



A Letter From Dr. Hugh Hildebrandt

I am writing to you with this inaugural issue as I stare out a hotel window at the snow and fog. I have taken over the research news letter from Dr. Oldfield and hope to be able to get information out to you as he has in the past. As I battle canceled flights

and boredom I would like to get everyone up to speed on currently funded research projects. I have given talks about the different projects but for those of you who have not been able to attend, I will as part of this issue list them. Over the next issues I will try to keep you updated on the projects and get results to you.

The research committee has been very active in the last few years and we are blessed with many very skilled researchers and continue to get increased numbers of research proposals to evaluate for funding. For those of you wondering about the snow and flights, it is a mink research coordination meeting on AD that has me stranded. Many of our projects are done in cooperation with the Canadian Mink Breeders Association's research committee. The Canadian research is now coordinated by Dr. Bruce Hunter and the meeting was set up to get AD researchers together.

We should again thank Dr. Oldfield for the work on the news letter and bear with the new editor during the transition.

Dr. Hugh Hildebrandt

CURRENT RESEARCH PROJECTS

- Dr. Kirsti Rouvinen-Watt
Nova Scotia Agriculture College
Body Condition and Reproductive Longevity
- Dr. Robert Stephon
Anti-AD IgM & Anti-AD Antibody (IgY)
- Dr. Steve Bursian
Michigan State University
Feed Studies & Research Ranch Maintenance
- Dr. John Gorham
Washington State University
Various Follow Up Studies on Viral Diseases
- Dr. Jack Rose
Idaho State University
Uterine Fertility Studies
- Fur Breeders Agriculture Co-op
Feed Studies
- Dr. Bernhard Benkel
Nova Scotia Agriculture College
Mink Genome Sequencing

AD RESEARCH UPDATE

While attending the AD meeting there was some terminology clarification and standardization that was agreed upon. These changes in the terminology were agreed upon by researchers to use when presenting information to more clearly define classes of mink when it comes to AD. The need for standardization is due to the search for resistant mink.

The group agreed that the terms we have heard like persistent, permissive, non-progressive and non-persistent were somewhat confusing. It was agreed that there would be two basic classifications of mink when discussing AD:

Progressive and Resistant

The progressive mink is one with classical AD which progresses to hypergammaglobulinemia (high antibodies) and body system failure. As the name implies, a mink if exposed, progresses to end stage disease.

The Resistant mink are divided into two categories or sub sets that are referred to as R1 & R2. These mink are resistant to the virus but in different ways. R1 mink are resistant to disease. R2 mink are resistant to infection. Resistant to disease means they can be infected with the virus but survive due to non damaging antibody levels. These mink are seen on positive ranches as surviving for a long period of time. Resistant to infection means mink are unable to become infected with the virus and will not shed virus to others.

The Progressive and R1 mink are a focus of research to see if we can differentiate them. Many

ideas were discussed using current technology to help by more accurately identify them. The R2 mink as one researcher put it are “a concept of hope” and will require genetic mapping and identifying genes. It is important that we remember different AD strains or color types alter the progressive / resistant ratio we may see on a ranch.

We ask the industry to try to adopt these classifications:

Progressive

Resistant --- R1

--- R2

FEED

Evaluation of Sodium Bisulfate as a Replacement for Phosphoric Acid in a Conventional Mink Ranch Diet

Phosphoric acid has been used in conventional mink ranch diets and in commercial mink and fox pellet formulations for preservation of feed, for prevention of urinary calculi in mink and fox and to reduce the incidence of “wet belly” disease in mink. The recommended level of inclusion is 1.0% of 75% feed-grade phosphoric acid in conventional ranch diets employing 15 to 20% fortified cereal. Prevention of urinary calculi composed of magnesium ammonium phosphate by addition of phosphoric acid to the diet is accomplished through a reduction in urinary pH.

