



Short communication

Newcastle disease virus from domestic mink, China, 2014



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ABSTRACT

Newcastle disease virus (NDV) is a pathogen that most often infects poultry species. In investigating a 2014 outbreak of encephalitis and death among farmed mink (*Mustela vison*), we found pathological and later experimental evidence that NDV can infect and cause severe encephalitic and pneumonic disease in these animals. Our findings confirm the host range of NDV.

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1. Introduction

Newcastle disease virus (NDV) or avian paramyxovirus type 1 virus (APMV-1) belongs to the family *Paramyxoviridae* and subfamily *Paramyxovirinae*. An enveloped, negative-sense single-stranded RNA virus, it possesses approximately 15,000 nucleotides and causes explosive outbreaks of severe diseases among poultry species (Bogoyavlenskiy et al., 2005). According to the pathological variation seen in chickens, NDV strains have been divided into 3 pathotypes: velogenic, mesogenic, and lentogenic (Alexander and Senne, 2008). Velogenic pathotypes are further subdivided into viscerotropic and neurotropic velogenic. Viscerotrophic velogenic pathotypes are highly pathogenic and may cause hemorrhagic intestinal lesions. Neurotropic velogenic pathotypes are highly virulent and often cause respiratory distress and neurologic signs. Occasionally, sudden death occurs with few or no symptoms in velogenic pathotypes. Mesogenic pathotypes are moderately virulent, often have a lower mortality rate, and may cause respiratory distress and occasionally neurologic signs. Lentogenic or respiratory pathotypes are the least virulent pathotype and often cause subclinical infection or mild respiratory disease.

NDV was first recognized in 1926 and is now thought to be endemic in multiple countries. NDV has been isolated from naturally infected mink populations and experimental inoculation

of mink with NDV showed a low pathogenicity (Haagsma et al., 1975). Here we report our investigation into an outbreak of severe illness among domestic mink (*Mustela vison*) with hemorrhagic-encephalitis and pneumonia in Heilongjiang Province, China. Morphological and virological findings of a NDV strain in mink from China are described, and artificial infection of mink with the virus resulted in the same lesions as were observed in naturally infected ones. These findings are of great interest to the study of the evolution of NDV in China and confirm the host range of NDV.

2. The study

In October 2014, a severe outbreak was reported in a population of three-month-old mink on a small breeding mink farm in Heilongjiang Province, China. Four hundred fifty-six young mink became ill with shaking mink syndrome, which is an encephalitic disease of unknown etiology in young mink. The illness rate was 9.12% and among the ill the case fatality rate was greater than 95%. Clinical signs included unkempt fur, loss of appetite, emaciation and significant head muscle tremors. Upon necropsy gross lesions were observed on hemorrhagic brain and lung tissues. PCR excluded all classical endemic viruses (Appendix A), aleutian mink disease virus, orthoreovirus, hepatitis E, and influenza virus (Jepsen et al., 2009; Krog et al., 2013; Lian et al., 2013). To identify the cause of disease, 456 brain and lung tissues were collected from deceased mink and were homogenized in sterile phosphate-buffered saline. NDV was identified by RT-PCR in brain and lung tissue samples using pan-Paramyxovirinae primers (Tong et al., 2008) (Fig. 1). Moreover, the homogenate was also inoculated into

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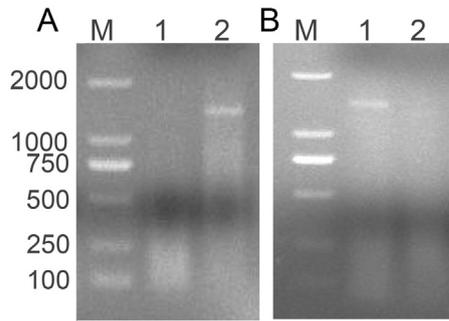


Fig. 1. PCR identification of mNDV F gene from infected mink in A) brain tissue, B) lung tissue. A1) negative control, A2) brain tissue; B1) lung tissue, B2) negative control.

