Research Foundation, Fur Council USA), Agriculture and Agri-Food Canada through the CARD Councils of Ontario, British Columbia and Nova Scotia (Agri-Futures NS), Atlantic Innovation Fund of ACOA and the Research Acceleration Program of the Nova Scotia Department of Agriculture is gratefully acknowledged.

Conflict of interest

AHF has no competing interest. JS is an employee of Profur.

References

1. Cotmore SF, Agbandje-McKenna M, Chiorini JA, Mukha DV, Pintel DJ Qiu, et al. (2014) The family Parvoviridae, Arch Virol 159: 1239-1247.
2. Alexandersen S (1986) Acute intestinal pneumonia in mink kits: experimental reproduction of the disease. Vet Pathol 23: 579-588.
3. Broll S, Alexandersen S (1996) Investigation of the pathogenesis of transplacental transmission of Aleutian mink disease parvovirus in experimentally infected mink. J Virol 70: 1455–1466.
4. Hansen M, Lund E (1988) Pregnancy rate and foetal mortality in Aleutian disease virus infected mink. Acta Vet Scand 29: 271-272.
5. Padgett GA, Gorham JR, Henson JB (1967) Epizootiologic studies of Aleutian disease. 1. Transplacental transmission of the virus. J Infect Dis 117: 35-38.
6. Porter DD, Larsen AE, Porter HG (1972) The pathogenesis of Aleutian disease of mink. II. Enhancement of tissue lesions following the administration of a killed virus vaccine or passive antibody. J Immunol 109: 1-7.
7. Aasted B, Alexandersen S, Christensen J (1998) Vaccination with Aleutian mink disease parvovirus (AMDV) capsid proteins enhances disease, while vaccination with the major non-structural AMDV protein causes partial protection from disease. Vaccine 16:1158-1165.
8. Castelruiz Y, Blixenkrone-Moller M, Aasted B (2005) DNA vaccination with the Aleutian mink disease virus NS1 gene confers partial protection against disease. Vaccine 23:1225-1231.
9. Farid AH, Zillig ML, Finley GG, Smith GC (2012) Prevalence of the Aleutian mink disease virus infection in Nova Scotia, Canada. Prevent Vet Med 106:332-338.
10. Bloom ME, Kanno H, Mori S, Wolfinbarger JB (1994) Aleutian mink disease: puzzles and paradigms. Infect. Agents Dis 3:279-301.
11. Aasted B, Hauch H (1988) Studies on the progression of Aleutian disease in mink. Acta Vet Scand 29: 315-321.
12. Hadlow WJ, Race RE, Kennedy RC (1984) Royal pastel mink respond variously to inoculation with Aleutian disease virus of low virulence. J Virol 50:38-41.
13. Henson JB, Gorham JR, Leader RW (1963) The familial occurrence of hypergammaglobulinemia in mink. Tex Rep Bio Med 21:37-42.
14. Larsen AE, Porter DD (1975) Pathogenesis of Aleutian disease of mink: identification of nonprogressive infections. Infect Immun 11:92-94.
15. Henson JB, Gorham JR, Leader RW (1962) A field test for Aleutian disease. National Fur News 34:8-9
16. Wright PF, Wilkie BN (1982) Detection of antibody in Aleutian disease of mink: comparison of enzyme-linked immunosorbent assay and counterimmunoelectrophoresis. Am J Vet Res 43:865-868.
17. Knuuttila A, Aronen P, Saarinen A, Vapalahti O (2009) Development and evaluation of an enzyme-linked immunosorbent assay based on recombinant VP2 capsids for the detection of antibodies to Aleutian mink disease virus. Clin Vaccine Immunol 16:1360-1365.
18. Dam-Tuxen R, Dahl J, Jensen TH, Dam-Tuxen T, Struve T, et al. (2014) Diagnosing Aleutian mink disease infection by a new fully automated ELISA or by counter current immunoelectrophoresis: A comparison of sensitivity and specificity. J Virol Method 199:53-60.
19. Andersson, AM, Wallgren P (2013) Evaluation of two enzyme-linked immunosorbent assay for serodiagnosis of Aleutian mink disease virus infection in mink. Acta Veterin Scand 55:86

20. Cho HJ, Greenfield J (1978) Eradication of Aleutian disease of mink by eliminating positive Counterimmunoelectrophoresis test reactors. J Clinical Microbiol 7:18-22.
21. Farid AH (2013) Aleutian mink disease virus in furbearing mammals in Nova Scotia, Canada. ACTA Veterin Scand 55: 10
22. SAS (2008) SAS for the Microsoft Window Environment, Version 9.2. SAS Institute Inc. Cary, NC, USA.
23. Florkowski CM (2008) Sensitivity, specificity, receiver-operating characteristic (ROC) curve and likelihood rations: Communicating the performance of diagnostic tests. Clin Biochem Rev 29(Suppl. 1): 83-837.
24. Cho HJ, Ingram DG (1973) Antigen and antibody in Aleutian disease in mink. II- The reaction of antibody with the Aleutian disease agent using immunodiffusion and immunoelectrophoresis. Can J Comp Med 37:217-223.
25. Aasted B, Cohn A (1982) Inhibition of precipitation in counter current electrophoresis. A sensitive method for detection of mink antibodies to Aleutian Disease Virus. Acta Path Microbiol Immunol Scand Sect C 90:15-19.
26. Uttenthal A (1992) Screening for antibodies against Aleutian disease virus (ADV) in mink. Elucidation of dubious results by additive counterimmunoelectrophoresis. Appl Theor Electroph 3:83-84.
27. Bloom ME, Race RE, Hadlow WJ, Chesebro B (1975) Aleutian disease of mink: the antibody response of sapphire and pastel mink to Aleutian disease virus. J Immunol 115:1034-1037.
28. Bloom ME, Race RE, Wolfinbarger JB (1982) Identification of a nonvirion protein of Aleutian disease virus: mink with Aleutian disease have antibody to both virion and nonvirion proteins. J Virol 43:608-616.
29. Aasted B, Tierney GS, Bloom ME (1984) Analysis of the quantity of antiviral antibodies from mink infected with different Aleutian disease virus strains. Scand J Immunol 19:395-402.
30. Henson JB, Leader RW, Gorham JR (1961) Hypergammaglobulinemia in mink. Proc Soc Exper Biol Med 107:919-920.
31. Kenyon AJ, Trautwein G, Helmboldt CF (1963) Characteristics of serum proteins from mink with Aleutian disease. Am J Vet Res 24:168-172.
32. Gershbein LL, Spencer KL (1964) Clinical chemical studies in Aleutian disease of mink. Can J Comp Med Vet Sci 28: 8-12.
33. An SH, Ingram DG (1977) Detection of inapparent Aleutian disease virus infection in mink. Am J Vet Res 38: 1619-1624.
34. Hahn EC, Hahn PS (1983) Autoimmunity in Aleutian disease: contribution of antiviral and anti-DNA antibody to hypergammaglobulinemia. Infect Immun 41: 494–500.
35. Aasted B, Bloom ME (1983) Sensitive radioimmune assay for measuring Aleutian disease virus antigen and antibody. J Clinic Microbiol 18:637-644.
36. Mouritsen S, Aasted B, Hoier-Madsen M (1989) Mink with Aleutian disease have autoantibodies to some autoantigens. Vet Immunol Immunopathol 30: 179-86.