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Isolation of a Recent Korean Epizootic Strain of Newcastle Disease Virus from Eurasian Scops Owls Affected with Severe Diarrhea

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ABSTRACT: Velogenic Newcastle disease virus (NDV) was recovered from two dead Eurasian Scops Owls (*Otus scops*) from a wildlife rescue center in Korea during 2005. Phylogenetic analysis based on the sequence of the partial fusion (F) protein revealed that the isolates had the highest level of homology to recent Korean NDV strains from poultry.

Key words: Fusion protein, Newcastle disease virus, owl, phylogenetic analysis.

Newcastle disease (ND) has a worldwide distribution and is caused by Newcastle disease virus (NDV), which is the sole member of avian paramyxovirus type 1 (APMV-1) belonging to the *Avulavirus* genus of the Paramyxoviridae family (Mayo, 2002). Newcastle disease virus has a negative-sense, single-stranded RNA genome of about 15 kb. This genome contains six genes (3′-NP-P-M-F-HN-L-5′), which code for six proteins, including a nucleoprotein (NP), phosphoprotein (P), matrix (M) protein, fusion (F) protein, hemagglutinin-neuraminidase (HN), and large (L) protein, respectively (Lamb and Kolakofsky, 2002).

Newcastle disease virus exists in two distinct classes, class I and class II, within a single serotype. Class I viruses are not commonly reported and are found in waterfowl, live bird markets, and domestic poultry (Alexander et al., 1992; Aldous et al., 2003; Seal et al., 2005; Kim et al., 2007). The class II viruses are categorized into genotypes I to IX (Ballagi-Pordany et al., 1996; Lomniczi et al., 1998; Herczeg et al., 1999; Yang et al., 1999; Liu et al., 2003). In Korea, genotype VII first emerged in 1984 and then reemerged in 1995 (Lee et al., 2004). Most recent Korean isolates (since 2000) belong to sublineage d of genotype VII (VIId) (Lee et al., 2004). The prevalence of VIId in Korea is similar to that in neighboring countries, including China (Liang et al., 2002; Liu et al., 2003; Zou et al., 2005), Japan (Mase et al., 2002), and Taiwan (Kou et al., 1999; Yang et al., 1999; Ke et al., 2001; Tsai et al., 2004). Newcastle disease virus strains can be classified into three pathotypes (lentogenic, mesogenic, and velogenic) on the basis of in vivo pathogenicity test parameters such as the mean death time (MDT) in specific pathogen-free (SPF) chicken embryos and the intracerebral pathogenicity index (ICPI) in day-old SPF chickens.

Two Eurasian Scops Owls (*Otus scops*) with injuries were taken to the Korean Association for Bird Protection (KABP) in June and July 2005, in the Cheorwon district of Gangwon Province. One owl was an adult, and the other one was a young bird, several days old. The KABP cared for the birds in an in-house cage. The KABP staff fed the captive Eurasian Scops Owls on mealworm and often some pieces of fresh chicken meat obtained from a wholesaler in a market close to the center. Approximately two months later, the two Eurasian Scops Owls died of severe diarrhea that was unrelated to their previous injuries.

An autopsy of the Eurasian Scops Owls showed hemorrhaging of their intestines and proventriculi, a characteristic feature

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1    ATGGGCTCC AAACCTTCT ACCAGGATC CCAGCACCT CTGATGCTG ACCACCCGG ATTACGCTG
     M  G  S   K  P  S   T  R  I   P  A  P   L  M  L   T  T  R   I  T  L

64   ATATTGAGC TGTATCCGT CCGACAAGC TCTCTTGAC GGCAGGCCT CTTGCAGCT GCAGGAATT
     I  L  S   C  I  R   P  T  S   S  L  D   G  R  P   L  A  A   A  G  I

127  GTAGTAACA GGAGATAAG GCAGTCAAT GTATACACC TCGTCTCAG ACAGGGTCA ATCATAGTC
     V  V  T   G  D  K   A  V  N   V  Y  T   S  S  Q   T  G  S   I  I  V

190  AAGTTGCTC CCGAATATG CCCAGGGAT AAAGAGGCG TGTGCAAAA GCCCCATTA GAGGCATAT
     K  L  L   P  N  M   P  R  D   K  E  A   C  A  K   A  P  L   E  A  Y

253  AACAGAACA CTGACTACT TTGCTCACT CCTCTTGGC GACTCCATC CGCAAGATC CAAGGGTCT
     N  R  T   L  T  T   L  L  T   P  L  G   D  S  I   R  K  I   Q  G  S

316  GTGTCCACG TCTGGAGGA AGGAGACAA AAACGCTTT ATAGGTGCT GTTATTGGC AGTGTAGCT
     V  S  T   S  G  G   R  R  Q   K  R  F   I  G  A   V  I  G   S  V  A

379  CTTGGGGTT GC
     L  G  V
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FIGURE 1. Nucleotides and predicted amino acid sequences of the first 389 nucleotides of the coding region of the fusion (F) gene of Korean owl strain Kr-owl/6/05. The F protein cleavage site sequence from position 110 to 119 is boxed, and the F protein N-terminal variable region is underlined.

of viscerotropic ND (Alexander, 2003). Various tissues from the dead owls were sampled and sent to the Conservation Genome Resource Bank (CGRB) of Seoul National University, Korea, where they were deposited with the designations cgrb2287 and cgrb2289. Three months later, kidney samples from the dead birds were sent to the National Veterinary Research and Quarantine Service (NVRQS), Korea, for virus isolation. Except for kidney, other tissues were not available for NDV testing.

