JOURNAL OF
WILDLIFE DISEASES

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DOI: 10.7589/2013-10-286

Journal of Wildlife Diseases, 50(3), 2014, pp. 689–693
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ABSTRACT: Aleutian mink disease virus (AMDV) occurs in the American mink (*Neovison vison*) in wild populations and on mink farms and can cause illness and death. The North American river otter (*Lontra canadensis*) may be exposed to AMDV because of shared space and habitat with mink. Using serology and real-time PCR, we tested river otters across Ontario for AMDV infection. We found no evidence of infection in otters, a surprising finding given the sympatric distribution, niche overlap, and close phylogenetic relationship of the river otter and the American mink. Our results are consistent with the hypothesis that the major point of spillover of AMDV between mink farms and wildlife is manure and composting carcasses on mink farms. Mink farms in Ontario are generally in agricultural landscapes; it is unlikely that river otter use these habitats and thus are likely not exposed to AMDV. We found no evidence that AMD is an important disease for the river otters in Ontario.

Key words: Aleutian mink disease virus, American mink, hybridization, mink farming, parvovirus, river otter, spillover.

Aleutian mink disease (AMD) is a pathogenic parvovirus infection caused by Aleutian mink disease virus (AMDV) which occurs primarily in the American mink (*Neovison vison*) (Bloom et al. 1994), a mustelid species that has been domesticated for fur markets since the late 1800s. The disease occurs in wild and domesticated mink (Bloom et al. 1994; Nituch et al. 2011, 2012), and has been reported in many jurisdictions where domestic mink are raised; it is apparent that the commercial trade of domestic mink contributes to the spread of the virus (Bloom et al. 1994; Nituch et al. 2012). Aleutian mink disease virus can be highly prevalent in wild mink populations (Nituch et al. 2011; Farid 2013) and mink farms can be point sources of disease (Nituch et al. 2011), but the extent to which wild and domestic mink transmit AMDV, and other wildlife act as reservoirs, is unknown (Nituch et al. 2012; Farid 2013).

Wild mink, or other wildlife, could spread AMDV to mink farms via contact of infected wild individuals with mink on farms. Conversely, AMDV could spread from mink farms into the wild via the escape of infected individuals from farms, or through contact between wildlife and infected material on farms. For example, wildlife may visit composting mink carcasses or mink manure piles on farms (Nituch et al. 2011). The virus can survive for more than 2 yr in soil and improperly composted manure or carcasses (Bloom et al. 1994).

Wildlife other than American mink can become infected with or exposed to AMDV, including striped skunk (*Mephitis mephitis*), ermine (*Mustela erminea*), European mink (*Mustela lutreola*), raccoon dog (*Nyctereutes procyonoides*), and raccoon (*Procyon lotor*) (Alexandersen et al. 1985; Mañas et al. 2001; Pennick et al. 2007; Farid 2013). Given demonstrated global problems with mink farm biosecurity (Bonesi and Palazon 2007; Bowman et al. 2007), there is potential for mink farms to be sources of cross-species spillover of AMDV (Nituch et al. 2011, 2012). Wildlife other than mink may also be sources of disease on farms (Farid 2013). Thus, evaluating the prevalence and distribution of AMDV in a range of species is important to understand and manage this disease.

The North American river otter (*Lontra canadensis*) is a mustelid that is sympatric with wild American mink over most of its range. Like mink, otters are semi-aquatic carnivores, often occupying similar habitats (Melquist et al. 1981). Given the degree of sympatry, niche overlap, and phylogenetic similarity of these two species, and thus the potential for spillover of AMDV from mink, we hypothesized that AMDV would be prevalent in the otter. There have been occasional observations of AMDV or AMDV-like symptoms in European otters (*Lutra lutra*; Wells et al. 1989; Mañas et al. 2001), but only two surveys for AMDV in North American river otters (Gaydos et al. 2007; Farid 2013), with inconclusive results. Gaydos et al. (2007) used an antibody test and found no evidence of disease exposure, whereas Farid (2013) used PCR for viral DNA, and found two of 11 individuals (18%) positive for AMDV. Experimental inoculation of one river otter with AMDV failed to elicit an immune response (Kenyon et al. 1978), and it remains unclear whether otters could be important reservoirs of AMDV. We have extensively surveyed local free-ranging mink populations in Ontario, Canada for AMDV (Nituch et al. 2011, 2012), and here used similar methods (an antibody test and real-time PCR for viral DNA) to survey sympatric river otters for AMDV.

We collected otter carcasses from fur trappers during the winters of 2011 and 2012 in the same geographic area as Nituch et al. (2011) who found prevalence of AMDV antibody in mink to be 29% (Fig. 1). We collected 59 otters (17 females, 42 males). The majority of individuals were adults; there were five juveniles (two female, three male).

We tested for AMDV antibody in collected otters following the methods of Nituch et al. (2011). During necropsy, we collected blood samples via cardiac puncture and analyzed samples for ADMV antibodies using counterimmunoelectrophoresis (CIEP) at the University of Guelph's Animal Health Lab (Guelph, Ontario). For mink, the CIEP test has a sensitivity of 98%, specificity of 86–91% (9–14% false positives), and repeatability of 98–99% (Animal Health Lab, University of Guelph, unpubl. data).

