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ALEUTIAN MINK DISEASE VIRUS IN STRIPED SKUNKS (*MEPHITIS MEPHITIS*): EVIDENCE FOR CROSS-SPECIES SPILLOVER

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ABSTRACT: Aleutian mink disease virus (AMDV) causes a parvovirus infection, initially characterized in American mink (*Neovison vison*), that may have harmful effects on wild populations of susceptible animals. In North America, where American mink are native, the origin, host range, and prevalence of AMDV in wild species is not clear. We studied striped skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*) to determine whether species sympatric with mink are potential reservoirs in the transmission of AMDV to wild mink and mink farms. Antibodies to AMDV were detected in 41% of skunk serum samples (143/347) and AMDV nucleic acids were detected in 32% (14/40) of skunk spleen samples by PCR, indicating that AMDV exposure and infection were frequent in skunks. We detected no AMDV antibodies in 144 raccoon blood samples. Phylogenetic analysis revealed a newly identified AMDV haplogroup consisting of isolates from Ontario skunks and a free-ranging domestic mink from Ontario. Our findings of frequent AMDV infection in skunks, close genetic similarity between skunk and mink AMDV isolates, and evidence of AMDV transmission from skunks to mink support the hypothesis that skunks may be acting as alternative hosts and reservoirs of AMDV to wild mink through cross-species virus spillover.

Key words: Aleutian mink disease virus, American mink, disease, raccoons, spillover, striped skunks.

INTRODUCTION

Generalist pathogens can play an important role in disease emergence in wildlife and domestic animals (Cleaveland et al. 2001; Dobson and Foufopoulos 2001). Seventy-seven percent of mammalian livestock pathogens and 91% of domestic carnivore pathogens are known to infect multiple hosts, and of the 70 most important animal diseases worldwide, 57 affect multiple hosts (Cleaveland et al. 2001). Parvoviruses of wildlife and domestic animals are especially variable pathogens capable of rapid adaptation to novel hosts (McDonald and Larivière 2001). For example, domestic dogs have been implicated in the transmission of feline panleukopenia virus to captive large cats (Steinel et al. 2000). Despite this, few ecologic studies have explored the dynamics and effect of single pathogens across multiple susceptible host species (Begon et al. 1992; Woolhouse et al. 2001).

Aleutian mink disease virus (AMDV) causes a chronic, persistent parvovirus infection of American mink (*Neovison vison*). Progressive Aleutian disease in adult mink is characterized by immune complex glomerulonephritis, hypergammaglobulinemia, and plasmacytosis (Bloom et al. 1994). Apart from direct mortality, AMDV infections in mink can also contribute to population declines through decreased fertility, spontaneous abortions, and increased susceptibility to other pathogens (Bloom et al. 1994). Aleutian mink disease (AMD) is the most important infectious disease affecting domestic mink on fur farms and can lead to large economic losses due to decreased reproduction and pelt value (Hunter 2008).

In the wild, anti-AMDV antibodies have been reported in feral domestic American mink in southern England (Yamaguchi and Macdonald 2001), France (Fournier-Chambrillon et al. 2004), and Spain

(Mañas et al. 2001). In Canada, where American mink are native, AMDV exposure and infection are widespread in the free-ranging wild and feral domestic mink populations (Nituch et al. 2011; Farid 2013), and antibody prevalence is higher in populations in close proximity to mink farms (Nituch et al. 2011, 2012).

Although AMD is considered primarily a disease of mink, serologic investigations have reported anti-AMDV antibodies in several other Mustelidae, including fishers (*Martes pennanti*), American martens (*Martes americana*), European otters (*Lutra lutra*), and domestic ferrets (*Mustela putorius furo*), as well as in non-mustelids, such as striped skunks (*Mephitis mephitis*), red foxes (*Vulpes vulpes*), and raccoons (*Procyon lotor*), suggesting that species other than mink are reservoirs of AMDV (Ingram and Cho 1974; Kenyon et al. 1978; Wells et al. 1989; Oie et al. 1996; Farid 2013). The potential for cross-species pathogen transmission is a particular concern in AMD control, both in fur farms and in the wild. Reservoir species tend to be widespread, highly abundant, and share similar habitat with other hosts; they can therefore maintain relatively high prevalence of infection, thus acting as continual sources of infection to smaller, more susceptible wildlife populations (Daszak et al. 2000; Funk et al. 2001; Cleaveland et al. 2002). As well, the presence of abundant and widespread wildlife reservoirs may impede AMD control measures on fur farms, especially if biosecurity is lax. The only effective means of eradicating the virus on mink farms involves repeated AMDV antibody testing followed by culling of positive animals (Cho and Greenfield 1978). Frequent reoccurrence of AMD on previously "AMD-clean" farms has been noted, however, and may be at least partially the result of reinfection of farms by contact with infected wildlife, ultimately rendering AMD control programs ineffective (Oie et al. 1996). The importance of reservoir identification is illustrated by challenges in controlling multihost pathogens, such as *Mycobacterium bovis* (Macdonald et al. 2006), phocine distemper virus in seal populations (Hall et al. 2006), and canid pathogens in endangered Island foxes (*Urocyon littoralis*; Clifford et al. 2006).

Although AMDV infection is common and well characterized in domestic mink on fur farms, there is a paucity of data regarding AMDV prevalence among wild species, and it remains unclear whether AMDV isolates from wild species are closely related to known AMDV isolates or whether they are pathogenic in mink.

Anecdotal evidence suggests that wild mink and scavengers, such as other mammals, birds, rodents, and flies, may be attracted to waste piles at mink ranches (B. Hunter unpubl. data), where they may contract and spread Aleutian mink disease virus. In several severe AMD outbreaks in Utah, raccoons and skunks entering mink sheds were implicated as potential reservoirs of AMDV to farmed mink. Viral DNA isolated from two raccoons caught near these mink farms matched the viral isolates from the farms (Oie et al. 1996).