10-day-old specific pathogen free (SPF) embryonated chicken eggs. Transmission electron microscopy of purified allantoic fluid from brain tissues inoculated showed an enveloped oval paramyxovirus-like particle. The diameter of the nucleocapsid was between 90 and 210 nm (Fig. 2A). Cytopathic effects observed in CEF included cell shrinking and cytoplasmic stranding (Supplementary material). Histologic findings demonstrated diffuse glial cells infiltrated in the cerebrum and cerebellum, meningeal hemorrhage, and hemorrhagic pneumonia (Fig. 2B–D). NDV was the only pathogen identified in the infected SPF chicken allantoic fluid using RT-PCR. After direct sequencing of the purified PCR products, a BLAST search (www.ncbi.nlm.nih.gov/blast/Blast.cgi) showed the sequences to be identical to NDV sequences, with the closest similarity to NDV strain mallard/China/HLJ-78-06. A hemagglutination inhibition test was carried out using allantoic fluid from the injected eggs to agglutinate 0.5% (vol/vol) chicken erythrocytes (1:2⁸). The allantoic fluid was then mixed with specific NDV-antiserum (Harbin Veterinary Research Institute, China) and no longer agglutinated erythrocytes suggesting that NDV alone was replicating in the allantoic fluid. These initial findings provide the molecular evidence that a mink NDV (mNDV) existed in the brain tissues, confirming a previous report suggesting NDV as an agent of meningo-encephalitis in mink (Haagsma et al., 1975).

The intracerebral pathogenicity index (ICPI), intravenous pathogenicity index (IVPI) and chicken embryo mean death time (MDT) were carried out and calculated according to OIE recommendations and the results were 1.45, 1.93 and 63.6 h, respectively (Alexander, 1998, 2004). These results further demonstrated that our mNDV isolate was of the velogenic pathotype. The pathogenicity of mNDV was tested by intramuscularly inoculating four 3-month-old mink at a dose of $2.0 \times 10^{2.0}$ EID₅₀. Two mink also served as negative controls. The clinical signs and gross lesions found upon necropsy were similar to those of

naturally infected mink. Inoculated mink experienced head muscle tremors after two days post inoculation (dpi) and all four mink died of brain hemorrhage three to six dpi. No clinical symptoms or gross lesions were observed in controls.

To further identify the virus and its phylogeny, we amplified and sequenced the entire genome of the mNDV (accession no. KT889365). Ten pairs of primers were used to clone all six genes of mNDV in the order of 3'-NP-P-M-F-HN-L-5'. The nucleotide sequences obtained from the entire genome, the F gene and HN gene were aligned with sequences of NDV from various species in GenBank using DNASTAR5.0 software. Two multi-species phylogenetic trees based on the nucleotide sequences of various NDV's were constructed with MEGA4.0 software by using a maximum likelihood tree method (Fig. 3). The F gene showed 100% identity with a Heilongjiang isolate from a mallard (accession no. EF592508). The HN gene of mNDV shared 99% identity with an isolate from wild birds in Heilongjiang (accession no. GU573794). The full genome of mNDV shared the highest homology (99%) with a chicken isolate (accession no. KC542902) from Beijing, China (Fig. 2, panel A). For genotyping, the phylogenetic analysis of the F gene compared with NDV strains from Genbank showed that the isolated mink-HLJ-01-2015 strain belonged to genetic group VII (Fig. 2, panel B). Its amino acid sequence analysis showed that the virulent fusion cleavage site (¹¹²RRQKR/^{F117}) belonged to the velogenic pathotype. It had 13 Cys amino acid residues at positions 25, 27, 76, 199, 338, 347, 362, 370, 394, 399, 401, 424, and 523 including 8 relatively conserved sites at positions 76, 338, 362, 370, 394, 399, 424, and 523, which play an important role in the structural framework of fusion proteins. It also had 5 relatively conserved glycosylation sites at positions 85–87, 191–193, 366–368, 447–449, 471–473 and an additional glycosylation site at position 541–543. We speculate that the function of the fusion protein may have changed to easily infect mink and other mammals.

3. Conclusions

Newcastle disease virus infection has been found in both domestic and wild bird populations. The severity of the infection varies across different species. Virulent strains cause either acute diarrhea or dyspnea and up to 90% of the birds die with hemorrhagic enteritis, tracheitis, and neurological signs (Perozo et al., 2008). However, there have been a total of three reports of the isolation of NDV from naturally infected non-avian and non-human hosts, including calves, mink, and sheep (Yates et al., 1952; Haagsma et al., 1975; Sharma et al., 2012). We documented infection with NDV in farmed mink in China during 2014, which showed similar gross changes to those mink who died of NDV-induced meningo-encephalitis in the Netherlands in 1972. NDV