Inclusion of sodium bisulfate into the diet

of cats has been shown to effectively reduce urinary pH, thus reducing the incidence of urinary calculi. It was of interest to determine if sodium bisulfate could be used as a replacement for phosphoric acid in a conventional mink ranch diet. This would benefit the mink rancher in terms of safe material handling (dry product compared to wet product) as well as a reduction in feed costs.

Objectives

The objectives of the study were to: (1) compare the effectiveness of sodium bisulfate and phosphoric acid for lowering urinary pH of mink; (2) compare the phosphorus content of diets containing phosphoric acid or sodium bisulfate; (3) compare the phosphorus content of feces from mink fed diets containing phosphoric acid or sodium bisulfate; (4) compare body weight gain of mink fed diets containing phosphoric acid or sodium bisulfate.

Methods

Thirty-one adult female mink from the Michigan State University Experimental Fur Farm herd were assigned to one of three treatment groups fed a conventional ranch mink diet containing 1% of 75% feed-grade phosphoric acid (PA, 13 females), the same diet with the phosphoric acid replaced by 1% sodium bisulfate (SBS, 10 females) or a diet containing neither phosphoric acid or sodium bisulfate (NoA, 8 females). Treatment diets were fed to adults from approximately one week prior to initiation of whelping through weaning of kits. At weaning, kits remained on their respective dietary treatments until December 8, 2009.

Animals were housed individually in wire

Table 1. Diets for evaluation of sodium bisulfate (SBS) as a replacement for phosphoric acid (PA) in conventional mink ranch diet

Ingredient	No Acid % of diet	Sodium Bisulfate % of diet	Phosphoric Acid % of diet
Cereal – National GNF-20	20.00	20.00	20.00
Whole ground chicken	20.00	20.00	20.00
Spray-dried liver	6.00	6.00	6.00
Spray-dried egg	7.00	7.00	7.00
Spray-dried blood protein	2.00	2.00	2.00
Fish meal	6.00	6.00	6.00
Soy oil	3.00	3.00	3.00
Water	36.00	36.00	36.00
MSU vitamin premix	0.43	0.43	0.43
MSU mineral premix	0.43	0.43	0.43
Biotin	0.029	0.029	0.029
Phosphoric Acid	0.00	0.00	1.00
Sodium Bisulfate	0.00	1.00	0.00
Larvadex	60 ml/1000 lb	60 ml/1000 lb	60 ml/1000 lb

Table 2. Effect of sodium bisulfate (SBS) as a substitute for dietary phosphoric acid (PA) on pre-weaning mink kit weight

	Mean kit weight at birth (g)	Mean kit weight at 3 wk (g)	Mean kit weight at 6 wk (g)
NoA	9.8	128	313
SBS	9.0	91	220
PA	9.9	106	270
SE	0.44	8.04	20.77
P-value	0.6829	0.2201	0.2319

mesh cages within an open-sided pole barn. Feed was provided daily by placing approximately 250 g on the top of the cage. Unconsumed feed was scraped off the cage the next day and replaced with fresh feed. Water was available ad libitum.

Adult females and their kits were weighed at whelping and at 3 and 6 weeks of age. Kits were then weighed on a monthly basis until the end of the trial. Urine samples for pH determination were collected at approximately 14, 23 and 32 weeks of age. Incidence of urinary calculi will be determined in the event of mortalities during the trial.

Results

Pre-weaning body weight gains of male and female mink fed diets containing 1% phosphoric acid or 1% sodium bisulfate were comparable (Table 2). This indicates that milk production of females and pre-weaning growth of mink kits is adequate in animals fed diets containing sodium bisulfate.

Figures 1 and 2 display body weight gain of juvenile female and male mink from 10 to 32 weeks of age. Gain during the period of 10 to 32 weeks post-weaning was greater for female mink fed the SBS diet than for those fed either NoA or PA. This increase in gain compared to the NoA treatment was particularly evident during the period of 10 to 14 weeks post-weaning. Body weight gain of males (Figure 2) followed the same trend as females with the period from 10 to 19 weeks of age having the greatest increase in gain in SBS animals compared with the other treatments.