In the striped skunk, AMDV infection has been identified in a small number of free-ranging individuals in South Dakota and British Columbia, Nova Scotia, and Ontario, Canada (Ingram and Cho 1974; Oie et al. 1996; Britton et al. 2010). Reports of suspected AMDV in pet striped skunks have noted hyperglobulinemia, anti-AMDV antibodies, and histologic changes consistent with AMDV infection in mink (Pennick et al. 2007; Allender et al. 2008). Viral DNA from three skunks shared 90% homology (Allender et al. 2008) and was 92% homologous with known mink AMDV viral sequences (Pennick et al. 2007). Although reports of AMDV antibody in striped skunks have been few, little is known about AMDV infection in skunks.

Because of the persistent nature of AMD and its potential negative effects on reproduction and survival, the disease

may have harmful effects on wild populations of susceptible animals. Furthermore, the potential for cross-species AMDV transmission highlights a critical need to identify whether additional AMDV reservoirs or alternative hosts exist that could lead to transmission of the virus among wild mink, other wildlife, and farmed mink. We investigated whether skunks and raccoons are potential reservoirs in the transmission of AMDV to wild mink and mink farms. We first tested for AMDV antibodies in skunks and raccoons and assessed antibody prevalence in relation to the density of mink farms. We hypothesized that if skunks and raccoons were becoming infected with AMDV from mink farms, then AMDV antibody prevalence in skunks and raccoons would be highest in proximity to mink farms. Second, we sought to characterize the AMDV in skunks and compare it with published AMDV sequences from domestic mink and wild mink. If cross-species transmission occurs from mink and mink farms to skunks (or vice versa), similar isolates should occur among samples from these different sources.

MATERIALS AND METHODS

Sample collection

We obtained serum samples from raccoons and skunks collected by the Ontario Ministry of Natural Resources between 2006 and 2008 as part of a rabies surveillance program. Animals were collected from SW Ontario, which had the highest mink farm density within the province, as well as eastern Ontario, which had few mink farms (Statistics Canada 2006). In eastern Ontario, 118 skunk serum samples were obtained, and 229 skunks were sampled in SW Ontario. We also obtained spleen samples from 40 of the SW Ontario skunks during routine postmortem examination. Sera from 304 raccoons were acquired from SW Ontario. Serum samples were tested for the presence of anti-AMDV antibodies by counterimmunoelectrophoresis (CIEP) at University of Guelph's Animal Health Lab (Ontario, Canada). All animals were handled according to guidelines and protocols approved by the Animal Care and Use Committees of Trent University (Peterborough, Ontario, Canada) and the Ontario Ministry of Natural Resources (OMNR), or, in the case of samples obtained through fur harvest, in accordance with OMNR protocols and regulations.

AMDV PCR and sequencing

We extracted whole genomic DNA from an approximately 10-mg spleen tissue sample using a QIAGEN DNeasy kit (Qiagen Inc., Valencia, California, USA). Samples were extracted according to the manufacturer's protocol, except for the last step, when the samples were eluted with 70 C $TE_{0.1}$ (1.0 M Tris-HCl, 0.5 M ethylenediaminetetraacetic acid) rather than with Buffer AE (Qiagen).

Nucleotide fragments of the hypervariable regions of the AMDV NS1 and VP2 genes were amplified from the isolated DNA by PCR using previously described primers (Oie et al. 1996; Olofsson et al. 1999). The PCR was performed in 20-μL reaction mixtures containing 5 μL template DNA, 1× PCR buffer with $(NH_4)_2SO_4$, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 0.5 μM of each primer, and 1 unit of *Taq* polymerase (Fermentas, Burlington, Ontario, Canada). We amplified all samples under the following conditions: 94 C for 5 min; 35 cycles of 94 C for 30 s, 65 C for 1 min (NS1) or 60 C for 1 min (VP2), and 72 C for 1 min; with a final 45-min elongation at 60 C. Positive and negative controls were included in each set of amplifications. Samples from all skunk spleen samples, regardless of CIEP antibody result, were also tested for AMDV using PCR. All DNA and reagents were processed and manipulated in a remote laboratory suite where no AMDV molecular biology had been performed.

We subjected PCR products (10 μL) to agarose gel electrophoresis and visualized them under ultraviolet light after ethidium bromide staining to determine presence and relative quality of extracted AMDV DNA. A positive PCR result was indicated by the presence of a DNA fragment of the expected size (~500 base pairs [bp]). The AMDV DNA product from positive PCR reactions was purified with ExoSAP (USB Corporation, Cleveland, Ohio, USA), and precipitated in ethanol to remove excess salts. Sequencing reactions were performed using BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems Foster City, California, USA). Samples then were resuspended in 15 μL of Hi-Di Formamide (Applied Biosystems), and both strands of the labeled DNA samples were run on an ABI 3730 automated sequencer (Applied Biosystems) at the Ontario Ministry

TABLE 1. Aleutian mink disease virus isolates ($n=25$) from striped skunks (*Mephitis mephitis*) included in a phylogenetic analysis. All samples were isolated in 2008 from counties in Ontario, Canada.

Isolate	Isolate region	County	GenBank accession
S2	VP2	Wellington	HM623397
S4	VP2	Perth	HM623405
S5	VP2	Perth	HM623406
S14	VP2	Huron	HM623393
S19	VP2	Oxford	HM623394
S22	VP2	Wellington	HM623395
S29	VP2	Oxford	HM623396
S32	VP2	Wellington	HM623398
S36	VP2	Oxford	HM623399
S37	VP2	Perth	HM623400
S38	VP2	Perth	HM623401
S40	VP2	Perth	HM623402
S41	VP2	Perth	HM623403
S43	VP2	Perth	HM623404
S4	NS1	Perth	HM623354
S5	NS1	Perth	HM623355
S14	NS1	Huron	HM623345
S19	NS1	Oxford	HM623346
S22	NS1	Wellington	HM623347
S29	NS1	Oxford	HM623348
S36	NS1	Oxford	HM623349
S37	NS1	Perth	HM623350
S38	NS1	Perth	HM623351
S40	NS1	Perth	HM623352
S43	NS1	Perth	HM623353

of Natural Resources genetics laboratory at Trent University.

