# Molecular epidemiological analysis of viscerotropic velogenic Newcastle disease viruses

*Youn Jeong Lee**
*Avian Disease Division, National Veterinary Research and Quarantine Service*

## Abstract

The study, using sequence analysis and phylogenetic relationship of the fusion protein gene, divided the Korean epizootic isolates of Newcastle disease virus (NDV) into several lineages to determine the molecular epidemiology of the virus. A 695 base pair fragment was amplified by polymerase chain reaction between matrix protein gene and fusion protein gene of 30 Korean NDV isolates, which were isolated from field outbreaks of Newcastle disease between 1949 and 2002. All isolates showed the amino acid sequence 112 R-R-Q/R-K-R116 at the C-terminus of the F2 protein and phenylalanine (F) at the N-terminus of the F1 protein, residue 117. These amino acid sequences were identical to a known virulent motif. The region of the F gene between nucleotides 47 and 435 was compared by phylogenetic analysis. Based on nucleotide sequence, the Korean NDV isolates belonged to genotype III, V, VI and VII corresponding to isolates in 1949, 1982 to 1984, 1988 to 1997, and 1995 to 2002, respectively. These data showed that genotypes of five Korean Newcastle disease epizootics had replaced each other serially (III, V, VI and VII) in chronological order. Further, the five Korean Newcastle disease epizootics were closely related with the Necastle disease panzootics or Newcastle disease epizootics in other countries. Present study showed that the Korean genotype V isolated before 1984 was related with European Newcastle diseaseepizootics in the 1970s, whereas the Korean genotype VI and VII isolated after 1988 were more closely related with Far East Newcastle disease epizootics, especially Newcastle disease3 epizootics in Japan, Taiwan and China. Since 1988, the genotype VI and VII of Far East origin were dominant in South Korea. That might be due to the increased trade of agricultural products including poultry among Far East Asian countries.

## Introduction

Newcastle disease (ND) causes one of the most serious diseases in commercial poultry. ND can be divided into five pathotypes based on severity of the disease in chickens. These are viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic or respiratory, and asymptomatic enteric type (Alexander, 1997). ND has continued to cause serious losses to the poultry industry, and it is defined as a list A disease by the Office International des Epizooties. Numerous ND live and inactivated vaccines have been developed however, the number of ND outbreaks in commercial poultry has been increased rather than being controlled (Alexander, 2001).

The causative agent of the disease, Newcastle disease virus (NDV), is classified as a member of the genus Avulavirus, in the family Paramyxoviridae (Mayo, 2002a, b). The enveloped virus has a negative-sense single-stranded genome of 15,186 nucleotides

* Avian Disease Division, National Veterinary Research and Quarantine Service, Ministry of Agriculture and Forestry, 480 Anyang 6-dong, Anyang, Gyeonggi-do, 430-016, Korea

(Krishnamurthy & Samal, 1998; de Leeuw & Peeters, 1999; Römer-Oberdörfer et al., 1999). The viral genome codes for six proteins, including an RNA-directed RNA polymerase (L gene), hemagglutinin-neuraminidase (HN gene), fusion (F gene), matrix (M gene), phosphoprotein (P gene) and nucleocapsid (NP) proteins, in that order, from the 5′ terminus to the 3′ terminus (Alexander, 1997). The fusion protein encoded by the F gene is cleaved into F2-F1 protein by post-translational cleavage by host proteinases. The virulent type of NDV has multiple basic amino acid sequence at the C-terminus of the F2 protein. These multiple basic amino acid sequences can be recognized by ubiquitous host proteinases. Pathotype prediction and diagnosis of the virulence of NDV can be performed using the fusion protein cleavage site sequence analysis (Collins et al., 1993; Seal et al., 1995; Marin et al, 1996; Gould et al., 2001).

Various methods have been introduced for grouping and analysis of NDV including differences in pathogenicity (Alexander, 1997), antigenicity (Russell & Alexander, 1983 Alexander et al., 1987, 1997), and genomes (Collins et al., 1993 Jarecki-Black& King, 1993 Seal et al., 1995, 1996, 2000 Ballagi-Pordany et al, 1996 Marin et al., 1996 Kant et al. 1997 Lomniczi et al., 1998; Herczeg et al., 1999, 2001; Yang et al., 1999; Nanthakumar et al., 2000 Aldous et al., 2001, 2003; Gould et al., 2001; Ke et al., 2001; Yu et al., 2001; Liang et al., 2002; Mase et al., 2002; Liu et al., 2003; Wehmann et al., 2003a, 2003b). Several epidemiological studies of NDV have been carried out using these molecular-based methods.