Hemagglutinating agents were isolated from both kidney samples using embryonated SPF eggs; these were identified as NDV by reverse transcription–polymerase chain reaction (RT-PCR) and hemagglutination inhibition (HI) testing using a reference Paramyxovirus antiserum panel (National Veterinary Service Laboratory, Iowa, USA). The two isolates were designated Kr-owl/6/05 and Kr-owl/7/05, respectively. The abbreviation of the isolates represents country-host/month/year. No other pathogens were detected in these owls.

Because the Eurasian Scops Owl is currently listed on the 2006 International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (see http://www.iucnredlist.org/), the virulence of the isolates Kr-owl/6/05 and Kr-owl/7/05 was evaluated using in vivo pathogenicity tests. Specific pathogen-free (SPF) chickens and embryonated SPF chicken eggs were used for in vivo pathogenicity tests, median death time (MDT) and intracerebral pathogenicity index (ICPI; Alexander, 1998). Both isolates had a MDT of <60 in embryonated chicken eggs and an ICPI of >1.80 in day-old chickens. These results indicate that the isolates Kr-owl/6/05 and Kr-owl/7/05 are velogenic (Alexander, 1998), and they indicate that the Eurasian Scops Owls may be highly susceptible to NDV.

The origin of both owl isolates was investigated using phylogenetic analysis based on the partial fusion (F) gene. For this purpose, viral RNA was extracted from both isolates propagated in chicken embryos using the RNeasy mini-kit in accordance with the manufacturer's protocols (Qiagen, California, USA). A genomic region of 695 nucleotides (nt) between nucleotides 1055 of the matrix gene