The effectiveness of sodium bisulfate to maintain urinary pH within the appropriate range (6.0-6.6) to prevent cystitis and urinary calculi is presented in Figure 3. While the pH of urine collected from mink fed the diet containing 1% phosphoric acid was slightly lower than pH of urine collected from mink fed the diet containing 1% sodium bisulfate throughout the trial, pH in both groups was lower than 6.6. None of the mink on this trial developed urinary calculi. These data indicate that sodium bisulfate is as effective as phosphoric acid in terms of keeping urinary pH within the appropriate range for prevention of cystitis and urinary calculi.

Dietary pH and dietary and fecal dry matter were determined at the end of the trial (Table 3). These results indicate similar dry matter content in both feed and feces between the SBS and PA treatments. This finding differs from our previous report in which adult female mink fed the SBS diet had greater fecal moisture content than those fed the PA diet (74.63 vs. 69.28 %, respectively). Dietary pH was slightly higher in the SBS treatment, which may explain the slightly greater urine pH in mink fed the SBS diet.

FIGURE 1

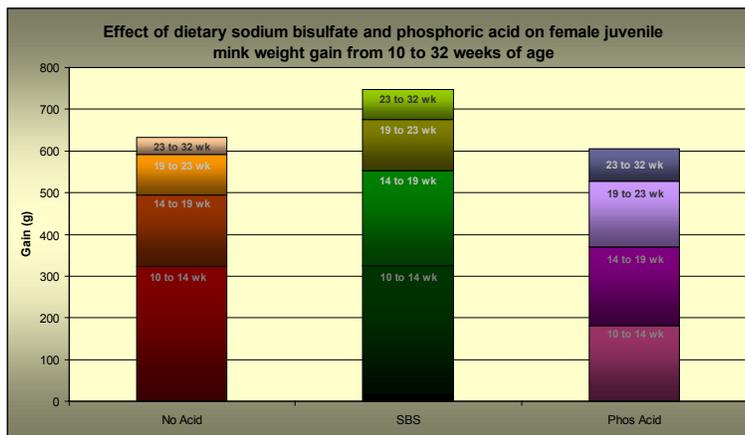


FIGURE 2

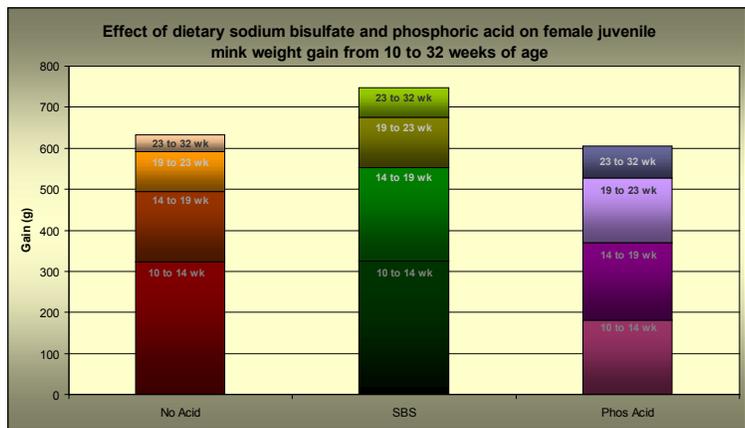


FIGURE 3

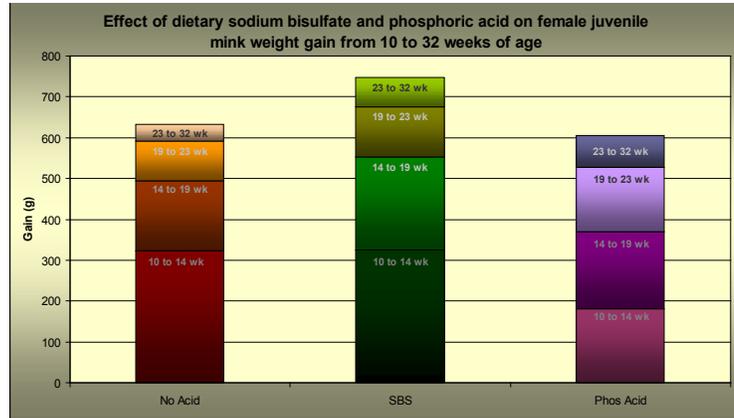


Table 3. Effect of sodium bisulfate (SBS) as a substitute for dietary phosphoric acid (PA) on mink diet pH and diet and fecal dry matter