The first confirmed outbreaks of ND occurred in 1926, in Java, Indonesia, and in Newcastle-upon-Tyne, UK, but the disease may have been present in Korea as early as 1924 (Konno et al., 1929; Alexander, 1997). There have been three main panzootics of ND (Alexander, 1997 Lomniczi et al., 1998). Major panzootic strains of NDV could be divided into three lineages (Sakaguchi et al., 1989; Toyoda et al., 1989) or seven genotypes (Ballagi-Pordany et al., 1996; Lomniczi et al., 1998) by comparing the nucleotide sequences and phylogenetic analysis. Genotypes II-IV were involved in the first panzootic of ND, genotypes V and VI might have caused the second and third panzootics (Ballagi-Pordany et al., 1996; Lomniczi et al., 1998). It was also suggested that genotype VI and VII, isolates of Middle East in late 1960s and isolates of Indonesia in the late 1980s, caused epizootic infection of Europe in the 1990s. Recently, there were several reports concerning molecular characterization of NDV in East Asia (Yang et al., 1999; Ke et al., 2001; Yu et al., 2001; Liang et al., 2002 Mase et al., 2002; Liu et al., 2003).

The present study was initiated to carry out the genetic characterization of NDV isolates derived from five ND epizootics in South Korea and to unravel epidemiological relationships of ND outbreaks.

## Materials and Methods

### *Viruses*

NDV isolates have been isolated in the National Veterinary Research and Quarantine Service since 1949. Thirty epizootic isolates in South Korea and two isolates from China, which were detected during the quarantine process in 2001/02, were selected for sequencing and phylogenetic relationship analysis. The origin of the NDV isolates and nucleotide sequences are summarized in Table 1.

### *Preparation of viral RNA, reverse transcription-polymerase chain reaction*

NDV isolates were grown in 10-day-old embryonated eggs, and infective allantoic fluids were stored at -70°C until use. RNA was extracted directly from allantoic fluid inoculated with NDVs. Sodium dodecyl sulfate (2%; w/v) was added to 500l of allantoic fluid and incubated at 55°C for 5 min. An equal volume of phenol: chloroform: isoamylalcohol (25:24:1) was added and mixed. The mixture was centrifuged at 12,000 x g for 10 min and the upper phase was collected, and the RNA was further purified with a Rnaid kit (BIO 101, La Jolla, CA,USA) according to the manufacturer's recommendations.

To obtain cDNA from a genomic RNA template, 1 l primer (M1055; 50pmol) was added to 10 l template RNA and boiled for 5 min. After standing on ice for 10 min, 4 l of 5x reverse transcription buffer, 2 l of

0.1 M dithiothreitol, 1 l of dNTP (each 10mM), 1 l of Rnasin (40 u/ l; Promega), and 1 l of Moloney murine leukaemia virus RT (200 u/ l; GibcoBRL) were added and incubated at 37 °C for 90 min.

Two primers, the primer M1055 (sense primer, 5′-GCT GAT CAT GAG GTT ACC TC-3′) and the primer F508 (anti-sense primer, 5′-AGT CGG AGG ATG TTG GCA GC-3′), were used to amplify the fusion protein gene, including the fusion protein cleavage site and the matrix protein gene. The two primers generated a 695 base pair (bp)fragment between nucleotides 1055 of the matrix (M) gene and 508 of the fusion (F) gene. For the polymerase chain reaction (PCR) amplication, 5 l cDNA, 5 l of 10x PCR buffer, 6 l 25mM MgCl2, 1 l dNTP (10mM), 1 l Taq DNA polymerase (2.5 u/l; promega), 1 l sense primer (50pmol), and 1 l anti-sense primer (50pmol) were mixed to a final volume of 50 l. Amplification was conducted in a capillary DNA thermal cycler (DNA AMP UNITDMS, Korea). The PCR was performed by 30 cycles at 94°C for 10 sec, 47°C for 30 sec, and 72°C for 40 sec. The products were analyzed on a 1.5% agarose gel.

### *Sequencing of the PCR products*

The amplified DNA fragments were purified using the GENE CLEAN II kit (BIO 101, Vista, CA, USA). Purified DNA fragments were cloned into a TA cloning vector (Invitrogen). The DNA sequence was determined using the Dye Terminator Cycle Sequencing method and analyzed by ABI 377 autosequencer (Applied BiosystemsInc.). The nucleotide sequences have been deposited in the GenBank under accession numbers (Table 1).

### *Analysis of sequence data*

Assembly of sequencing contigs and translation of the nucleotide sequence into the protein sequence were performed with DNASIS. The sequence data were aligned using the MegAlign program in the Lasergene package (DNASTAR Inc, Madison, WI, USA) by clustal multiple alignment algorithm. Phylogenetic relationships were established with the computer program TREECON for windows, version 1.1(Van de Peer & de Wachter, 1997). Briefly, a distance matrix was created by the Kimmura two parameter model and the tree was constructed by the neighbor-joining algorithm. The robustness of the groupings were assessed by bootstrap resampling of 1,000 replicate trees.