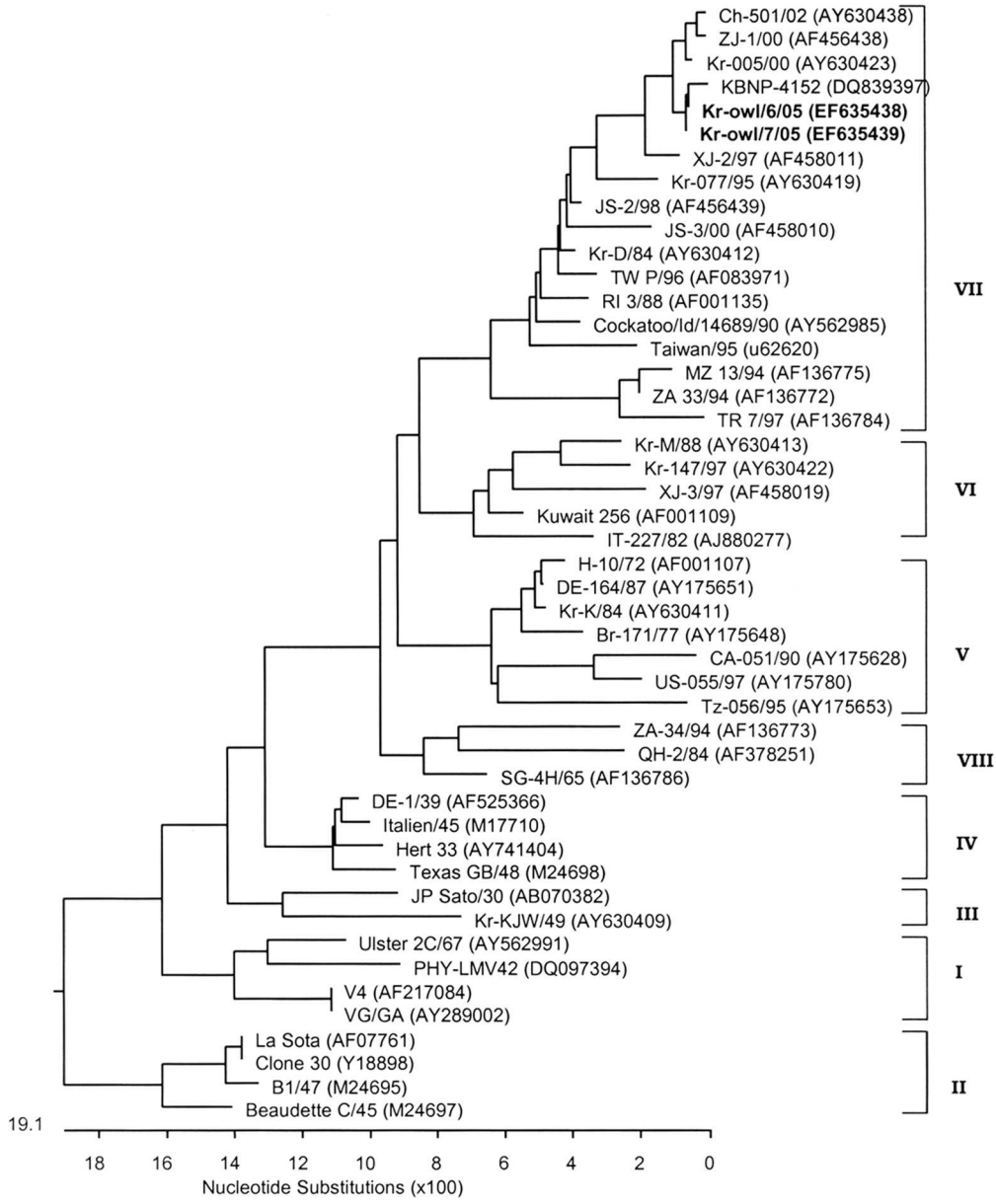

FIGURE 2. Phylogenetic tree of the nucleotide sequences of Newcastle disease virus (NDV) isolates based on the first 389 nucleotides of the coding region of the F gene. Sequences of reference strains were obtained from the GenBank database. Genbank accession numbers for all NDV strains are included in parenthesis. The sequences of NDV strains were aligned using the Clustal W method available in MEGALIGN program (Lasergene ver. 7.0, DNASTAR, Wisconsin, USA), and phylogenetic analysis was performed using neighbor-joining method with 1,000 bootstrap replicates, visualized by the TREEVIEW program. The provisional designations, including genotypes, are indicated on the right.

and 508 of the fusion gene was amplified by reverse transcription PCR according to a method described previously (Lee et al., 2004). Amplified DNA products were subjected to direct sequencing. The F gene sequence of the Kr-owl/6/05 was identical to that of Kr-owl/7/05. The cleavage site of the F protein possessed

the amino-acid sequence ^{112}R-R-Q-K-R-F^{117}, which is a motif characteristic of virulent NDV strains (Toyoda et al., 1987; Collins et al., 1993; Nagai, 1993; Yang et al., 1999; Seal, 2004) as shown in Figure 1. These sequence results support the biologic properties of the isolate presented here with regard to virulence.

Nucleotide similarities of the first 389 nucleotides of the F gene and predicted amino-acid sequences of 129 residues were compared with the corresponding sequences of representative strains. The F gene sequences of 45 NDV strains from the Genbank database were used for comparison with both Korean owl isolates (see Fig. 2). Kr-owl/6/05 and the Kr-owl/7/05 were placed in the genotype VII and showed the highest degree of similarity to strains of NDV (Ch-501/02, ZJ-1/00, Kr-005/00, and KBNP-4152) belonging to subgenotype d of the genotype VII (VIId). This indicates that these Korean owl isolates belong, genetically, to the VIId group, which is prevalent in poultry in eastern Asia, including Korea, Japan, China, and, in more recent years, Taiwan (Ke et al., 2001; Yu et al., 2001; Mase et al., 2002; Lee et al., 2004; Zou et al., 2005). Interestingly, the owl isolates Kr-owl/6/05 and the Kr-owl/7/05 showed the highest nucleotide similarities (99.5% over 389 nucleotides) to Korean isolates Kr-021/04, which was isolated from a layer chicken in December 2004 (Cho et al., 2007). This indicates that the Korean owl isolates may be related to the epizootic strain circulating among affected poultry in Korea, coincident with the isolation of NDV from owls.

This is the first report of virulent NDV in captive birds of prey from Korea. However, it is unclear as to how the Eurasian Scops Owls became exposed to NDV during the recent epizootic in Korea. This species normally feeds on small insects (Marchesi and Sergio, 2005). It is unlikely that the owls were infected with NDV in their natural habitat two months prior to their rescue, as the incubation period for this virus in susceptible fowl is 2 to 15 days (average 5 to 6 days) (Alexander, 2003). In addition, the KABF staff had no connections to the poultry industry. Thus, in view of the NDV epizootic in Korea during the time that these were captive at the KABP, it is plausible that they were exposed to NDV infection through the diet of domestic chicken meat, which is not routinely tested for NDV.

Interestingly, KABF staff also reared ten captive Eurasian Eagle Owls (*Bubo bubo*) with injuries in different cages within the same house as the affected Eurasian Scops Owls. The Eurasian Eagle Owls were also fed fresh chicken meat but did not show any clinical signs of disease. Unfortunately, they were not tested for NDV as the NDV diagnosis was made 3 mo after the disease was detected at the KABP center.

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