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Short communication

## Canine distemper of vaccine origin in European mink, *Mustela lutreola*—a case report

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### Abstract

Cases of canine distemper (CD) related to vaccination of exotic carnivores extend over three decades and have been described in at least nine different species. Our report describes a case of acute CD in a European mink, *Mustela lutreola*, vaccinated with live attenuated CD vaccine licensed for use in fur-farmed mink. The male mink died of an acute grey matter disease with an unusually long incubation period. A female vaccinated at the same time showed no obvious signs of illness. The diagnosis was confirmed by reverse transcriptase–polymerase chain reaction (RT–PCR) and by subsequent sequencing of the PCR products. The sequenced products of the virus isolated from the mink and of the vaccine batch showed 100% identity. This is the first report in which molecular methods were used to confirm that the disease was caused by the vaccine strain. Based on our findings, it is clearly evident that current CD vaccines cannot be safely used in exotic species.

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### 1. Introduction

Members of the *Canidae* and *Mustelidae* are susceptible to canine distemper virus (CDV) which belongs to the *Morbillivirus* genus within the *Paramyxovirus* family. The viral genome consists of a negative-sense single-stranded unsegmented RNA 15–16 kbp in

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size. Canine distemper (CD) is controlled by vaccination in domestic dogs and fur-farmed mink, *Mustela vison*, but vaccination of exotic animals has often proved to be complicated since few vaccines are licensed for them (Montali et al., 1994). The following is a report of vaccine-induced CD in a European mink, *Mustela lutreola*, at the Helsinki Zoo in 1998.

Two 4-month-old European minks, non related male and female, were imported to the Helsinki Zoo. The male came from the Tallin Zoo in Estonia and the female was on breeding loan from a Polish Zoo. The animals were quarantined 4 weeks and no signs or evidence of infectious diseases were found. The nutritional state of the animals was also normal. Before arrival they had not been vaccinated against CD. In Helsinki, 10 weeks after arrival, both minks were vaccinated against CD with a modified live-virus (MLV) vaccine (avian origin), Distemink. Thirty days postvaccination, the male showed clinical signs while the female remained healthy. The male was ill with purulent oculonasal discharge, dermatitis and otitis externa, but remained alert. During the following day, the clinical status worsened, the animal developed respiratory distress, neurological signs and died. The mink was submitted to the National Veterinary and Food Research Institute in Helsinki for necropsy.

## 2. Materials and methods

The tissue samples obtained at necropsy were fixed in 10% buffered formalin (pH 7.4), processed routinely, cut into 4- $\mu$ m sections and stained with haematoxylin and eosin. The formalin-fixed and paraffin-embedded tissue sections of nictitating membrane, haired skin, lung, urinary bladder and brain were immunostained with anti-CDV-nucleoprotein (NP) monoclonal antibody (mAb) (VMRD Inc.) using the EnVision<sup>TM</sup> system (Dako) and diaminobenzidine for visualization.

For the indirect immunofluorescence assay (IFA) for CDV, epithelial cells were scraped from unfixed tracheal and urinary bladder samples, air-dried on slides and fixed in acetone. A mixture of anti-NP-mAb clones 4.100 and 3851 (kindly supplied by Dr. Claes Örvell) was used as a primary antibody and the rabbit anti-mouse IgG fraction conjugated with fluorescein isothiocyanate (Dako) as a secondary antibody.

A 10% organ suspension was prepared from lung, trachea and urinary bladder for reverse transcriptase–polymerase chain reaction (RT–PCR). Total RNA was extracted from the suspension and also from the vaccine batch (Distemink, United Vaccines. Lot. 30729) used for immunization of the European mink. A volume of 50 pmol of each primer, published by Barret et al., 1993, and the GeneAmp RNA PCR kit were used for RT–PCR amplification of phosphoprotein (P) gene fragments of 429 base pairs (bp). Amplification consisted of 35 cycles of denaturation at 94 °C for 45 s, annealing at 52 °C for 1 min and extension at 72 °C for 1 min. PCR products were purified with MicroSpin S-400HR columns (Amersham Pharmacia Biotech, USA) and sequenced in both directions with primers used in the PCR. The automated sequencing of amplicons was done at the DNA Synthesis and Sequencing Laboratory, Institute of Biotechnology, University of Helsinki.