## Results

### *Virus isolation and sequence analysis*

Thirty virulent field NDV isolates were used for the examination of molecular epidemiological relationshipsof ND outbreaks in South Korea since 1949(Table 1). The 695bp fragment between nucleotides 1055 of the matrix gene and 508 of the fusion gene was amplified including the fusion protein cleavage site, and the sequence was analyzed (Data not shown). Nucleotide similarities of the first 389 bp and predicted amino acid sequences of 129 residues of Korean isolates were compared with the corresponding sequences of the representative strains. Representative strains of each genotype showed similarities of 81.8 to 92.8% for the nucleotide sequence and 79.8 to 95.2% for amino acid sequence. Korean isolates of genotypes III, V, VI and VII showed the highest similarity with representative strain of each genotype: 91.3, 99.5, 92.3, and 93.6 to 97.7%, respectively. Korean isolates and Chinese isolates of genotype VII showed similarities of 95.4 to 99.5% for nucleotide, and 93.0 to 99.2% for amino acid. Recent epizootic isolates, genotype VII, showed the lowest similarity with a vaccine strain like VG/GA and LaSota (genotypes I and II): 82.3 o 84.8% for the nucleotide and 79.1 to 84.5% for the amino acid.

### *Alignment of the deduced amino acid sequences*

All of isolates showed the amino acid sequence 112 R-R-Q/R-K-R 116 at the C-terminus of the F2 protein and phenylalanine (F) at the N-terminus of the F1 protein, residue 117. These amino acid sequences were identical with a virulent motif in previous reports on NDV isolates. Kr-KJW/49 belonged to genotype III and was characterized by

Table 1. *Origin of Newcastle Disease Virus strains and nucleotide sequences*

| Abbreviation | | Year | Country | Host | Genetic group | Accession number |
|---|---|---|---|---|---|---|
| This study | Original | | | | | |
| Kr-KJW/49 | - | 1949 | Korea | Chicken | III | AY630409 |
| Kr-48/82 | - | 1982 | Korea | - | V | AY630410 |
| Kr-1129/83 | - | 1983 | Korea | - | V | AY648305 |
| Kr-K/84 | - | 1984 | Korea | Chicken | V | AY630411 |
| Kr-D/84 | - | 1984 | Korea | peafowl | VII | AY630412 |
| Kr-M/88 | - | 1988 | Korea | Quail | VI | AY630413 |
| Kr-12A/89 | - | 1989 | Korea | Chicken | VI | AY630414 |
| Kr-102/89 | - | 1989 | Korea | - | VI | AY630415 |
| Kr-163/90 | - | 1990 | Korea | - | VI | AY630416 |
| Kr-9/91 | - | 1991 | Korea | - | VI | AY630417 |
| Kr-104/92 | - | 1992 | Korea | - | VI | AY630418 |
| Kr-077/95 | - | 1995 | Korea | Chicken (broiler) | VII | AY630419 |
| Kr-146/95 | - | 1995 | Korea | Chicken (broiler) | VII | AY630420 |
| Kr-279/95 | - | 1995 | Korea | Chicken (broiler) | VII | AY630421 |
| Kr-147/97 | - | 1997 | Korea | Chicken (broiler) | VI | AY630422 |
| Kr-005/00 | - | 2000 | Korea | Chicken (Layer) | VII | AY630423 |
| Kr-009/00 | - | 2000 | Korea | Chicken (Layer) | VII | AY630424 |
| Kr-010/00 | - | 2000 | Korea | Chicken (Layer) | VII | AY630425 |
| Kr-011/00 | - | 2000 | Korea | Chicken (broiler) | VII | AY630426 |
| Kr-014/00 | - | 2000 | Korea | Chicken (Layer) | VII | AY630427 |
| Kr-017/00 | - | 2000 | Korea | Chicken (broiler) | VII | AY630428 |
| Kr-018/00 | - | 2000 | Korea | Chicken (Layer) | VII | AY630429 |
| Kr-019/00 | - | 2000 | Korea | Chicken (Layer) | VII | AY630430 |
| Kr-021/00 | - | 2000 | Korea | Chicken (Layer) | VII | AY630431 |
| Kr-029/00 | - | 2000 | Korea | Chicken (Layer) | VII | AY648304 |
| Kr-352/00 | - | 2000 | Korea | Chicken | VII | AY630432 |
| Kr-400/00 | - | 2000 | Korea | Chicken (broiler) | VII | AY630433 |
| Kr-401/00 | - | 2000 | Korea | Chicken | VII | AY630434 |
| Kr-420/00 | - | 2000 | Korea | Ostrich | VII | AY630435 |
| Kr-188/02 | - | 2002 | Korea | Chicken(broiler) | VII | AY630436 |
| Ch-77/01 | - | 2001 | China | Chicken meat | VII | AY630437 |
| Ch-501/02 | - | 2002 | China | Chicken meat | VII | AY630438 |
| DE-164/87 | ADEHU87164 | 1987 | Germany | human | V | AY175651 |
| CA-140/75 | BCACO75140 | 1975 | Canada | cormorant | V | AY175670 |
| CA-051/90 | ACAGL90051 | 1990 | Canada | gull | V | AY175628 |
| CA-053/90 | ACACO90053 | 1990 | Canada | cormorant | V | AY175630 |
| CA-095/92 | -CACO92095 | 1992 | Canada | cormorant | V | AY175681 |
| US-098/92 | 1USCO92098 | 1992 | USA | cormorant | V | AY175638 |
| US-055/97 | AUSCO97055 | 1997 | USA | cormarant | V | AY175780 |
| Br-171/77 | ABRCK77171 | 1977 | Brazil | Domestic fowl | V | AY175648 |
| Br-160/83 | ABRCK83160 | 1983 | Brazil | Domestic fowl | V | AY175649 |
| Tz-056/95 | ATZCK95056 | 1995 | Tanzania | Domestic fowl | V | AY175653 |
| Tz-080/95 | ATZDK95080 | 1995 | Tanzania | Duck | V | AY175656 |
| Tz-094/95 | GTZCK95094 | 1995 | Tanzania | Domestic fowl | V | AY175728 |

