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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 (VII_d) (Lee et al., 2004). The prevalence of VII_d 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

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						

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

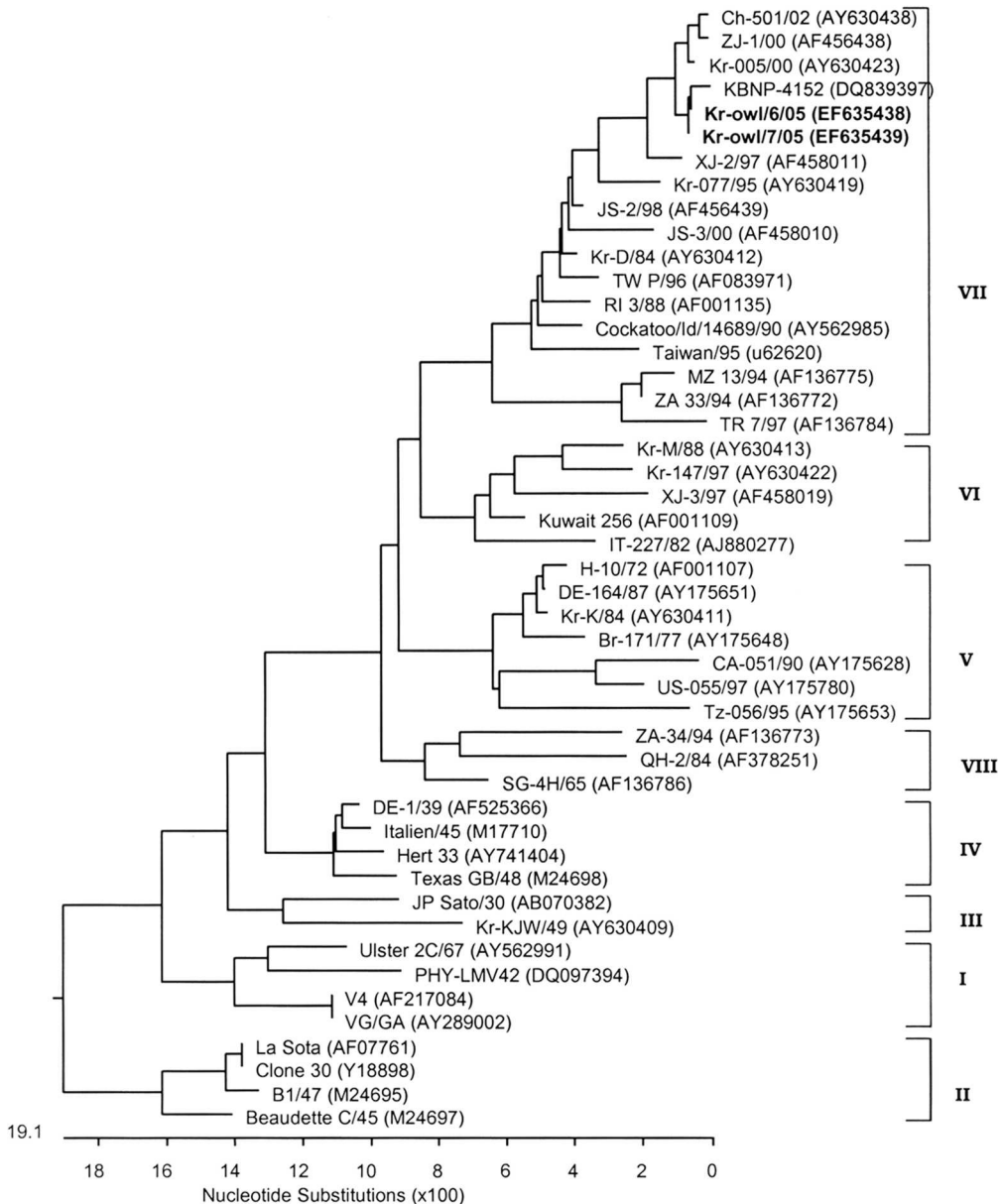


FIGURE 2. Phylogenetic tree of the nucleotide sequences of Newcastle disease virus (NDV) isolates based on the first 389 nucleotides of the coding region of the F gene. Sequences of reference strains were obtained from the GenBank database. Genbank accession numbers for all NDV strains are included in 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 $^{112}\text{R-R-Q-K-R-F}^{117}$, 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 (VII_d). This indicates that these Korean owl isolates belong, genetically, to the VII_d 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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LITERATURE CITED