The nucleotide alignment of the partial P-gene sequences (388 bp, PCR primer sequences are excluded) was created with the PILEUP program and the nucleotide identities calculated with the BESTFIT program from the Genetics Computer Group



software (GCG; Devereux et al., 1984). Phylogenetic tree was created using the Clustal method with the MegAlign/DNASTAR program. Some of the sequence data used here were extracted from the GenBank: three strains (u53713, u53714, u53715) isolated in Serengeti National Park from *Panthera leo*, *Otocyon megalotis* and *Canis familiaris*, respectively; wild-type CDV strain A75-17 (af164967); three strains originating from Japan (ab028914, ab028915 and ab028916); the Onderstepoort vaccine strain (af305419). Two German strains, one isolated from a dog and the other from a ferret, were reported by Harder et al. (1996).

### 3. Results

The animal was dehydrated but otherwise in good bodily condition. There was dry mucopurulent exudate around the nostrils and eyes and multifocal brownish crusts with numerous adult *Demodex sp.* mites and larvae in the scrapings. The lungs were oedematous with multifocal dark red mottling.

In histological examination, specific changes were found in the trachea, urinary bladder, conjunctiva, lung, brain and haired skin. Nonsuppurative inflammation with hydropic degeneration of the epithelial cells was present in the conjunctiva, trachea, and urinary bladder. Marked multifocal bronchointerstitial pneumonia was present in the lung with numerous syncytial cells. Numerous intraepithelial eosinophilic intracytoplasmic inclusion bodies were found in all these tissues. Nonsuppurative encephalitis with intraneuronal eosinophilic inclusion bodies, neuronal degeneration and multifocal gliosis was present in the brainstem, most prominent caudally.

There were widespread lesions in the haired skin. In the epidermis extending to the infundibula, the keratinocytes were multifocally necrotic, either singly or in clusters, with exocytosis of neutrophilic leukocytes and numerous eosinophilic intracytoplasmic inclusion bodies. The epidermis was moderately hyperplastic with marked parakeratotic and orthokeratotic hyperkeratosis extending also to the infundibula. In some sections, there

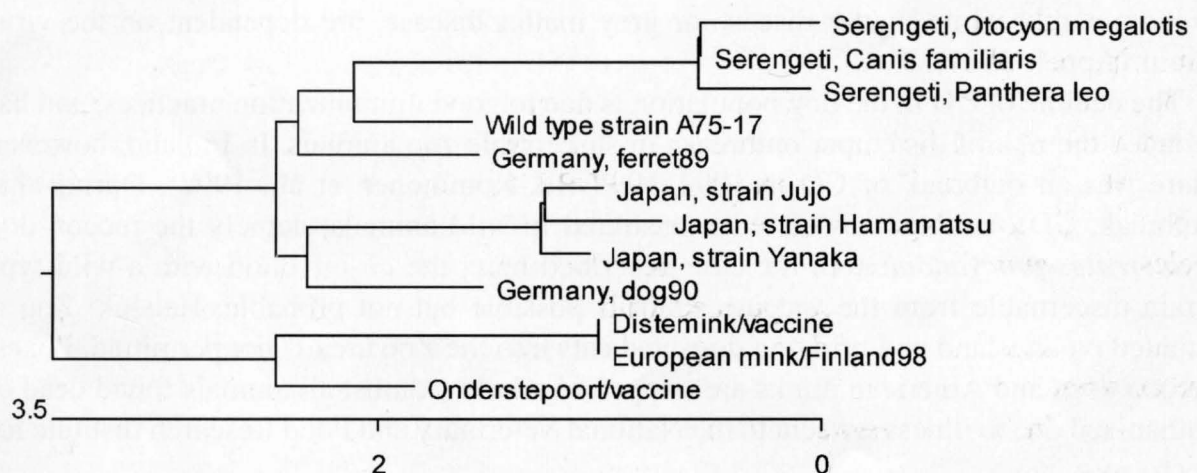


Fig. 1. Phylogenetic tree showing the genetic relationships of the European mink/Distemink CDV strain to the other CDV strains.

were adult *Demodex* sp. mites and larvae in the hair follicles without significant inflammatory changes.