	Diet pH	Diet DM (%)	Fecal DM (%)*
NoA	6.18	45.29	25.92
SBS	5.49	46.20	24.47
PA	5.21	46.24	24.87

*Composite fecal sample from 5 animals per treatment taken at 32 weeks of age.

Summary

Incorporation of 1% sodium bisulfate in a conventional mink diet as a replacement for phosphoric acid resulted in urinary pH values that were comparable to those resulting from use of phosphoric acid. Fecal moisture content did not differ between juvenile mink fed 1% sodium bisulfate and those fed 1% phosphoric acid. Overall body weight gain between 10 and 32 weeks of age was greater in juvenile mink fed 1% sodium bisulfate compared with those fed 1% phosphoric acid.

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HEALTH

Effects of Moderate Diet Restriction on Reproductive Longevity in Mink Females

The overall goal of this research program is to examine the effects of moderate diet restriction on the body condition, health and reproductive longevity of mink breeder females. This multi-year multi-disciplinary program will investigate the impacts of moderate caloric restriction during

the fall on the seasonal body condition and subsequent reproductive success in mink females over four reproduction cycles. The health of the mink dam and her kits, and the growth performance of the kits will be monitored. We will also estimate population genetic indicators of maternal traits, and will employ molecular genetic markers of genomic health, oxidative stress and aging, such as telomere length and DNA strand breaks. The first two years of this research program will be

carried out as the M.Sc. thesis research of Laura Boudreau. This research project began in the fall 2009 with 100 mink females in the control group (CTRL) and 100 females in the moderate diet restriction group (MDR). Prior to breeding there were two mortalities in the CTRL group. During breeding, 84 females were double mated in the CTRL group and 86 for the MDR group, while 9 CTRL females were single mated and 7 of the MDR females were single mated. Of the CTRL females 76 whelped and 72 weaned one or more kits, while of the MDR females 74 whelped and 66 weaned one or more kits. In the CTRL group, a total of 474 kits were born, 410 of which were born alive, whereas in the MDR group, 526 kits were born, 470 of which were born alive. The CTRL females weaned a total of 309 kits, while the MDR females weaned a total of 356 kits. Females were culled during the study if she or her sister died, was not mated, did not whelp, had no kits remaining at weaning, or if health problems were apparent. In total, 43 sister pairs remained to continue in the trial. The 43 continuing CTRL females produced a total of 188 kits by weaning, while the 43 continuing MDR females produced a total of 251 kits by weaning. On average, the continuing CTRL females weaned 4.37 kits, while the MDR females weaned 5.84 kits. By the end of June, 7 females in the CTRL group and 9 females in the MDR group had died. Based on our preliminary results of the first year of the study, it is evident that moderate diet restriction of yearling females during September-December resulted in larger litter size at birth and at weaning. In addition, the MDR females that met the selection criteria to continue on the study had an average a litter size

about 1.5 kits larger than the continuing CTRL females. This project will enable us to develop a better understanding of the impacts of body condition on genomic health and reproductive longevity in mink females as very little information exists about these effects in mink. We anticipate that this research will enhance the reproductive performance of mink breeder females and will result in significant economic advancement in the fur industry. Given that mink nursing sickness and fatty liver disease are two leading causes of morbidity and mortality in adult female mink, this research is also expected to markedly improve the health and welfare of the mink.