Sequence analysis

We compared our skunk AMDV sequences with AMDV NS1 and AMDV VP2 sequences from our previous mink AMDV study (Nituch et al. 2012) and from the GenBank database (NCBI 2014) (Table 1). We edited all nucleotide sequences in BioEdit 7.0.5.3 (Hall 1999) and conducted multiple sequence alignment using Molecular Evolutionary Genetics Analysis (MEGA) 4.0 (Tamura et al. 2007). The acquired NS1 sequences were edited to a length of 322 bp corresponding to nucleotide positions 601–922 of the AMDV-G genome, and the VP2 sequences were edited to a length of 531 bp corresponding to nucleotide positions 2,725–3,255 of AMDV-G.

Nucleotide divergence values were calculated using MEGA. Median-joining networks for both the NS1 and the VP2 regions were created using R (R Development Core Team 2014) and Cytoscape (Lopes et al. 2010). Phylogenetic trees were constructed using the maximum likelihood method in R, with the use of bootstrap analyses of 1,000 replicates, and visualized in FigTree (Rambaut 2007).

RESULTS

Serology

All 304 raccoon sera were negative for anti-AMDV antibodies. In eastern Ontario, 36% (43/118) of skunk sera were positive for antibody to AMDV in 2007. Of skunks sampled in SW Ontario, 27% (11/41) were AMDV antibody–positive in 2006, 49% (71/144) were positive in 2007, and 41% (18/44) were positive in 2008. Overall, AMDV antibody prevalence in skunks from SW Ontario across all years was 44% (100/229), and prevalence in skunks across all years and locations was 41% (143/347). Overall antibody prevalence did not differ between high (SW Ontario) and low (eastern Ontario) mink farming regions ($\chi^2=1.68$, $P=0.195$).

PCR and sequencing

Using PCR, we detected AMDV in 32% (14/40) of skunk spleen samples from SW Ontario. The acquired NS1 sequences were edited to a length of 322 bp corresponding to nucleotide positions 600–922 of the complete sequence of the culture-adapted AMDV-G strain. The acquired VP2 sequences were edited to 528 bp corresponding to nucleotide positions 2,727–3,255 of the complete sequence of the AMDV-G.

The NS1 gene nucleotide sequences of the newly sequenced skunk AMDV isolates 93–99% similarity with each other and 84–87% similarity to AMDV-G. They shared between 82% (isolates skunk S40 and Ontario mink ON11, and isolates skunk S36 and Ontario mink ON15) and 91% (skunk S22 and Sweden mink SE22) identity with previously characterized mink sequences. Skunk AMDV isolates, however, shared as much as 98% identity with one escaped domestic-wild hybrid

mink AMDV isolate (mink ON8; Essex, Ontario).

The VP2 gene nucleotide sequences of the newly sequenced skunk AMDV isolates shared 94–100% similarity with each other and 90–93% similarity with AMDV-G. The AMDV isolates from skunks S40 and S38 from Perth County, Ontario, were identical. The skunk sequences shared 88% (skunk S43 and Chinese mink CH4) to 95% (skunk S5 and Ontario mink ON3) identity with mink AMDV sequences, with the exception of Ontario mink ON8, which shared up to 99% identity with skunk isolates.

Phylogenetic and network analyses

Both the AMDV NS1 and VP2 minimum-spanning networks (Figs. 1, 2) and maximum likelihood phylogenies (Figs. 3, 4) indicated that Ontario skunk AMDV isolates were closely related to, but somewhat separate from mink AMDV isolates. In the VP2 network, the skunk haplogroup formed a starlike pattern characteristic of a recent bottleneck with subsequent expansion (Slatkin and Hudson 1991). One mink AMDV isolate (isolate ON8; Essex, Ontario) was contained within the skunk haplogroups in both networks.

DISCUSSION

We identified widespread exposure to AMDV and presence of AMDV in striped skunks. Skunk AMDV prevalence was comparable in regions with both high and low densities of mink farms, suggesting that mink farms may not be important sources of AMDV transmission to skunks, as they appear to be for wild mink (Nituch et al. 2011, 2012). The AMDV antibody prevalence in skunks was higher than the prevalence previously observed in free-ranging mink in Ontario (Nituch et al. 2011), however, suggesting that skunks may be reservoirs of AMDV infection to mink and other sympatric species with overlapping ecologic niches. Conversely, despite previous reports of AMDV antibodies (Ingram and Cho 1974; Oie et al. 1996; Farid 2013) and PCR-positive AMDV results in raccoons, our study indicated that raccoons in Ontario had either not been exposed to the AMDV, that AMDV exposure in raccoons was so infrequent that antibody-positive individuals were not sampled, or that AMDV antibodies in raccoons were not detected by the CIEP test. Thus, AMDV in raccoons warrants further investigation.

Our phylogenetic analyses revealed a newly identified AMDV group consisting of isolates from Ontario skunks and an escaped domestic mink from Ontario. The AMDV skunk isolates formed a sister clade with AMDV mink isolates in trees constructed from both AMDV sequences. The NS1 skunk AMDV clade had high bootstrap support (97%), whereas the VP2 skunk clade was less well resolved (bootstrap value of 66%). Slightly dissimilar topology between trees and networks constructed from different AMDV proteins may be due to differential selective pressures on these two regions. As well, fewer isolates have been characterized using the VP2 region; therefore, more gaps exist in the VP2 tree because mink isolates from Denmark, Sweden, and The Netherlands were not available for this region. Mink AMDV isolates from the Essex, Niagara, and Wellington regions of Ontario shared high nucleotide similarity (92–99%) with skunk AMDV isolates and, in some cases, exhibited higher identity with skunk AMDV isolates than with mink AMDV isolates from other regions in Ontario and abroad. Therefore, we suspect that skunk isolates from other regions in Ontario may form similar geographic clusters with Ontario mink AMDV isolates.