- ALDOUS, E. W., J. K. MYUN, J. BANKS, AND D. J. ALEXANDER. 2003. A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. *Avian Pathology* 32: 239–256.
- ALEXANDER, D. J. 1998. Newcastle disease and other avian paramyxoviruses. In: *A laboratory manual for the isolation and identification of avian pathogens*, 4th ed., D. E. Swayne, J. R. Glisson, M. W. Jackwood, J. E. Pearson and W. M. Reed (eds.). American Association of Avian Pathologists, Kennet Square, Pennsylvania, pp. 156–163.
- . 2003. Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. In: *Diseases of poultry*, 11th ed., Y. M. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald and D. E. Swayne (eds.). Iowa State Press, Ames, Iowa, pp. 63–99.
- , G. CAMPBELL, R. J. MANVELL, M. S. COLLINS, G. PARSONS, AND M. S. McNULTY. 1992. Characterization of an antigenically unusual virus

- responsible for two outbreaks of Newcastle disease in the Republic of Ireland in 1990. *Veterinary Record* 130: 65–68.
- BALLAGI-PORDANY, A., E. WEHMANN, J. HERCZEG, S. BELAK, AND B. LOMNICZI. 1996. Identification and grouping of Newcastle disease virus strains by restriction site analysis of a region from the F gene. *Archives of Virology* 141: 243–261.
- CHO, S. H., S. J. KIM, AND H. J. KWON. 2007. Genomic sequence of an antigenic variant Newcastle disease virus isolated in Korea. *Virus Genes* 35: 293–302.
- COLLINS, M. S., J. B. BASHIRUDDIN, AND D. J. ALEXANDER. 1993. Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variation in antigenicity and pathogenicity. *Archives of Virology* 128: 363–370.
- HERCZEG, J., E. WEHMANN, R. R. BRAGG, P. M. TRAVASSOS DIAS, G. HADJIEV, O. WERNER, AND B. LOMNICZI. 1999. Two novel genetic groups (VIIb and VIII) responsible for recent Newcastle disease outbreaks in Southern Africa, one (VIIb) of which reached Southern Europe. *Archives of Virology* 144: 2087–2099.
- KE, G. M., H. J. LIU, M. Y. LIN, J. H. CHEN, S. S. TSAI, AND P. C. CHANG. 2001. Molecular characterization of Newcastle disease viruses isolated from recent outbreaks in Taiwan. *Journal of Virological Methods* 97: 1–11.
- KIM, L. M., D. J. KING, D. L. SUAREZ, C. W. WONG, AND C. L. AFONSO. 2007. Characterization of class I Newcastle disease virus isolates from Hong Kong live bird markets and detection using real-time reverse transcription-PCR. *Journal of Clinical Microbiology* 45: 1310–1314.
- KOU, Y. T., L. L. CHUEH, AND C. H. WANG. 1999. Restriction fragment length polymorphism analysis of the F gene of Newcastle disease viruses isolated from chickens and an owl in Taiwan. *Journal of Veterinary Medical Science* 61: 1191–1195.
- LAMB, R. A., AND D. KOLAKOSKY. 2002. Paramyxoviridae: The viruses and their replication. In: *Fundamental virology*, B. B. Fields, D. M. Knipe and P. M. Howley (eds.). Lippincott-Raven Publishers, New York, pp. 1305–1340.
- LEE, Y. J., H. W. SUNG, J. G. CHOI, J. H. KIM, AND C. S. SONG. 2004. Molecular epidemiology of Newcastle disease viruses isolated in South Korea using sequencing of the fusion protein cleavage site region and phylogenetic relationships. *Avian Pathology* 33: 482–491.
- LIANG, R., D. J. CAO, J. Q. LI, J. CHEN, X. GUO, F. F. ZHUANG, AND M. X. DUAN. 2002. Newcastle disease outbreaks in western China were caused by the genotypes VIIa and VIII. *Veterinary Microbiology* 87: 193–203.