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Effect of moderate diet restriction on body condition,
health and reproductive longevity in mink females;
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ALEUTIAN DISEASE

Dual Identification of Aleutian Disease Virus (ADV) IgG and IgM Antibodies in Mink Blood by Lateral Flow Immunoassay With Two Test Lines:

The ability to distinguish ADV-specific IgG and IgM has a three-fold advantage in the control of ADV: First, detection of IgM provides an advanced, early warning signal that will alert the mink rancher to an ADV infection at the earliest possible time. Second, the detection of high levels of IgG indicates a chronic, potentially deadly and persistent ADV infection that could decimate an entire mink ranch population. It is known in the industry that, depending on factors such as the virus strain and genetic makeup of the animal, not all mink that contract ADV need to be culled, and this can often be assessed by the degree of antibody development. For mink ranchers in geographic areas that traditionally have a low prevalence of ADV the major benefit would be the early warning signal of a positive ADV IgM test, thereby helping to forestall potential outbreaks of ADV.

Specific immunoglobulin G (IgG) tends to increase linearly and remain in high concentrations over the course of a persistent

ADV infection. An increase in ADV IgM occurs within the earliest phase of the disease and, although transient in nature (generally dissipating after 85 days) appears as early as 6 days post infection while ADV IgG does not appear until a minimum of 12 days following infection. Several

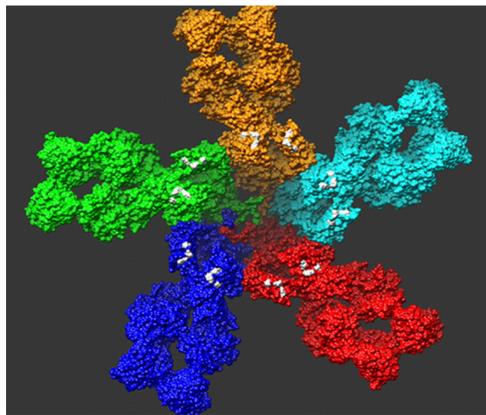
techniques have been developed to identify ADV positive animals by antibody detection, including counter immunoelectrophoresis (CIEP), enzyme-linked immunosorbent assay (ELISA), and lateral flow immunoassay (LFIA). None distinguishes between IgG, IgM (or IgA) but, rather, they detect any and all agglutinating ADV-related antibodies. Because the overabundance of

ADV immunoglobulins present in chronic infections IgG the tests are useful in the diagnosis and management of persistent ADV.

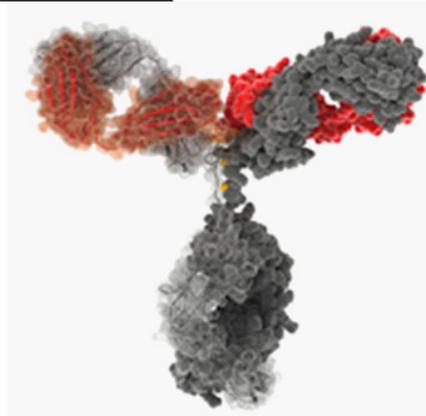
Results

The ADV infection profiles for each mink in this study were previously determined by PCR, ADV antibody dipstick (run on the

same day as collection), and by an ELISA technique for the qualitative detection of ADV antibodies; these specimens were obtained from the anti-ADV



ADV-specific IgM



ADV-specific IgG



IgY study. Those mink that did get infected by ADV generally showed an increase in absorbance over the negative control in the ELISA test beginning with specimens collected on 10/21/10 and showed an even greater increase in absorbance with specimens collected on 10/28/10. These results were corroborated by the lateral flow and PCR tests.