Our comparison of skunk AMDV sequences with AMDV sequences from free-ranging mink (Nituch et al. 2012) and from sequences retrieved from GenBank showed nucleotide divergence of 2–18% between skunk and mink AMDV sequences in the NS1 region, and 1–12% difference in the VP2 region. Within host

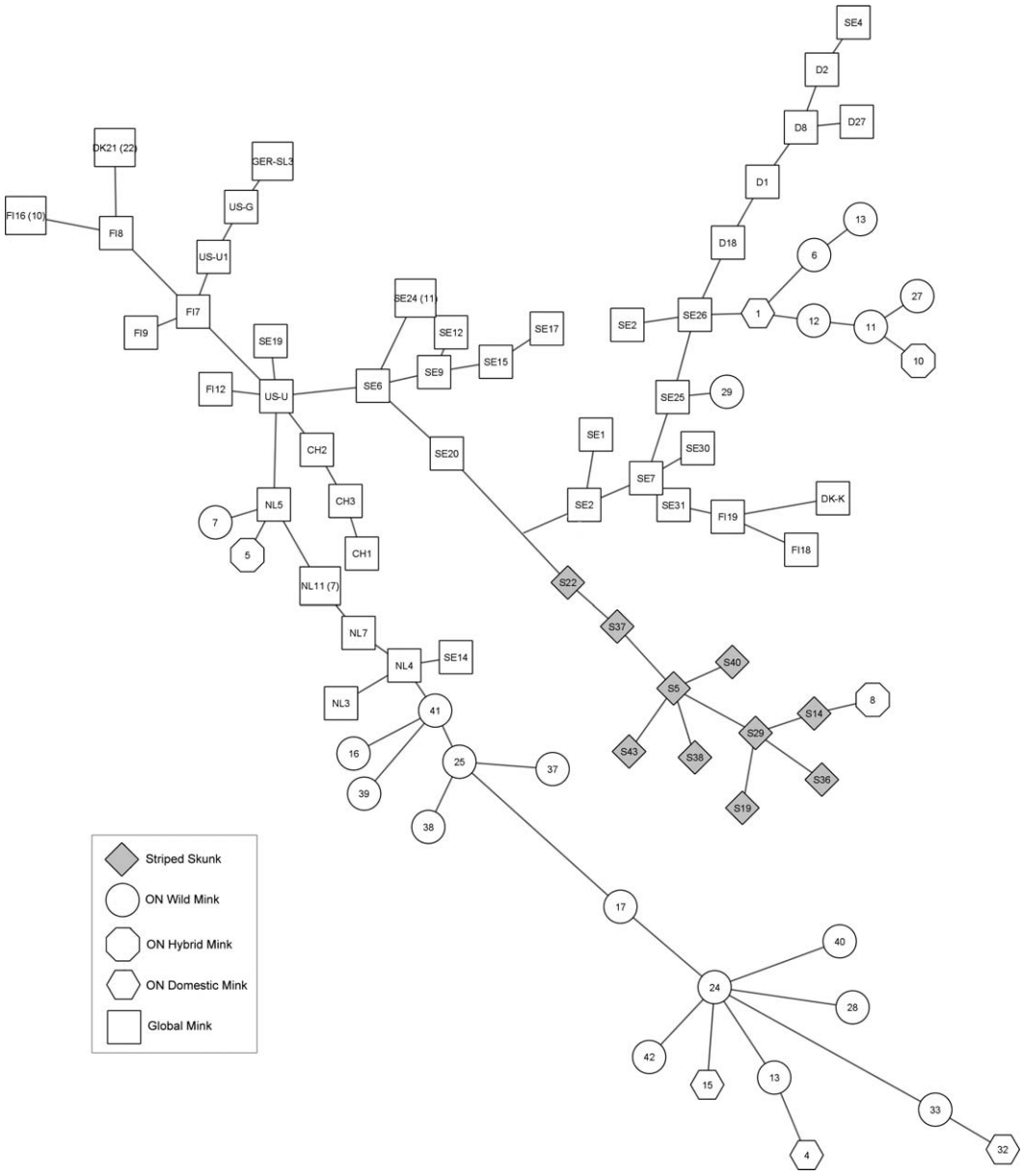

FIGURE 1. Minimum-spanning network of 135 Aleutian mink disease virus (AMDV) isolates based on alignment of the 322-nucleotide fragment of the AMDV NS1 gene. Isolates were obtained from striped skunks (*Mephitis mephitis*; nodes colored grey). Isolates with only numeric labels were sampled in free-ranging mink (*Neovison vison*) from Ontario (ON), Canada, with different genetic ancestries (domestic, wild, and domestic-wild hybrid). Skunks were also sampled in Ontario. Isolates indicates by square nodes are domestic mink sampled in different countries. The number of haplotypes per node (>1) is listed in brackets. Country codes: CH=China; DK or D=Denmark; FI=Finland; GER=Germany; IE=Ireland; NL=Netherlands; SE=Sweden; US=United States. See Table 1 and Nituch et al. (2012) for isolate descriptions.

