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Reduced severity of histopathological lesions in mink selected for tolerance to Aleutian mink disease virus infection

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ABSTRACT

The objective of this study was to measure the effect of selection for tolerance on the severity of the Aleutian disease (AD) lesions in mink. Sensitivity and specificity of antibody detection in the blood by counter-immunoelectrophoresis (CIEP) relative to the presence of Aleutian mink disease virus (AMDV) in the spleen by PCR in naturally infected farmed mink were also estimated. Carcasses of 680 sero-positive (CIEP-P) black mink from 28 farms in Nova Scotia, Canada, and from 132 sero-negative (CIEP-N) mink from 14 of these farms were collected at pelting time. A total of 116 of the CIEP-P mink were from three farms where animals have been selected for tolerating AD for almost 20 years. The severity of the AD lesions was assessed by histopathological examination of kidneys, lungs, heart, brain and liver on a scale of 0 to 4. Sensitivity and specificity of CIEP relative to PCR were 0.97 and 0.85, respectively, and 16.5% of CIEP-N mink were PCR positive, which could be one of the reasons for the failure of virus eradication by CIEP in Canada. The CIEP-N and tolerant CIEP-P animals had 9.39 and 6.23 greater odds of showing lower lesion severity, respectively, than the CIEP-P animals ($P < 0.01$). The CIEP-N mink had a slightly higher chance ($P = 0.07$) of showing lower lesion severity (odds ratio 1.51) compared with tolerant CIEP-P mink. The results suggested that tolerant mink had significantly reduced severity of AD lesions despite having anti-viral antibodies and carrying the virus.

1. Introduction

The Aleutian mink disease virus (AMDV, *Carnivore amdoparvovirus* 1) is a major concern for the mink industry worldwide. Chronically infected adult mink exhibit a persistent antiviral antibody production, hypergammaglobulinemia, generalized plasmacytosis and immune complex-mediated glomerulonephritis and arthritis (reviewed in Bloom et al., 1994). Cases of severe nonsuppurative meningoencephalitis have also been reported in mink infected with AMDV (Dyer et al., 2000; Jahns et al., 2010). Severity of the Aleutian disease (AD) symptoms varies greatly between Aleutian and non-Aleutian mink and among individuals within color types (Bloom et al., 1975; Hadlow et al., 1985; Johnson et al., 1975; Larsen and Porter, 1975), and is influenced by age of the mink at the time of exposure, strain of the virus as well as the time elapsed after infection (Alexandersen, 1990; Hadlow et al., 1983; Henson et al., 1976; McCrackin Stevenson et al., 2001; Oie et al., 1996; Porter et al., 1969). Some non-Aleutian mink can tolerate the virus, showing persistent non-progressive infection, characterized by persistent antibody production, low serum gamma globulin and no clinical symptoms, whereas some mink show non-persistent non-progressive infection in which virus replication is ceased (Bloom et al., 1994). The frequency of sero-positive mink that did not succumb to AD was 25% of 24 naturally infected farmed mink and 80% of 30 feral mink (Cho and Greenfield, 1978), 33.8% of 74 naturally infected pastel mink (An and Ingram, 1977), 29% of 140 pastel mink intraperitonealy inoculated and tested at 66 and 500 days post-inoculation (Larsen and Porter, 1975), 46% of 195 naturally infected wild-type farmed mink (Aasted and Hauch, 1988) and 85% of 20 pastel mink subcutaneously inoculated with the Pullman strain and tested 24 weeks after inoculation (Hadlow et al., 1984), indicating that the presence of non-progressive infection is not a rare event in non-Aleutian mink.

Because AD has no effective treatment or vaccine (Aasted, 1985; Aasted et al., 1998; Castelruiz et al., 2005) the accepted control strategy across the globe has been the elimination of sero-positive mink identified by the counter-immunoelectrophoresis (CIEP) (Cho and Greenfield, 1978) or recently by the enzyme-linked immunosorbent assays (ELISA) (Andersson and Wallgren, 2013; Dam-Tuxen et al., 2014; Knuuttila et al., 2014). This strategy, in combination with disinfection practices and implementation of biosecurity measures, has been followed in Nova Scotia (NS), Canada, since the mid-1970s, but has not been effective in permanent viral eradication from many farms (Farid et al., 2012). False negative CIEP tests could be one of the reasons for the failure of the virus eradication programs in NS. Yet, the extent of false

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negative CIEP tests in mink herds that have been practicing the test-and-cull strategy for a long period of time is not known.

A few farmers in NS have selected their mink for tolerance to the virus solely based on phenotypic assessment of animal health and production traits or in combination with the iodine agglutination test (IAT), which is an in-house method for detecting animals with high amounts of serum gamma globulin (Henson et al., 1962). The primary objective of this field survey was to assess the effect of selection for tolerance on the severity of the AD lesions in black mink in NS. Results of CIEP and polymerase chain reaction (PCR) tests for detecting AMDV infection in naturally infected captive mink were also investigated. This study is the first to investigate the effect of long-term selection for tolerance on histopathological lesions of AD.