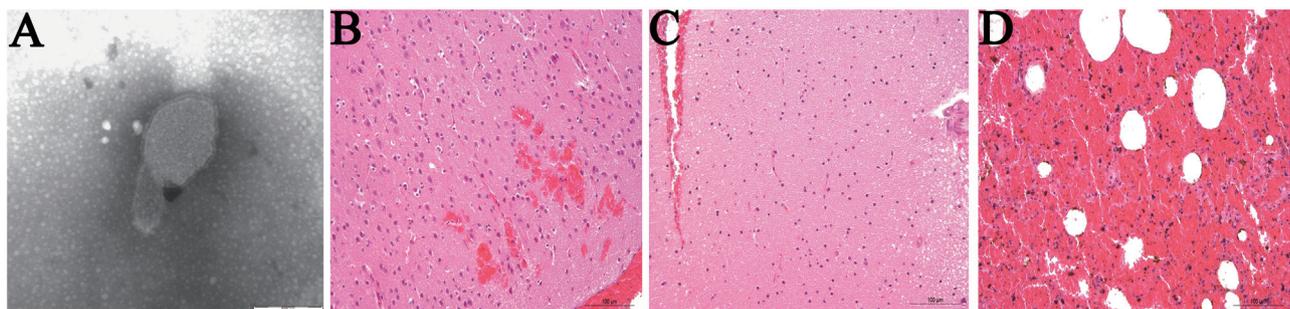


Fig. 2. Transmission electron microscopy of mNDV and histological changes of brain tissues from mink infected with mNDV. A) Mature enveloped oval mNDV particles with a diameter of approximately between 90 and 210 nm in purified allantoic fluid supernatant collected from specifically pathogen free (SPF) eggs after injection with homogenised brain and lung tissues from ill mink in China. Bar = 200 nm; B) Cerebrum with under-meningeal hemorrhage, macular bleeding in the cortex, and a great number of glial cell infiltrations; C) cerebellum with a great number of glial cell infiltrations in the cortex; D) lung with diffused and severe hemorrhagic pneumonia.