The sequences of the strains were obtained from GeneBank (Aldous *et al.*, 2003). Original designation was changed in this study.

two unique amino acids (104 Glu and 110 Arg). Genotype III and V NDV isolates possess Arg, Thr, and Val at the residues 101, 107, and 118, whereas genotype VI and VII possess Lys, Ser, and Ile. The residue 114Arg was unique for genotype VI except for the Kr-400/00 isolate. Substitution at the position 121, Ile to Val, was characteristic of genotype VII. Among genotype VII, recent isolates (after 2000) showed substitutions 52 Ile to Val and 71 Lys to Arg. Two Chinese NDV isolates had similar amino

acid characteristics with Korean genotype VII viruses.

### *Phylogenetic analysis*

The region of the F gene between nucleotides 47 and 435 was compared by phylogenetic analysis. A phylogenetic tree of NDV isolates based on sequence analysis of the first 389 nucleotides of the F gene coding region is shown in Figure 1~4. Corresponding sequences of representative strains available from the GenBank were used. Lineages designated I to VII in this study correspond to those described by Lomniczi et al. (1998).

Based on the topology of tree, the isolates have been separated into eight genotypes, genotype I to VIII (Figure 1). Korean NDV isolates belonged to genotype III, V, VI, and VII corresponding to isolates of epizootics in 1949, in 1982 to 1984, in 1988 to 1997, and in 1995 to 2002, respectively. Genotype III included Kr-KJW/49 and three representative viruses, JP-Sato/30, Ch-F48E9/44 and Aus Victoria/32. NDV isolates emerged in the early 1980s in South Korea belonged to genotype V and obtained in the late 1980s to the middle 1990s belonged to genotype VI. Most of South Korea isolates after 1995 and two isolates from China, which were detected during quarantine process in 2001/02, belonged to genotype VII.

To unravel the origin of Korean isolates, genotypes V to VII were analyzed as a separate dataset and trees generated accordingly (Figure 2 - 4). Genotype V contains 39 isolates, which can be separated into four putative sublineages, Va, Vb, Vc and Vd (Figure 2). Genotype V was largely composed of isolates derived from or considered to be from 'second' NDV panzootic in the 1970s (Alexander, 2001), which was influenced by trade in exotic birds. Sublineage Va was composed of three Korean isolates, Kr-48/82, Kr-1129/83 and Kr-K/84, and isolates from Western Europe in the 1970s and 1980s. There was divergence in Korean isolates compared with European isolates at the root of this sublineage. Sublineage Vb was largely composed of isolates of Eastern Europe. Sublineage Vc was composed of cormorant viruses isolated between 1975 and 1997 in Canada and USA. Sublineage Vd was composed of viruses from Tanzania.

Genotype VI contains 58 isolates, which can be separated into five sublineages, VIa (VIa+VIe), VIb, VIc, VId and VIf (Figure 3). In previous a report, the sublineage VIa and VIe were described as separated sublineages (Liu et al., 2003). However, in present study, the sublineage VIa included largely 'ancient' isolates originating in the Middle East (Kuwait 256, Iraq AG68), Japan (JP-Narashino/67, JP-Chiba/69) and UK (Warwick/66) according to the result of Aldous et al.(2003). Sublineage VIb was largely composed of isolates from Japanese pigeon and Chinese isolates. Sublineage VIc contained an Italien isolate and a Japanese isolate. Sublineage VId contained isolates obtained from Europe, China and Japan in the 1980s and 1990s. Seven Korean isolates as well as the other isolates from Japan and China were divided into the new sublineage VIf of Far East Asia origin.