Because otters may have been exposed to AMDV without expressing antibodies, we used real-time (RT)-PCR to test for AMDV DNA. We used known AMDV-positive mink (Nituch et al. 2012) as positive controls. Otter and mink spleen tissues were extracted using QIAGEN DNeasy blood and tissue kit (Qiagen Inc., Mississauga, Ontario, Canada). Quality of extracted DNA was assessed by visualizing on 1.5% agarose gel stained with ethidium bromide (EtBr).

We used VP2 primers (Oie et al. 1996) to amplify a portion of the AMDV capsid genes in eight positive mink samples. The PCR was performed in 50 μL reactions containing 5 μL mink DNA, 1× PCR buffer, 1 μg/mL bovine serum albumin, 0.2 mM dNTP, 1.5 mM MgCl2, 0.5 mM of each primer, and 1 unit of Taq polymerase (Invitrogen, Life Technologies, Burlington, Ontario, Canada). Amplification was completed using Eppendorf mastercyclers under the following conditions: 94 C for 5 min, 35 cycles of 94 C for 30 sec, 60 C for 1 min, and 72 C for 1 min, with a final 45 min extension at 60 C. Negative controls were used in each set of amplifications. Amplified product was visualized on 1.5% agarose gel stained with EtBr. A positive result was indicated by a DNA fragment of approximately 500 base pairs. Confirmed positive amplicons were quantified using Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Eugene, Oregon, USA) using a Fluorstar Galaxy. Serial dilutions were generated from 10×10^9 to 1 copy per 5 μL for positives. Serial dilutions were run on a StepOne Plus™ real-time PCR (Applied Biosystems, Life Technologies, Burlington, Ontario, Canada), using the Fast SYBR® Green protocol. Reagents included 10 μl (2×) Fast SYBR® Master mix (Applied Biosystems),

FIGURE 1. Counties in Ontario, Canada, where river otters (*Lontra canadensis*) were sampled and tested for Aleutian mink disease virus (AMDV). Shaded counties were previously found to contain American mink (*Neovison vison*) that were positive for AMDV (Nituch et al. 2012), and river otters in the labeled counties were sampled during this study.

0.2 mM forward and reverse primers and 5 μL DNA. The PCR was then repeated with the otter samples to be tested for virus, concurrently with the mink positives, that were used to produce the standard curve, which was only accepted as an accurate reference if the R^2 value was ≥ 0.99.

We tested all of the otters for AMDV antibodies using the CIEP test, and found 0/59 individuals positive. The initial RT-PCR test of positive control samples displayed a standard curve $R^2=0.998$ and the test using positive controls as standards against the unknown otter samples had a standard curve $R^2=0.991$. Capsid proteins of AMDV were detected in all mink samples used as positive controls. In contrast, we detected no capsid proteins in any of the otter samples. Evidence of a

surplus of DNA was observed, as concentrations of template DNA were high, however the melt curves confirmed a lack of target amplification in the otter samples.

Despite the known prevalence of AMDV infection in free-ranging mink in Ontario (29%), we found no evidence for either the virus or antibodies to the virus in river otters from the study area. Thus, we found no evidence of AMDV spillover from mink to otters. Our finding is consistent with that of Gaydos et al. (2007), who found no AMDV in otters in Alaska or Washington, where prevalence in free-ranging mink was unknown; however, our result contrasts with a study in Nova Scotia, where two of 11 (18%) otters were PCR positive for AMDV (Farid 2013). The prevalence of AMDV in mink in the Nova Scotia study was 93%.

It appears that North American river otters can be exposed to AMDV (Wells et al. 1989; Mañas et al. 2001; Farid 2013); however, it remains to be confirmed whether otters can mount an immune response (i.e., exhibit AMDV antibodies), and express clinical symptoms of disease (Kenyon et al. 1978; Wells et al. 1989; Farid 2013). Nevertheless, our finding of no evidence of AMDV in otters in a location with known infection in mink may be indicative of barriers to spread between mink and otter. Such a barrier could exist if spread principally occurs at or around mink farms (Nituch et al. 2011). Mink farms in Ontario are generally in agricultural landscapes, and because otters tend to be restricted to aquatic habitats (Melquist et al. 1981), it would be unlikely that otters would visit compost and manure piles on mink farms. Alternatively, if the virus is principally spread through social contacts of free-ranging animals, it could be that there are few social contacts between mink and otter and little opportunity for viral spread. Several studies support the idea that social contacts between American mink and otter are rare (Melquist et al. 1981; Ben-David et al. 1996; Bonesi et al. 2004).

It is possible that survivorship of otters exposed to AMDV is very low, such that there were no active, exposed individuals to sample. Progression of this disease in other species suggests, however, that such a high mortality rate would be unlikely (Alexandersen et al. 1985; Bloom et al. 1994; Farid 2013). Instead, our findings suggest that, at least in Ontario, the North American river otter is not an important reservoir or transmitter of AMDV. Furthermore, we observed no evidence to suggest that AMD is an important disease for the river otter, despite sympatry and niche overlap of the otter with the American mink.

We acknowledge the Wildlife Research and Monitoring Section of the Ontario Ministry of Natural Resources for funding. We also thank the fur harvesters who participated in the study.

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Submitted for publication 30 October 2013.
Accepted 25 February 2014.