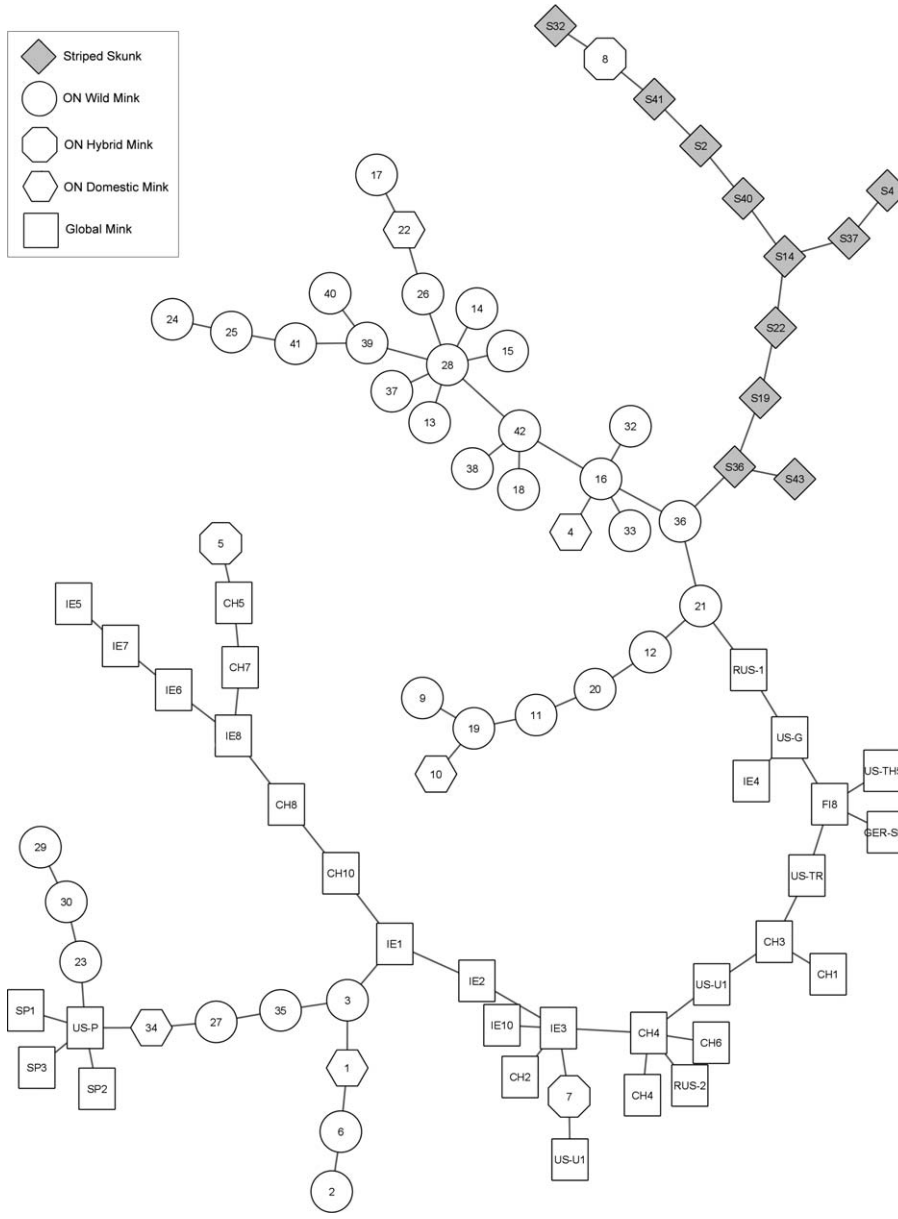

FIGURE 2. Minimum-spanning network of 88 Aleutian mink disease virus (AMDV) isolates based on alignment of the 528-nucleotide fragment of the AMDV VP2 gene. Isolates were obtained from striped skunks (*Mephitis mephitis*; nodes colored grey) and American mink (*Neovison vison*). Isolates with only numeric labels were sampled in free-ranging mink from Ontario (ON), Canada, with different genetic ancestries (domestic, wild, and domestic-wild hybrid). Skunks were also sampled in Ontario. Isolates indicated by square nodes are domestic mink sampled in different countries. Country codes: CH=China; FI=Finland; GER=Germany; IE=Ireland; RUS=Russia; SP=Spain; US=United States. See Table 1 and Nituch et al. (2012) for isolate descriptions.

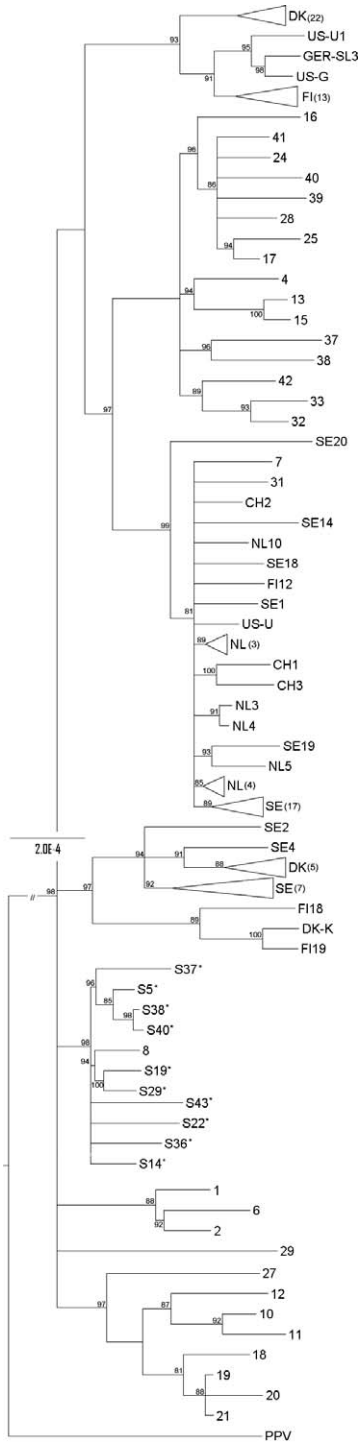

FIGURE 3. Maximum likelihood phylogeny of 135 Aleutian mink disease virus (AMDV) isolates based on alignment of the 322-nucleotide fragment of the AMDV NS1 gene. Isolates marked with S* were obtained from striped skunks (*Mephitis mephitis*) sampled in Ontario, Canada, and numeric isolates were sampled in free-ranging American mink (*Neovison vison*) with different genetic ancestries (domestic, wild, and domestic-wild hybrid), also from Ontario. Isolates with other alphanumeric labels originated from the global trade of domestic mink. The country codes are CH=China; DK=Denmark; FI=Finland; GER=Germany; NL=Netherlands; SE=Sweden; US=United States. Porcine parvovirus (PPV) was used as an outgroup. See Table 1 and Nituch et al. (2012) for isolate descriptions. For illustrative purposes, the branch leading to the outgroup is broken.