Genotype VII contains 102 isolates, which can be separated into five sublineages, VIIa to VIIe (Figure 4). Sublineage VIIa was composed of isolates originating from Europe in the 1990s and isolates originating from Indonesiain 1988. Sublineage VIIb contained isolates from Europe, South Africa and Mozambique in the late 1980s and 1990s. Sublineage VIIc contained isolates originating from Taiwan, Japan and China. NDV isolates in sublineage VIIc in 1984 were the earliest isolates of the genotype VII. One Korean isolate (Kr-D/84),isolated from peafowl reared in the zoo in 1984, was also included sublineage VIIc. Sublineage VIId was composed enormous isolates originating from Far East Asia (Taiwan, Japan, China and South Korea) in the middle 1990s to 2002. Among the sublineage VIId, Korean isolates were more closely .related to Chinese isolates rather than Japanese and Taiwan isolates.

## Discussion

Sequence analyses of fusion protein cleavage site were used for determining the pathotype of NDV instead of conventional methods such as mean death time and intracerebral pathogenic index tests (Collins et al., 1993; Seal et al., 1995; Marin et al, 1996; Gould et al., 2001). All virulent field isolates of Korean NDV showed the amino acid sequence 112 R-R-Q/R-K-R

116at the C-terminus of the F2 protein and phenylalanine (F) at the N-terminus of the F1 protein, residue 117. These amino acid sequences were identical with a virulent motif on reported NDV isolates. (Alexander, 1997)

Since 1980, the epidemiological situation of ND in South Korea was characterized by periodic epizootics, whereas enzootic infections usually occurred, especially in winter season in Korea (Kim & Song, 1992). The isolates from each different periodic epizootics in Korea were not identical in origin, based on the sequence analysis and phylogenetic relationships. In the presentstudy, the phylogenetic analysis of partial F gene sequence produced similar tree topology compared with those from entire gene sequences as shown in previous studies (Sakaguchi et al., 1989; Toyoda et al., 1989; Seal et al., 1995; 1996; Lomniczi et al., 1998; Yang et al., 1999 Yu et al., 2001).

Lomniczi et al. (1998) proposed that at least three different genotypes (II, III and IV) were responsible for the epizootics during the first panzootic (before the 1960s), each one being restricted to a specific geographical region (Herczeg et al., 1999). During the first panzootic, there was epizootic infection of ND in Korea. The Korean representative NDV challenge strains, Kr-KJW/49, was isolated in 1949. The old Japanese (JP-Sota/30) and Chinese (Ch-F48E9/44) NDV strains were isolated in 1930 and 1944, respectively. These three isolates from Far East belong to genotype III. Furthermore, Yang et al. (1999) recently reported that one of the Taiwan isolate of NDV isolated in 1969 was similar to the genotype III viruses. Consequently, it was assumed that there were genotype III viruses in the Far East at the time of the first panzootic in the 1930s to 1960s.

Compared with these early viruses, two new genotypes were emerged during the second panzootic (after the 1960s). The one genotype that wasresponsible for the outbreaks in England, California and in some other European countries in the early 1970s was influenced by trade ofexotic birds (genetic group V), and the other genotype caused epizootics in the Middle East and Greece during the late 1960s (genetic group VI) (Herczeg et al., 1999 Alexander, 2001). Based on the present study, the Korean NDV isolates in the early 1980s belonged to genotype V, sublineage Va (Figure 2). Three Korean isolates, Kr-48/82, Kr-1129/83 and Kr-K/84, were clustered with Western European isolates in the 1970s, but there was some divergence in Korean isolates compared with European isolates at the root of this sublineage. These data suggest that Korean isolates were the same origin with European isolates in the 1970s, but these viruses seemed to be introduced before 1980s and changed within the country. It was the first report of NDV outbreaks by genotype V viruses in the Far East. Sublineage Vb was largely composed of Italy and Eastern Europe isolates between 1977 and 2002, as reported previous studies (Herczed et al., 2001; Czeglédi et al., 2002; Wehmann et al., 2003b). Like a previous study (Aldous et al. 2003), cormorants isolates in the US and Canada in the 1990s (putative sublineage Vc) and Tanzanian isolates (putative sublineage Vd) differed from sublineage Va, in which Korean isolates were included.