The epithelial cells from trachea and urinary bladder were positive for CDV in IFA. Formalin-fixed and paraffin-embedded tissue sections of nictitating membrane, haired skin, lung, urinary bladder and brain also stained positively with anti-CDV-NP antibody.

The RT-PCR resulted in products of expected size (429 bp) for both the clinical sample and the vaccine. Furthermore, sequence analysis showed 100% nucleotide identity between the strains amplified from the Distemink vaccine and from the European mink. These two identical strains differed by 2.1–5.7% from the other corresponding sequences used in analysis (Fig. 1).

The sequences determined in this paper have been deposited in the GenBank with accession numbers AY130856 and AY130857.

#### 4. Discussion

In this case, the clinical signs, necropsy and histological findings were typical for CD with acute grey matter disease in the brain (Summers et al., 1984). The diagnosis was confirmed by immunohistochemical staining for CDV NP, indirect IFA and RT-PCR. The lesions were typical for an acute distemper infection; however, the presumed incubation period following vaccination was fairly long. The incubation period of acute CD is usually about 1 week. Postvaccination disease in dogs due to the use of inadequately attenuated virus is characterized by the presence of acute grey matter disease, and the lesions are usually restricted to the pontine area of the brainstem (Hartley, 1974). Postvaccination disease usually develops in 7–10 days after vaccination and results in death in a few days.

Sutherland-Smith et al. (1995) reported vaccine-induced distemper in European mink vaccinated with a multivalent MLV against CDV (avian origin), canine parvovirus, canine adenovirus and canine parainfluenza. The first clinical signs were noted 16 days post-vaccination, and 10 days later all four animals succumbed. In our case, the long incubation period may simply be explained by the fact that the first clinical signs may not have been observed. The incubation period, clinical signs and central nervous system lesions, either predominantly white matter disease or grey matter disease, are dependent on the virus strain (Appel, 1987).

The decline of CD in the dog population is due to good immunization practices, and has reduced the risk of distemper outbreaks in susceptible zoo animals. In Finland, however, there was an outbreak of CD in 1994–1995 (Ek-Kommonen et al., 1997). During that outbreak, CDV antigen was also demonstrated in wild animals, namely the racoon dog *Nyctereutes procyonoides*. In the case described here, the co-infection with a wild-type strain discernable from the vaccine strain is possible but not probable. Helsinki Zoo is situated on an island and bringing dogs and cats into the Zoo area is not permitted. Foxes, racoon dogs and American minks are trapped regularly. Almost all animals found dead or euthanized due to illness are sent to the National Veterinary and Food Research Institute for necropsy.

The major cause of distemper in exotic carnivores has been the use of MLV vaccines licensed for other species (Montali et al., 1994). Thus the majority of carnivores at the

Helsinki Zoo, including skunks *Mephitis mephitis*, wolverines *Gulo gulo*, weasels *Mustela nivalis* and ermines *Mustela erminea* are not routinely vaccinated against CD. In this case, the zoo wanted to ensure that the imported European mink, which is an endangered species, would be protected from CD and decided to vaccinate them with a vaccine licensed for farmed mink.

Sequence analysis based on partial P-gene sequences showed 100% nucleotide identity between the European mink strain and the Distemink vaccine strain indicating the presence of vaccine-induced infection. Our studies, of course, do not exclude a spontaneous mutation elsewhere in the viral genome increasing the virulence of the vaccine strain. The RT-PCR based sequencing of the entire genome, however, does not necessarily find that kind of mutation, because RNA virus populations consist of complex distributions of mutant genomes (quasispecies) and the virulent strain may form the extreme minority of them.

This report is the first confirming a vaccine-induced CD by using molecular biological methods. In addition to the improved diagnostic methods, the sequence data analysis forms the basis for understanding the molecular epidemiology of CDV and also indirectly benefits the development of CD vaccines. Our report clearly shows the risk involved in vaccinating exotic species even with vaccines designed for closely related species.

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