Mink Blood Specimens

1. Blood specimens were obtained from a total of sixteen (16) sapphire mink housed at the Michigan State University Department of Animal Sciences. Each mink was initially tested for ADV Antibodies by using the lateral flow dipstick test, and tested for the presence of the AD virus using PCR (University of Wisconsin Veterinary Diagnostic Test Laboratory). All mink were initially negative.
2. On September 8, 2010 (day 7, d7) all mink were injected intraperitoneally with 0.10 mL of the Utah-1 ADV strain diluted to a titer of 10⁻⁹ in sterile PBS, according to instructions from Dr. Marshall Bloom.
3. Blood samples were obtained on September 8, 2010 (d7), September 15, 2010 (d15), September 22, 2010 (d22), September 29, 2010 (d29), October 21, 2010, and October 28, 2010. On 10/14/10 all mink were re-injected with 0.10 mL of a 10⁻³ dilution of the ADV virus. On 10/21/10 all mink were re-injected with 0.080 mL of undiluted virus. For the purpose of the present study blood was collected using a swab technique. The swab was placed into a 12 x 75 mm conical plastic test tube, capped, and sent the same day as collection to Scintilla Development Company LLC. The samples were stored at -20 degrees centigrade until use. Swabs/tubes were not sent for the 10/21/10 collection; however,

plasma samples from that day were sent for ELISA testing.

4. Blood and plasma samples from each collection day were also analyzed by PCR, ELISA, and ADV Antibody lateral flow dipstick.

IgM and IgM/IgG Antibody Testing Formats

Dual ADV IgM/IgG Antibody Lateral Flow Immunoassay Dipstick – A membrane was striped with two test lines and one control line. The first test line consisted of goat anti-human IgM/ μ chain specific (closest to the bottom of the membrane) and the second test line was anti-ADV IgG. Anti-human IgM/ μ chain specific was chosen because of its use in previous immunoassay experiments for the detection of mink IgM and found to be superior to anti-mink IgM (Journal of Veterinary Diagnostic Investigation 3:3-9 (1991)). The conjugate was composed of black microspheres labeled with ADV antigen.

1. Enhanced ADV IgM Antibody Lateral Flow Immunoassay Dipstick – A membrane was striped with streptavidin. The conjugate was composed of black microspheres labeled with ADV antigen and mixed with biotin-labeled goat anti-human IgM/ μ chain specific before drying.
2. ADV IgM Antibody ELISA – The wells of a 96-well ELISA plate were coated with goat anti-human IgM/ μ chain specific. Diluted plasma specimens (1:100 in PBS) were added to each well and allowed to incubate at room temperature for 30 minutes. Following a wash step ADV antigen was added to each well, and again allowed to incubate for 30 minutes. The wells were washed, enzyme-labeled anti-ADV conjugate was added to each well,

and incubated for 30 minutes. The wells were washed and substrate solution added to each well and allowed to incubate in the dark for 30 minutes. Stopping solution was added to each well and the absorbances were read at 450 nm.

Results

All versions of the lateral flow test strips failed to detect anti-ADV IgM. However the ELISA results for ADV IgM indicate an increase in absorbances for all specimens tested, beginning around 10/21/10, or about one week after re-inoculating the mink with a higher titer of virus. Absorbances also increase uniformly for one week thereafter (the limit of our sampling), also following a second re-inoculation with even more virus.

Although IgM antibody never reaches the extremely high concentrations that IgG does following infection with ADV, it does increase after about 6 days and reaches its peak at around 18 days post infection. The amount of IgM then decreases, returning to pre-infection levels at around 85 days.

In a previous study anti-ADV IgM was mixed with an excess of anti-ADV IgG then tested by immunofluorescence. Although the intensity of the reaction was reduced in the presence of ADV IgG, the IgM activity was not entirely abolished, but was decreased. The authors speculate that IgG and IgM antibodies react with spatially different ADV determinants on the antigen (1). In the present study ADV IgM is found with the ELISA but not with the lateral flow strip. I suggest this is because IgM-specific determinants on the ADV antigen are hidden in the attachment points between the antigen and the chemical attachment “handle” on the microspheres. In the ELISA, the ADV antigen is added in solution and all determinants are unconstrained and thus free to bind.