It was determined that genotype VI was newly emerged in South Korea during the 1988 to 1992 epizootic infections (Figure 3). Genotype VI included isolates in the 1960 to 1970 Middle East epizootics of the second panzootic, pigeon isolates in the early 1980s of the third panzootic and isolates in the 1990s Western Europe epizootics (Alexander, 1997). In previous study, genotype VI was divided into several subtypes according to their geographical origins and hosts (Yu et al., 2001; Aldous et al. 2003; Liu et al., 2003). Also Aldous et al. (2003) divided genotype VI into four putative sublineages, VIa, VIb VIc and VId. In present study, the isolates from Middle East and Japan belong to the sublineage VIa and the 'Far East' and 'European' origin NDV isolates belong to the sublineage VIb, VIc and VId in agreement with the previous study. However, in the present study, all the Korean isolates belong to the new putative sublineage VIf. The putative sublineage VIf included NDV isolates from Japan, China and South Korea in the 1980s to 1990s. The chicken isolate, JP-Chiba/81, was the earliest isolate of putative sublineage VIf. The distribution feature of the genotype VI viruses in Far East was that only one sublineage existed in

South Korea, while a great diversity of sublineages existed in Japan and China.

Since 1984, several NDV outbreak cases causedby genotype VII have been reported in China, Taiwan, South Africa, Europe and Japan (Lomniczi et al., 1998; Herczeg et al., 1999, 2001; Yang et al., 1999; Ke et al., 2001 Liang et al., 2002; Yu et al., 2001; Mase et al., 2002; Aldous et al., 2003; Liu et al., 2003). The Taiwan isolates, TW/84P and TW/84C, were reported as the first genotype VII viruses (Yang et al., 1999). It was also proposed that genotype VII might have originated from the Far East similar to the proposal of Lomniczi et al.(1998). NDV isolates in sublineage VIIc in 1984 were the earliest isolates of the genotype VII. Interestingly, in the present study, one Korean NDV isolate, Kr-D/84, isolated from peafowl in the zoo in 1984, belonged to sublineage VIIc. Except Kr-D/84, no more NDV isolates belonging to genotype VII were observed in South Korea during the past 10 years, until re-emerged in 1995. These datasuggested that genotype VII virus might have been introduced into South Korea from neighboring countries through imported peafowl, but it did not spread into commercial poultry flocks within the country at that time. Genotype VII seemed to be re-introduced into South Korea through an unknown route in 1995. The sublineage VIId was composed of isolates originated from Far East (Taiwan, Japan, China and South Korea),in the middle 1990s to 2002. Among the sublineage VIId, Korean isolates were more closely related to Chinese isolates rather than Japanese and Taiwan isolates. Korean isolates of sublineage VIId were distinguishable from sublineage VIIa of European origin and sublineage VIIb of South Africa, European origin in the 1980s to 1990s.

In conclusion, the present study gave us a more clear understanding about the ND epizootics in Far East. Since the first ND outbreak in Korea in 1924, there have been five or more ND epizootics. Before this study, these five ND epizootics were believed to be caused by similar origin virus, and these seemed to have beeb circulating continuously in Korea since 1924. In the present study, however, we found that NDV genotypes hadreplaced each other serially (III, V, VI and VII) in chronological order. Furthermore, the fiveKorean ND epizootics were closely related with the ND panzootics or ND epizootics in other countries. The present study showed that the Korean genotype V isolated before 1984 was related with European ND epizootics in the 1970s, whereas the Korean genotype VI and VII isolated after 1988 were more closely related with Far East ND epizootics, especially ND epizootics in Japan, Taiwan and China. Since 1988, the genotype VI and VII of Far East origin were dominant in South Korea. That might be due to the increased trade of agricultural products including poultry among Far East Asian countries.

## 적 요


### 내장친화성 뉴캣슬병 바이러스 한국분리주의 분자역학적 분석

뉴캣슬병 바이러스 한국분리주의 유전자 염기서열과 아미노산 염기서열을 분석한 후 미국, 유럽, 일본, 대만과 중국 등지에서 보고된 뉴캣슬병 바이러스의 유전자와 비교분석을 실시하였다. 1949, 1982-1984, 1988-1997, 1995-2002년도에 분리된 뉴캣슬병 바이러스 한국분리주들은 각각 유전자형 III, V, VI, VII형에 속하는 것으로 나타났다. 이와 같은 결과는 다섯번의 한국 뉴캣슬병 유행기에 분리된 바이러스의 유전자형이 시대순으로 차례대로 III, V, VI, VII형으로 교체되어 왔음을 의미한다. 계통발생학적 분석 결과, 뉴캣슬병 바이러스 한국분리주 중 V유전자형에 속하는 바이러스는 1970년대의 유럽 유행주와 관련성이 있는 반면, 1988년 이후 분리된 VI과 VII 유전자형의 바이러스는 일본, 대만, 중국과 같은 극동아시아의 뉴캣슬병 발생과 관련성이 높은 것으로 나타났다. 따라서 최근에는 극동아시아 유래의 VI과 VII유전자형이 한국분리주의 주류를 이루고 있으며, 이는 극동아시아 국가들간의 축산물과 사람의 교역 및 교류의 증가 때문인 것으로 보인다.