species, however, nucleotide variation appears to be lower in skunk AMDV isolates (6% variation in both regions) compared with variation in mink AMDV isolates in the NS1 (18%) and VP2 (11%) regions. This lower within-group variation in skunk AMDV isolates may reflect a more rapid and recent radiation of AMDV in skunks or may merely be a consequence of our limited geographic sampling of skunk AMDV isolates in Ontario.

Recent radiation of AMDV in skunks may also be indicated by the starlike pattern of skunk haplotypes in the VP2 network. Alternatively, the occurrence of a mink infected with a skunk haplotype of AMDV (isolate ON8) suggests skunk-to-mink transmission. Given these various results, it is necessary to characterize skunk AMDV isolates from additional regions in Ontario and North America to help elucidate the exact relationship between mink and skunk AMDVs.

Cross-species pathogen transmission requires that the natural host come into biological proximity with a second species and that the spillover host be susceptible to infection (Childs et al. 2007). Skunks are more closely related to mink than are raccoons, often occupying similar habitat as mink (Ingram and Cho 1974), and from our results, they appear highly susceptible to AMDV, making them candidates for AMDV reservoir hosts. Additionally, our study is the first, to our knowledge, to report the likely infection of a mink with a

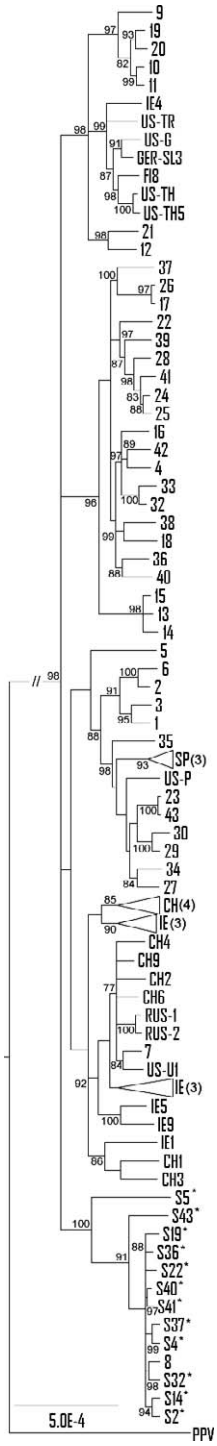

FIGURE 4. Maximum likelihood phylogeny of 88 Aleutian mink disease virus (AMDV) isolates based on alignment of the 528-nucleotide fragment of the AMDV VP2 gene. Isolates marked with S* were obtained from striped skunks (*Mephitis mephitis*) sampled in Ontario, Canada, and numeric isolates were sampled in free-ranging American mink (*Neovison vison*) with different genetic ancestries (domestic, wild, and domestic-wild hybrid), also from Ontario. Isolates with other alphanumeric labels originated from the global trade of domestic mink. The country codes are CH=China; DK=Denmark; FIN=Finland; GER=Germany; NL=Netherlands; RUS=Russia; SP=Spain; S=Sweden; US=United States. Porcine parvovirus (PPV) was used as an outgroup. See Table 1 and Nituch et al. (2012) for isolate descriptions. For illustrative purposes, the branch leading to the outgroup is broken.

skunk AMDV isolate, demonstrating the potential for cross-species transmission of AMDV from skunks to mink.

Pathogens can be more or less virulent in a secondary host than in the original host, and the emergence of a second host can cause an increase or a decrease in virulence in the first host (Woolhouse et al. 2001). Although AMDV appears to replicate and persist in skunks, and mink and skunk AMDV isolates are closely related antigenically and by DNA sequence (up to 99% similarity), it remains unclear whether AMDV pathogenicity in skunks differs markedly from the infectious process and outcome for mink, and whether skunk AMDV is virulent in mink (Oie et al. 1996; Childs et al. 2007). Two recent cases of suspected AMDV infection in pet striped skunks presented with hyperglobulinemia, anti-AMDV antibodies, and histologic changes consistent with mink AMDV (Pennick et al. 2007; Allender et al. 2008), however, supporting the hypothesis that skunks may also be negatively affected by AMDV infection.

Cross-species pathogen transmission presents several potential problems. Multihost disease models have shown that adding a second or third host species can often increase the number of infected individuals in the first host species (Craft et al. 2008). Individual wild carnivore species are unlikely to occur at high enough densities to attain population sizes

necessary to maintain most pathogens (Alexander et al. 2008). Different host species typically vary in their resistance and response to infection, have varying contact patterns based on social behavior, and have different spatial distributions across the landscape (Woolhouse et al. 2001; Dobson 2004; Craft et al. 2008). In multihost diseases, where cross-species transmission can occur, the presence of additional susceptible host populations could form a maintenance population sufficiently large and widespread to sustain persistently high infection prevalence and frequent transmission (Daszak et al. 2000; Alexander et al. 2008). For instance, spotted hyenas (*Crocuta crocuta*) and jackals (*Canis adustu, Canis aureus, Canis mesomelas*) have been implicated in the transmission of canine distemper virus to lions (*Panthera leo*), as the two species are more abundant than lions and commonly come into contact with lions at kill sites (Campbell and Borner 1986; Cleaveland et al. 2008).