In previous R/D incarnations of the ADV Antibody dipstick test the ADV antigen was attached to several different types of microspheres, including derivatized and non-derivatized models that bind antigens strictly through random hydrophobic interactions and presumably would leave exposed at least some IgM-specific determinants. The present ADV Antibody dipstick test uses derivatized microspheres and covalently binds the antigen, but most likely occludes the area on the antigen that binds to IgM. The present form of the ADV Antibody dipstick works adequately in the field for the detection of ADV antibodies and changing this test would not be advised. The ADV IgM ELISA test may be used upon request by mink farmers interested in knowing the stage of ADV infection, however cost and convenience factors from the mink rancher's perspective will have to be discussed.

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Selection For Low Blood Gamma Globulin In Mink Naturally Exposed To The Aleutian Mink Disease Virus

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Introduction

Aleutian disease is an important health issue for the mink industry worldwide. Infection of adult mink by the Aleutian mink disease virus (AMDV) causes enhanced production of antiviral antibodies, massive destruction of white blood cells, viral persistency, and formation of immune complexes that deposit in various organs (Alexandersen, S., Bloom, M.E., Wolfenbarger, J. et al. (1987), Bloom, M.E., Kanno, H., Mori, S. et al. (1994)). Currently, the control measure for the disease is regular testing of mink for antibody against the virus by Counter-immune electrophoresis (CIEP) (Cho, H.J. and Ingram, D.G. (1972)) and elimination of infected mink. Although this costly practice has been effective in reducing the prevalence of infected animals, it has not been effective in eliminating the virus from several regions in Nova Scotia (Farid, A., Finley, G.G. and Zillig, M.L. (2008)).

Development of the disease and severity of the disease symptoms depend on the genetic constitution of the mink, the strain of the virus and environmental conditions. While some mink color types can tolerate certain AMDV strains and do not develop clinical symptoms following infection, other color types suffer heavy losses (Porter, D.D., Larsen, A.E. and Porter, H.G. (1969), Hadlow, W.J., Race, R.E. and Kennedy, R.C. (1984)). Differences have been reported among individuals within color types in response to natural or experimental AMDV infection (Larsen, A.E. and Porter, D.D. (1975),

An, S.H. and Ingram, D.G. (1977, 1978), Cho, H.J. and Greenfield, J. (1978), Hadlow, W.J., Race, R.E. and Kennedy, R.C. (1984, 1985), Aasted, B. and Hauch, H. (1988)). These data suggest that the creation of genetically resistant mink to the AMDV infection may be a possibility. The objective of this study was to investigate the prevalence of seropositive animals on an infected ranch that has not been using CIEP as a selection tool.

Materials and Methods

This study was performed on a mink ranch that was established in 1944 in eastern Nova Scotia, Canada. Black mink have been kept on this ranch since its establishment, and some non-black mink that were kept on this ranch for several years were discarded in 1990. Since 1990, the breeding colony consisted of approximately 700 to 800 breeder females and 180 males. The herd has been closed to outside stock since 1995. The Iodine Agglutination Test (IAT) has been performed in December each year since 1966 without interruption. Animals that were positive on the IAT were discarded from the herd at pelting season. In addition to IAT results, selection has been primarily based on litter size and fur quality traits. Mink with high quality fur have sometimes been retained for breeding even if they were born into smaller litters or produced small litters. A sample of 61 mink was tested by CIEP in 2000, but CIEP test has not been used as a selection tool on this ranch.

A total of 454 breeder females, 99 breeder males and 220 female kits were sampled in November 2004. Two blood samples were taken into capillary tubes from each mink by toe-nail clipping. One sample was used to determine the presence of AMDV-specific antibody by the CIEP at the Animal Health Laboratories, Nova Scotia Department of Agriculture, Truro, Nova Scotia. The second sample was tested for IAT by the owner

on the ranch. Some samples showed inconclusive results by the CIEP test.

Results and discussion

The percentage of CIEP-positive mink was 84.4 (Table 1), which is comparable with the 84.7% positive cases on this ranch when 61 mink were tested in 2000, showing that the ranch has been heavily infected with the AMDV for at least six years. The percentage of positive mink on the IAT was 5.3. The proportion of IAT-positive animals has remained within the 5% to 10% range for almost two decades (personal communications). When serum gamma globulins rise over 20 to 22 % of total serum proteins, they precipitate in the presence of iodine. Therefore, the IAT test detects those mink that have an elevated level of gamma globulin, regardless of the source of infection.