2. Materials and methods

2.1. Source of animals

Mink farmers in NS test potential replacement kits and adults by CIEP every year and eliminate seroconverted mink. A total of 28 infected farms participated in this survey (F1 to F28), which were approximately 50% of all infected mink farms in this province. These farms were located in Digby and Yarmouth counties in south-western NS, except farms F6, F8 and F16, which were in the eastern part of the province. Three of the farms, F6 and F16 in eastern NS and F17 in the west, did not follow the test-and-removal strategy. Farm F6 used health status, litter size, pelt quality traits and the IAT for over 20 years for selecting replacements with a considerable success in establishing a tolerant herd (Farid, 2010). The prevalence of CIEP-positive (CIEP-P) animals on this farm varied between 80.2% and 84.7% from 2000 to 2005 (Farid et al., 2012). Selection of replacement animals on F17 has been solely based on health, litter size and pelt quality traits for >20 years and the prevalence of CIEP-P animals on this farm was 73.6% in a sample of 6060 mink tested in 2003 (Farid et al., 2012). The majority of mink on F16 originated from F6 and from another farm on which the mink were selected based on IAT results. This farm was in operation from 2004 to 2008, and prevalence of CIEP-P animals were 90.9% in 2006 and 76.5% in 2007. Mortality rate and reproductive performance of mink on these farms were comparable to those in the AMDV-free farms in the province (Farid, 2010 and unpublished data) and they will be referred to as tolerant.

Carcasses of 680 CIEP-P mink from the 28 farms and of 132 CIEP-negative mink (CIEP—N) from 14 of the same farms were collected during the pelting season (November to February) in 2003 to 2010, inclusive (Table 1, Fig. 1). CIEP-N animals were from the same shed as, and often in adjacent cages to, CIEP-P animals. Samples from 20 to 88 CIEP-P mink were collected from 14 of the farms and another nine farms were represented by 15 to 19 samples (Table 1). One farm (F16) ceased its operation in 2008 after the initial sampling. There were few CIEP-N mink on tolerant farms (F6, F16, F17) which were not sampled in this study.

2.2. Animal sampling

Arrangements were made with farmers to keep mink carcasses for this survey after they were killed according to the standard industry protocols. Information on the sampling procedures along with a sampling form were sent to participating farmers who either pelted the mink on their farms or sent carcasses to the Co-op Pelting Plant in Weymouth, NS. In some cases the sampling process was discussed with farmers face-to-face on their premises. Carcasses were identified prior to shipment to the Pelting Plant, and were manually pelted to avoid inaccurate identification. CIEP-N animals were pelted and processed ahead of CIEP-P animals on the farms and at the Pelting Plant. Each carcass was put into a labeled plastic bag and shipped to the provincial Pathology Laboratory, Veterinary Services, NS Department of Agriculture in Truro, NS, for necropsy within three days of killing.

2.3. Necropsy and histopathology

Necropsy was performed by an experienced veterinary pathologist (Dr. L.E. Ferns) at the provincial Pathology Laboratory. The spleen, brain, lungs, liver, kidneys, mesenteric lymph nodes and heart were examined for size, color, inflammation and necrosis that could be associated with AD, and samples of brain, lungs, liver, kidneys and heart were stored in 10% neutral buffered formalin for histopathology. Initially, pancreas and urinary bladder were also sampled for histopathology, but examination of these organs discontinued in 2004 because the AD-related lesions were not as apparent on these organs as on the other five.

Paraffin-embedded tissue sections were prepared and stained with hematoxylin and eosin and were examined under a light microscope for lesions characteristics of AD. Histopathological lesions were subjectively scored on a scale of 0 (no lesion) to 4 (very severe lesions of advanced AD) by the pathologist (Dr. L.E. Ferns) who was blinded to the source of the samples. Scoring was primarily based on the degree of accumulation of mononuclear cells (plasma cells, lymphocytes and macrophages) in the tissues with associated lesions (Henson et al., 1976; Johnson et al., 1975). A sample of the spleen from each animal was harvested aseptically at necropsy and stored at −80 °C for DNA extraction.

2.4. Laboratory analysis

DNA was extracted from spleen samples by the high-salt procedure (Aljanabi and Martinez, 1997) in 2003 to 2005 with the addition of an RNAse treatment step, using 2 μL of a 10 μg/μL RNAse-A and incubation at 37 °C for 30 min. PCR amplifications were performed using 60F-60R primers and four DNA solution volumes (2.55, 1.5, 0.15 and 0.075 μL)

Table 1
Distribution of CIEP positive and CIEP negative mink by farm.

Farm	Positive	Negative	Farm	Positive	Negative	Farm	Positive	Negative
F1	36	–	F11	15	6	F21	19	–
F2	47	–	F12	7	12	F22	25	6
F3	15	–	F13	15	10	F23	13	7
F4	28	–	F14	31	11	F24	20	–
F5	36	12	F15	32	–	F25	16	–
F6[a,b]	88	–	F16[a,b,c]	12	–	F26	20	5
F7	10	–	F17[a]	16	–	F27	16	8
F8[b]	49	20	F18	20	11	F28	9	3
F9	28	4	F19	20	–	–	–	–
F10	18	17	F20	19	–	Total	680	132

[a] All farms used CIEP for virus eradication, except F6, F16 and F17 where animals were tolerant and CIEP-positive.
[b] Farms are located in western Nova Scotia except F6, F8 and F16 which are in the east.
[c] F16 closed down in 2008.