Cross-species infection can also introduce pathogens into a host species from which it has been eliminated. Consequently, high AMDV antibody prevalence and viral replication in skunks, as well as anecdotal reports of skunks on mink farms (Oie et al. 1996), suggest that frequent reemergence of AMDV on some sanitized mink farms may be, at least in part, the result of AMDV transmission by skunks to the mink on farms. Because there is currently no treatment or vaccine for AMD, it is critical to reduce contact between susceptible hosts and reservoirs to limit further pathogen transmission. Mink farms appear to be reservoirs of AMDV infection for wild mink (Nituch et al. 2011, 2012); therefore, we recommend limiting the potential for skunks both to transmit and to acquire AMDV on mink farms through increased biosecurity measures, such as adequate fencing surrounding mink farms, to prevent access by skunks and other wildlife. Limiting access to mink farms by wildlife that serve as AMDV reservoirs could also improve the success of AMD control programs by preventing reinfection of "AMDV-clean" farms by AMDV-infected wildlife.

The estimation of pathogen exposure is a first step toward evaluation of the risk of spillover. Our results suggest that AMDV infection is prevalent in skunks; therefore, skunks may be acting as alternative hosts and reservoirs of the AMDV to wild mink through cross-species spillover. Our molecular analysis revealed that skunk AMDV isolates are closely related to mink AMDV isolates, and the presence of a skunk AMDV isolate in an escaped domestic mink suggests that skunks are able to transmit the AMDV to mink. Although skunk AMDV isolates are highly similar to mink AMDV isolates, a small number of nucleotide changes can sometimes greatly alter a parvovirus' biologic characteristics (Parrish 1999). Many issues need to be resolved about the specific details of this host-pathogen system, such as whether skunk AMDV isolates are pathogenic in skunks and mink and whether skunks are susceptible to mink AMDV isolates. Furthermore, Lawson et al. (1989) reported a suspected case of vaccine-induced rabies in an AMDV-infected skunk. We recommend that further AMD research in skunks is warranted to determine whether skunks infected with AMDV suffer the same immune complex disease as in AMDV-infected mink and whether this results in increased susceptibility to other infections.

Although most emerging diseases of domestic animals and wildlife infect multiple hosts, the epidemiology of multihost pathogens and cross-species pathogen transmission are poorly understood (Haydon et al. 2002). Identifying and recognizing the role of alternative host and reservoir species, such the newly discovered potential role of skunks in AMDV transmission, is essential to ensure the correct host is targeted with appropriate and effective disease control measures both in the wild and on farms.

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LITERATURE CITED

Alexander KA, McNutt JW, Briggs MB, Standers PE, Funston P, Hemson G, Keet D, van Vuuren M. 2008. Multi-host pathogens and carnivore management in southern Africa. *Comp Immunol Microbiol Infect Dis* 33:249–265.

Allender MC, Schumacher J, Thomas K, McCain S, Ramsay E, James E, Wise A, Maes R, Reel D. 2008. Aleutian mink parvovirus-like disease in a captive striped skunk (*Mephitis mephitis*). *J Am Vet Med Assoc* 232:742–746.

Begon M, Bowers RG, Kadianakis N, Hodgkinson DE. 1992. Disease and community structure: The importance of host self-regulation in a host-pathogen model. *Am Nat* 139:1131–1150.

Bloom ME, Kanno H, Mori S. 1994. Aleutian mink disease: Puzzles and paradigms. *Infect Agents Dis* 3:279–301.

Britton AP, Sojonky KR, Scouras AP, Bidulka JJ. 2010. Pandemic (H1N1) 2009 in skunks, Canada. *Emerg Infect Dis* 16:1043–1045.

Campbell KLI, Borner M. 1986. Census of predators on the Serengeti plains May 1986. *Serengeti Ecological Monitoring Programme. Report 62*. Arusha, Tanzania, 6 pp, http://www.catsg.org/cheetah/05_library/5_3_publications/C/Campbell_&_Borner_1986_Census_of_predators_on_the_Serengeti_plains.pdf. Accessed December 2014.

Childs JE, Richt JA, Mackenzie JS. 2007. Conceptualizing and partitioning the emergence process of zoonotic viruses from wildlife to humans. *Curr Top Microbiol Immunol* 315:1–31.

Cho HJ, Greenfield J. 1978. Eradication of Aleutian disease of mink by eliminating positive counter-immunoelectrophoresis test reactors. *J Clin Microbiol* 7:18–22.

Cleaveland S, Hess GR, Dobson AP, Laurenson MK, McCallum HI, Roberts MG, Woodroffe R. 2002. The role of pathogens in biological conservation. In: *The ecology of wildlife diseases*, Hudson PJ, Rizzoli A, Grenfell BT, Heesterbeek H, Dobson AP, editors. Oxford University Press, New York, New York, pp. 139–150.

Cleaveland S, Laurenson MK, Taylor LH. 2001. Diseases of humans and their domestic mammals: Pathogen characteristics, host range and the risk of emergence. *Philos Trans R Soc Lond B Biol Sci* 356:991–999.

Cleaveland S, Packer C, Hampson K, Kaare M, Kock R, Craft M, Lembo T, Mlengeya T, Dobson A. 2008. The multiple roles of infectious diseases in the Serengeti Ecosystem. In: *Serengeti III: Human impacts on ecosystem dynamics*, Sinclair ARE, Packer C, Mduma SAR, Fryxell JM, editors. University of Chicago Press, Chicago, Illinois, pp. 209–239.

Clifford DL, Mazet JAK, Dubovi EJ, Garcelon DK, Coonan TJ, Conrad PA, Munson L. 2006. Pathogen exposure in endangered island fox (*Urocyon littoralis*) populations: Implications for conservation management. *Biol Conserv* 131:230–243.

Craft ME, Hawthorne PL, Packer C, Dobson AP. 2008. Dynamics of a multihost pathogen in a carnivore community. *J Anim Ecol* 77:1257–1264.

Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging infectious diseases of wildlife—Threats to biodiversity and human health. *Science* 287:443–449.

Dobson A. 2004. Population dynamics of pathogens with multiple host species. *Am Nat* 164 (Suppl): S64–S78.

Dobson A, Foufopoulos J. 2001. Emerging infectious pathogens of wildlife. *Philos Trans R Soc Lond B Biol Sci* 356:1001–1012.

Farid AH. 2013. Aleutian mink disease virus in furbearing mammals in Nova Scotia, Canada. *Acta Vet Scand* 55:10.

Fournier-Chambrillon C, Aasted B, Perrot A, Pontier D, Sauvage F, Artois M, Cassiede J-M, Chauby X, Dal Molin A, Simon C, et al. 2004. Antibodies to Aleutian mink disease parvovirus in free-ranging European mink (*Mustela lutreola*) and other small carnivores from southwestern France. *J Wildl Dis* 40:394–402.

Funk SM, Fiorello CV, Cleaveland S, Gompper ME. 2001. The role of disease in carnivore ecology and conservation. In: *Carnivore conservation*, Gittleman JL, Funk SM, Macdonald DW, Wayne RK, editors. Cambridge University Press, Cambridge, UK, pp. 442–446.

Hall AJ, Jepson PD, Gooman SJ, Harkonen T. 2006. Phocine distemper virus in the North and European Seas—Data and models, nature and nurture. *Biol Cons* 131:211–220.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98.

Haydon DT, Cleaveland S, Taylor L, Laurenson MK. 2002. Identifying reservoirs of infection: A conceptual and practical challenge. *Emerg Infect Dis* 8:1468–1473.

Hunter B. 2008. Mink health and disease: Challenges and accomplishments, a Canadian perspective. *Scientifur* 32:200–204.

Ingram DG, Cho HJ. 1974. Aleutian disease in mink. *J Rheumatol* 1:74–92.

Kenyon AJ, Kenyon BJ, Hahn EC. 1978. Protides of the Mustelidae: Immunoresponse of mustelids to Aleutian mink disease virus. *Am J Vet Res* 39:1011–1015.

Lawson KF, Hertler R, Charlton KM, Campbell JB, Rhodes AJ. 1989. Safety and immunogenicity of ERA® strain of rabies virus propagated in a BHK-21 cell line. *Can J Vet Res* 53:438–444.

Lopes CT, Franz M, Kazi F, Donaldson SL, Morris Q, Bader GD. 2010. Cytoscape Web: An interactive web-based network browser. *Bioinformatics* 26:2347–2348.

Macdonald DW, Riordan P, Mathews F. 2006. Biological hurdles to the control of TB in cattle: A test of two hypotheses to explain the failure of control. *Biol Conserv* 131:268–286.

Mañas S, Ceaña JC, Ruiz-Olmo J, Palazón S, Domingo M, Wolfinbarger JB, Bloom ME. 2001. Aleutian mink disease parvovirus in wild riparian carnivores in Spain. *J Wildl Dis* 37:138–144.

McDonald RA, Larivière S. 2001. Diseases and pathogens of *Mustela* spp., with special reference to the biological control of introduced stoat *Mustela erminea* populations in New Zealand. *J Roy Soc New Zeal* 31:721–744.

National Center for Biotechnology Information (NCBI). 2014. GenBank, http://www.ncbi.nlm.nih.gov/genbank/. Accessed December 2014.

Nituch LA, Bowman J, Beauclerc KB, Schulte-Hostedde AI. 2011. Mink farms predict Aleutian disease exposure in wild American mink. *PLoS One* 6:e21693.

Nituch LA, Bowman J, Wilson P, Schulte-Hostedde AI. 2012. Molecular epidemiology of Aleutian mink disease virus in free-ranging domestic, hybrid, and wild mink. *Evol Apps* 5:330–340.

Oie KL, Durrant G, Wolfinbarger JB. 1996. The relationship between capsid protein (VP2) sequence and pathogenicity of Aleutian mink disease parvovirus (ADV): A possible role for raccoons in the transmission of ADV infections. *J Virol* 70:852–861.

Olofsson A, Mittelholzer C, Berndtsson LT, Lind L, Mejerland T, Beldk S. 1999. Unusual, high genetic diversity of Aleutian mink disease virus. *J Clin Microbiol* 37:4145–4149.

Parrish CR. 1999. Host range relationships and the evolution of canine parvovirus. *Vet Microbiol* 69:29–40.

Pennick KE, Latimer KS, Brown CA, Hayes JR, Sarver CF. 2007. Aleutian disease in two domestic striped skunks (*Mephitis mephitis*). *Vet Pathol* 44:687–690.

R Development Core Team. 2014. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org/. Accessed December 2014.

Rambaut A. 2007. *FigTree*, tree.bio.ed.ac.uk/software/figtree. Accessed December 2014.

Slatkin M, Hudson RR. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics* 129:555–562.

Statistics Canada. 2006. *2006 census of agriculture. Farm data and farm operator data*. Catalogue 95-629-XWE, http://www.statcan.gc.ca/pub/95-629-x/95-629-x2007000-eng.htm. Accessed December 2014.

Steinel A, Munson L, Van vuuren M, Truyen U. 2000. Genetic characterization of feline parvovirus sequences from various carnivores. *J Gen Virol* 81:345–350.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599.

Wells GAH, Keymer IF, Barnett KC. 1989. Suspected Aleutian disease in a wild otter (*Lutra lutra*). *Vet Rec* 125:232–235.

Woolhouse MEJ, Taylor LH, Haydon DT. 2001. Population biology of multihost pathogens. *Science* 292:1109–1111.

Yamaguchi N, Macdonald DW. 2001. Detection of Aleutian disease antibodies in feral American mink in southern England. *Vet Rec* 149:485–488.

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