IAT positive mink would be expected to either show clinical signs of Aleutian disease, i.e. they are really sick, or have other health problems, such as kidney malfunction. The IAT should therefore detect animals that are infected with AMDV in addition to those that are infected by other pathogens. IAT cannot, however, detect mink infected with the AMDV who fail to produce high levels of gamma globulins. Such animals will be detected by CIEP if they produce detectable levels of AMDV-specific antibody. The observations that only 5.3% of mink were positive on IAT while 84.4% were positive on CIEP suggest that

a large percentage of mink on this ranch were infected with the virus but were not developing the disease symptoms. The virus has very likely low pathogenicity, and the long-term selection for IAT-negative mink favored those individuals that could tolerate the virus, i.e. those that become infected with AMDV but did not become ill and thus remain IAT negative.

Litter size has rarely been below 4.5 during the past two decades on this ranch, and kit and adult mortalities have been low, particularly during the cold winter months. This is surprising in view of the fact that a large percentage of mink on this ranch were infected with AMDV. The disease is expected to reduce litter size and increase mortality, particularly at sub-zero temperatures. Survivability and reproductive performance have not been compromised on this heavily infected ranch because animals are tolerating the pathogen.

The percentages of adult females and males that were positive on IAT were 1.4 and 4.1, respectively, which were significantly lower than 13.6% for kits (Table 1). The corresponding

Table 1: Distribution of the IAT and CIEP test results by age and sex

Description	Young females	Breeder females	Breeder Males	Total
Number of mink sampled and tested	220	454	99	773
No. of samples inconclusive on CIEP	3	19	3	25
Number tested by CIEP	217	435	96	748
Number positive on CIEP	172	385	74	631
% positive on CIEP	79.3	88.5	77.1	84.4
No. positive on IAT	30	6	4	40
% positive on IAT	13.6	1.4	4.1	5.3
CIEP positive- IAT positive, %	13.3	1.1	3.1	4.9
CIEP positive – IAT negative, %	65.9	87.3	74.0	79.4
CIEP negative – IAT positive,	0.5	0.2	1.0	0.4
CIEP negative – IAT negative, %	20.3	11.2	21.9	15.2

figures for CIEP were 88.5%, 77.1% and 79.3%, respectively. These figures support the hypothesis that most animals become infected with AMDV during the first year of their lives, and only a small number of the clean mink become infected in later years. Those mink that had high levels of gamma globulin were detected by IAT and were removed from the herd. Only those that were not infected cleared the virus (CIEP negative) or those that were infected but were able to tolerate the virus were retained for breeding (CIEP positive and IAT negative).

The joint distribution of animals based on IAT and CIEP test results is presented in Table 1. Most animals (79.4%) were CIEP positive but IAT negative, which are ADV-infected mink with low levels of gamma globulin. The percentage of animals that were positive on both tests was 4.9. These were animals with high levels of gamma globulin, very likely as a result of infection with AMDV alone or in combination with other pathogens. Only 0.4% of the mink were CIEP negative but IAT positive.

These were possibly animals that were infected with pathogens other than AMDV.

Animals that were negative on both tests constituted the second largest group (15.2%). There are several plausible explanations for this group of animals: ii- they were exposed to the virus, but were not infected (resistant), ii- they were infected but cleared the virus from their bodies (resistant), iii- they were infected and were carrying the virus in their tissues, but the virus was not replicating. It seems that viral replication is required to trigger antibody production. These animals were thus tolerating the virus or iv- although it is unlikely, these animals may have never been exposed to the AMDV on this ranch by the Aleutian mink disease virus or may clear the virus after infection.

Conclusion

This study provided evidence that some mink may not become infected by the Aleutian mink disease virus or may clear the virus after infection.

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