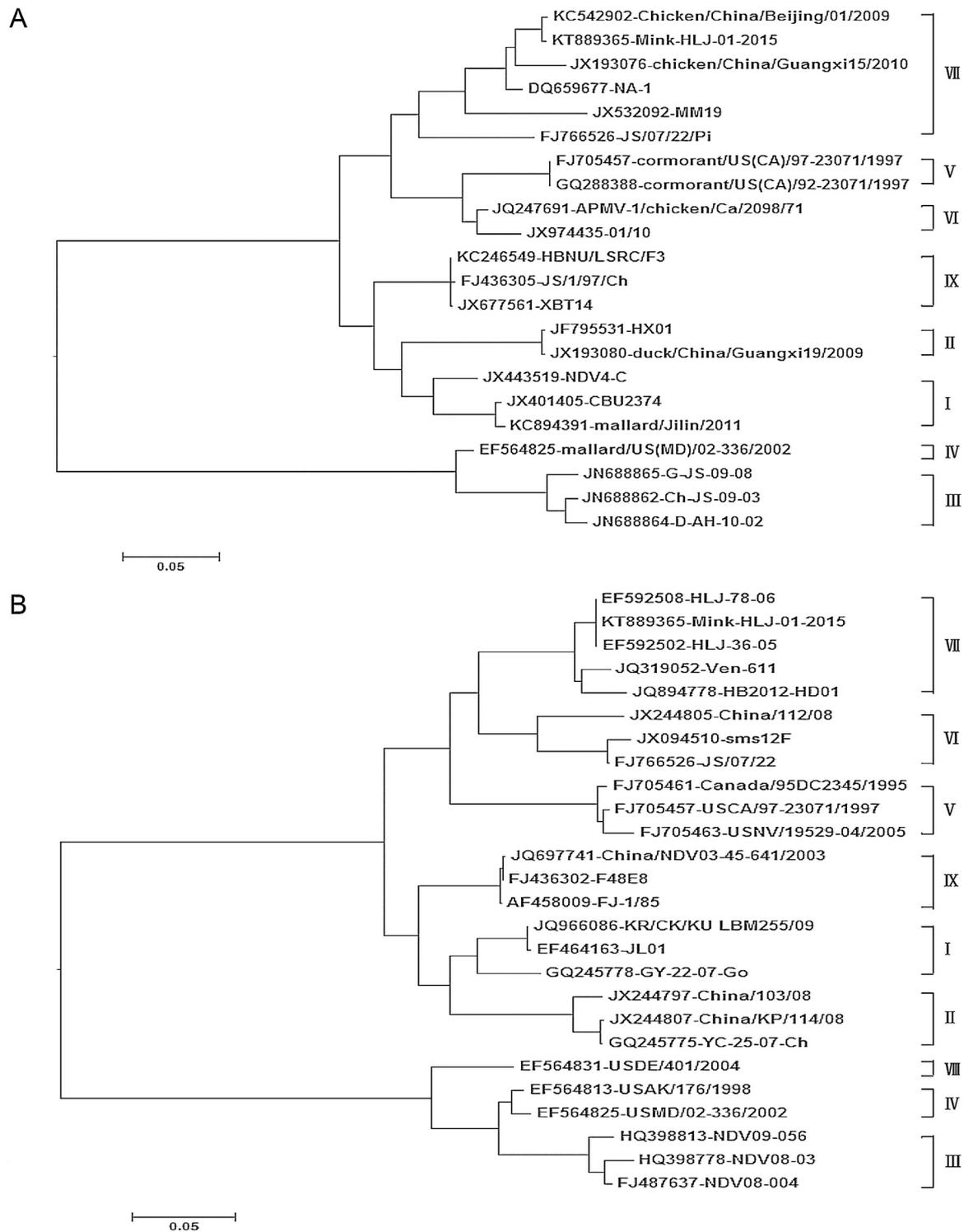


Fig. 3. Phylogenetic tree of mNDV isolated from domestic mink in China compared with NDV from other birds. A) nucleotide sequences of the entire genome; B) nucleotide sequences of F gene. The tree was constructed in the MEGA5 program using the maximum likelihood method. For each strain, the GenBank accession number is indicated.

infection was further confirmed after testing the pathogenicity of mNDV in mink. Our findings are of great concern to mink farmers. Our data suggest that NDV can cross species barriers and having adapted to new species, NDV may cause epizootics in the new species. These observations in mink, are concerning in that previous low dose NDV inoculations in pigs and sheep have similarly resulted in death suggesting other species have potential to experience NDV epizootics (Hofstad, 1950).

Concerning the possible mechanism of NDV transmission to mink, we postulate that this transmission could have resulted from feeding mink untreated chicken and duck meat. In Northeast China, untreated chicken and duck meat, including heads and internal organs, have been widely used to feed mink in many mink farms. We conclude that the source of the virus for the mink was the same as was concluded by Haagsma et al. in the Netherlands (Haagsma et al., 1975). Sequence analysis of mNDV isolated from a

hemorrhagic brain tissue sample revealed that it was nearly identical to the NDV circulating in chickens and ducks, which supports this hypothesis.

Mink farms are a major economic source in Northeast China. Our findings highlight the risk posed by NDV to mink and confirm the known host range of NDV. This finding has implications for NDV epidemiology in mink populations and economic loss in the farmed mink industry. To be prepared for the potential emergence of more virulent variants, we should carefully monitor NDV virus evolution in mammals.

Author bio

Dr. Xing is the Associate Professor in the Northeast Forestry University and doctor of Wildlife Conservation. Dr. Chai is the Associate Professor in the Northeast Forestry University and doctor of Wildlife Conservation.

Their research interests include animal toxicology and diseases of poultry.

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Appendix A.

See Table A1.

Table A1

Primers used for ruling out other possible mink pathogens.

Primers ^a	5'–3' sequence ^b
F: AMDV-s ^c	CTTGTCACGCTACTAGAAATGGT
R: AMDV-as ^c	AGCTTAAGGTTAGTTTACATGGTTTAC
R: MRV-s ^d	GCATCCATTGTAAATGACGAGTCTG
F: MRV-as ^d	CTTGAGATTAGCTCTAGCATCAGTTG
F: HEV-s ^e	TGCGGCATCACMTTYTTCCARAA
R: HEV-as ^e	GCCATGTTCCAGACDGTTRITCCA
F: M-s ^f	TTCTAACCGAGGTCGAAAC
F: M-as ^f	AAGCGTCTACGCTGCAGTCC
F: NP-s ^f	CAGRTACTGGGCHATAAGRAC
R: NP-as ^f	GCATTGTCTCCGAAGAAATAAG

^a F and R represent upstream primer and downstream primer, respectively.

^b Degenerate primers: H = A/T/C/R = A/G.

^c Primers AMDV-s and AMDV-as were used to detect aleutian mink disease virus.

^d Primers MRV-s and MRV-as were used to detect orthoreovirus.

^e Primers HEV-s and HEV-as were used to detect hepatitis E virus.

^f Primers M-s, M-as and NP-s, NP-as were used to detect influenza virus.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vetmic.2016.12.003>.

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