## References


1. Aldous, E.W., Collins, M.S., McGoldrick, A. & Alexander, D.J. (2001). Rapid pathotyping of Newcastle disease virus (NDV) using fluoregenic probes in a PCR assay. Veterinary Micorobiology, 80, 201-212.
2. Aldous,E.W., Mynn, J.K., Banks, J. & Alexander, D.J.

(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, 239-257.

3. Alexander, D.J., Manvell, R.J., Kem, P.A., Parsons, G., Collins, M.S., Brockman, S., Russell, P.H. & Lister, S.A. (1987). Use of monoclonal antibodies in the characterizationof avian paramyxovirus type 1 (Newcastle disease virus) isolates submitted to an international reference laboratory. Avian Pathology, 16, 553-565.
4. Alexander, D.J. (1997). Newcastle disease and other avian paramyxoviridae infections. In : B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald, Y.M. Saif (Ed.), Disease of poultry 10th edn(pp.541-569). Ames: Iowa State University Press.
5. Alexander, D.J., Manvell, R.J., Lowings, J.P., Frost, K.M., Collins, M.S., Russell, P.H. & Smith, J.E. (1997). Antigenic diversity and similarities detected in avian paramyxovirus type 1 (Newcastle disease virus) isolates using monoclonal antibodies. Avian Pathology, 26, 399-418.
6. Alexander, D.J. (2001). Newcastle disease. British Poultry Science, 42, 5-22.
7. Ballagi-Pordany, B., Wehmann, E., Herczeg, J., Belak, S. & Lomniczi, B. (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.
8. Collins, M.S., Bashiruddin, J.B. & Alexander, D.J. (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.
9. Czeglédi, A., Herczeg, J., Hadjiev, G.., Doumanova, L., Wehmann, E. & Lomniczi, B. (2002). The occurrence of five major Newcastle disease virus genotypes (II, IV, V, VI and VIIb) in Bulgaria between 1959 and 1996. Epidemiology & Infection, 129, 679-688.
10. De Leeuw, O.& Peeters, B. (1999). Complete nucleotide sequence of Newcastle disease virus : evidence for the existence of a new genus within the subfamily Paramyxovirinae. Journal of General Virology, 80, 131-136.
11. Gould, A.R., Kattenbelt, J.A., Selleck, P., Hansson, E., Della-Porta, A. & Westbury, H.A. (2001).Virulent Newcastle disease in Australia: Molecular epidemiological analysis of viruses isolated prior to and during the outbreaks of 1998-2000. Virus Research, 77, 51-60.
12. Herczeg, J., Wehmann, E., Bragg, R.R., Travassos Dias, P.M., Hadjiev, G., Werner, O. & Lomniczi, B. (1999). Two novel genetic groups (VIIb and VIII) responzible for recent Newcastle disease outbreaks in Southern Africa, one (VIIb) of which reached Southern Europe. Archives of Virology, 144, 2087-2099.
13. Herczeg, J., Pascucci, S., Massi, P., Luini, M., Selli, L., Capua, I. & Lomniczi, B. (2001). A longitudinal study of velogenic Newcastle disease virus genotypes isolated in Italy between 1960 and 2000. Avian Pathology, 30, 163-168.
14. Jarecki-Black, J.C. & King, D.J. (1993). An oligonucleotide probe that distinguishes isolates of low virulence from the more pathogenic strains of Newcastle disease virus. Avian Diseases, 37, 724-730.
15. Kant, A., Koch, G., van Roozelaar, D.J., Balk, F. & ter Huurne, A. (1997).Differentiation of virulent and non-virulent strains of Newcastle disease virus within 24 hours by polymerase chain reaction. Avian Pathology, 26, 837-849.
16. Ke, G.M., Liu, H.J., Lin, M.Y., Chen, J.H., Tsai, S.S. & Chang, P.C. (2001).Molecular characterization of Newcastle disease viruses isolated from recent outbreaks in Taiwan. Journal of Virological Methods, 97, 1-11.
17. Kim, J.H. & Song, C.S. (1992).Consideration of cause of recent severe outbreaks of Newcastle disease in Korea and a brief review of virological differences, serological diagnosis and administration of a vaccine. Korean Journal of Poultry Science, 19, 65-76.
18. Konno, T., Ochi, Y. & Hashimoto, K. (1929). [New poultry disease in Korea](German). Deutsche tierärztliche Wochenschrift, 37, 515-517.
19. Krishnamurthy, S. & Samal, S.K. (1998). Nucleotide sequence of the trailer, nucleocapsid protein gene and intergenic regions of Newcastle disease virus strain Beaudette C and completion of the entire genome sequence. Journal of General Virology, 79, 2419-2424.
20. Liang, R., Cao, D.J., Li, J.Q., Chen, J., Guo, X., Zhuang, F.F. & Duan, M.X. (2002). Newcastle disease outbreaks in western China were caused by the genotypes VIIa and VIII. Veterinary Microbiology, 87, 193-203.
21. Liu, X.F., Wan, H.Q., Ni, X.X., Wu, Y.T. & Liu, W.B. (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.