- LIU, X. F., H. Q. WAN, X. X. NI, Y. T. WU, AND W. B. LIU. 2003. Pathotypical and genotypical characterization of strains of Newcastle disease virus isolated from outbreaks in chicken and goose flocks in some regions of China during 1985–2001. *Archives of Virology* 148: 1387–1403.
- LOMNICZI, B., E. WEHMANN, J. HERCZEG, A. BALLAGI-PORDANY, E. F. KALETA, O. WERNER, G. MEULEMANS, P. H. JORGENSEN, A. P. MANTE, A. L. GIELKENS, I. CAPUA, AND J. DAMOSER. 1998. Newcastle disease outbreaks in recent years in Western Europe were caused by an old (VI) and a novel genotype (VII). *Archives of Virology* 143: 49–64.
- MARCHESI, L., AND F. SERGIO. 2005. Distribution, density, diet and productivity of the Scops owl *Otus scops* in the Italian Alps. *Ibis* 147: 176–187.
- MASE, M., K. IMAI, Y. SANADA, N. SANADA, N. YUASA, T. IMADA, K. TSUKAMOTO, AND S. YAMAGUCHI. 2002. Phylogenetic analysis of Newcastle disease virus genotypes isolated in Japan. *Journal of Clinical Microbiology* 40: 3826–3830.
- MAYO, M. A. 2002. A summary of taxonomic changes recently approved by ICTV. *Archives of Virology* 147: 1655–1663.
- NAGAI, Y. 1993. Protease-dependent virus tropism and pathogenicity. *Trends in Microbiology* 1: 81–87.
- SEAL, B. S. 2004. Nucleotide and predicted amino acid sequence analysis of the fusion protein and hemagglutinin-neuraminidase protein genes among Newcastle disease virus isolates. Phylogenetic relationships among the Paramyxovirinae based on attachment glycoprotein sequences. *Functional & Integrative Genomics* 4: 246–257.
- , M. G. WISE, J. C. PEDERSEN, D. A. SENNE, R. ALVAREZ, M. S. SCOTT, D. J. KING, Q. YU, AND D. R. KAPCZYNSKI. 2005. Genomic sequences of low-virulence avian paramyxovirus-1 (Newcastle disease virus) isolates obtained from wild-bird markets in North America not related to commonly utilized commercial vaccine strains. *Veterinary Microbiology* 106: 7–16.
- TOYODA, T., T. SAKAGUCHI, K. IMAI, N. M. INOCENCIO, B. GOTOH, M. HAMAGUCHI, AND Y. NAGAI. 1987. Structural comparison of the cleavage-activation site of the fusion glycoprotein between virulent and avirulent strains of Newcastle disease virus. *Virology* 158: 242–247.
- TSAI, H. J., K. H. CHANG, C. H. TSENG, K. M. FROST, R. J. MANVELL, AND D. J. ALEXANDER. 2004. Antigenic and genotypical characterization of Newcastle disease viruses isolated in Taiwan between 1969 and 1996. *Veterinary Microbiology* 104: 19–30.
- YANG, C. Y., H. K. SHIEH, Y. L. LIN, AND P. C. CHANG. 1999. Newcastle disease virus isolated from recent outbreaks in Taiwan phylogenetically related to viruses (genotype VII) from recent outbreaks in Western Europe. *Avian Disease* 43: 125–130.
- YU, L., Z. WANG, Y. JIANG, L. CHANG, AND J. KWANG.

2001. Characterization of newly emerging Newcastle disease virus isolates from the People's Republic of China and Taiwan. *Journal of Clinical Microbiology* 39: 3512–3519.

ZOU, J., S. SHAN, N. YAO, AND Z. GONG. 2005. Complete genome sequence and biological

characterizations of a novel goose Paramyxovirus-SF02 isolated in China. *Virus Genes* 30: 13–21.

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