22. Lomniczi, B., Wehmann, E., Herczeg, J., Ballagi-Pordany, A., Kaleta, E.F., Werner, O., Meulemans, G., Jorgensen, P.H., Mante, A.P., Gielkens, A.L.J., Capua, I. & Damoser, J. (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.
23. Marin, M.C., Villegas, Pedro, Bennett, J.D. & Seal, B.S. (1996).Virus characterization and sequence of the fusion protein gene cleavage site of recent Newcastle disease virus field isolates from the Southeastern United States and Puerto Rico. Avian diseases, 40, 382-390.
24. Mase, M., Imai, K., Sanada, Y., Sanada, N., Yuasa, N., Imada, T., Tsukamoto, K. & Yamaguchi, S. (2002). Phylogenetic analysis of Newcastle disease virus genotypes isolated in Japan. Journal of Clinical Microbiology, 40, 3826-3830.
25. Mayo, M.A. (2002a). Virus taxonomy-Houston 2002. Archives of Virology, 147(5), 1071-1076.
26. Mayo, M.A. (2002b). A summary of taxonomic changes recently approved by ICTV. Archives of Virology, 147(8), 1655-1656.
27. Nanthakumar, T., Tiwari, A.K., Kataria, R.S., Butchaiah, G., Kataria, J.M. & Goswami, P.P. (2000). Sequence analysis of the cleavage site-encoding region of the fusion protein gene of Newcastle disease viruses from India and Nepal. Avian Pathology, 29, 603-607.
28. Römer-Oberdörfer, A., Mundt, E., Mebatsion, T., Buchholz, U.J. & Mettenleiter, T.C. (1999). Generation of recombinant lentogenic Newcastle disease virus from cloned cDNA. Journal of General Virology, 80, 2987-2995.
29. Russell, P.H. & Alexander, D.J. (1983). Antigenic variation of Newcastle disease virus strains detected by monoclonal antibodies. Archives of virology, 75, 243-253.
30. Sakaguchi, T., Toyoda, T., Gotoh, B., Inocencio, N.M., Kuma, K., Miyata, T. & Nagai, Y. (1989).Newcastle disease virus evolution I. Multiple lineages defined by sequence variability of the hemagglutinin-neuraminidase gene. Virology, 169, 260-272.
31. Seal, B.S., King, D.J. & Bennett, J.D. (1995). Characterization of Newcastle disease virus isolates by reverse transcription PCR coupled to direct necleotide sequencing and development of sequence database for pathotype prediction and molecular epidemiological analysis. Journal of clinical microbiology, 33, 2624-2630.
32. Seal, B.S., King, D.J. & Bennett, J.D. (1996). Characterization of Newcastle disease virus vaccines by biological properties and sequence analysis of the hemagglutinin-neuraminidase protein gene. Vaccine, 14, 761-766.
33. Seal, B.S., King, D.J. & Meinersmann, R.J. (2000).Molecular evolution of the Newcastle disease virus matrix protein gene and phylogenetic relationships among the paramyxoviridae. Virus Research, 66, 1-11.
34. Toyoda, T., Sakaguchi, T., Hirota, H., Gotoh, B., Kuma, K., Miyata, T. & Nagai, Y. (1989). Newcastle disease virus evolution II. Lack of gene recombination in generating virulent and avirulent strains. Virology, 169, 273-282.
35. Van de peer Y. & de Wachter R. (1997). Construction of evolutionary distance trees with TREECON for4 windows: accounting for variation in nucleotide substitution rate among sites. Computer Application in the Biosciences, 13, 227-230.
36. Wehmann, E., Czeglédi, A., Werner, O., Kaleta, E.F. & Lomniczi, B. (2003a). Occurrence of genotypes IV, V, VI and VIIa in Newcastle disease outbreaks in Germany between 1939 and 1995. Avian Pathology, 32, 157-163.
37. Wehmann, E., Ujvári, D. Mazija, H., Velhner, M., Ciglar-Grozdanić, I., Savić, V. Jermolenko, G., Ćač, Ž., Prukner-Radovčić, E., Lomniczi, B. (2003b).Genetic analysis of Newcastle disease virus strains isolated in Bosnia-Herzegovina, Croatia, Slovenia and Yugoslavia, reveals the presence of only a single genotype, V, between 1979 and 2002. Veterinary Microbiology, 94, 269-281.
38. Yang, C.Y., Shieh, H.K., Lin, Y.L. & Chang, P.C. (1999). Newcastle disease virus isolated from recent outbreaks in Taiwan phylogenetically related to viruses (Genotype VII) from recent outbreaks in Western Europe. Avian Diseases, 43, 125-130.
39. Yu, L., Wang, Z., Jiang, Y., Chang, L. & Kwang, J. (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.