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Genetic and Filogenetic Characterization of some Newcastle Strains Isolated from Poultry in Albania

MARSEL BORAKAJ[1], LILJANA CARA[2], LULJETA QAFMOLLA[3], KRISTAQ BERXHOLI[3]

[1]"Aiba" Company, Durres-Albania

[2]Institute of Food Safety and Veterinary Research; Department of Virology and Bacteriology, Tirana-Albania

[3]Faculty of Veterinary Medicine, Agricultural University of Tirana, Tirana-Albania

Abstract

Abstract section. In this study, we present the molecular characterization and phylogenetic analysis of three strains of NDV, isolated from the Tirana region in Albania during the 2011-2014 years. Three strains with number 28, 29 and 31, isolated from a different farm of poultry in Tirana Region (Rural flocks), which were diagnosed clinically with the ND. The Intracerebral Pathogenicity Index in SPF bird one day old was determined by doing the proteolytic sequencing at the cleavage, and specifying the aminoacid motif at proteolytic cleavage site. More over we performed BLAST search and phylogenetic analysis of obtained RNA sequences. All strains replicated well in the SPF –chicken emryo eggs. The isolates displayed an aminoacid motif at the proteolytic cleavage site at the Fusion (F) protein with multiple basic amino acids as a well a Phenylalanine on position 117. For one isolate (28) numerous nucleotide positions had signals for at last two nucleotides, making it imposible to conclude on a specific sequence. The pathogenicity of all three isolates (28, 29 and 33), was assessed by the analysis of the F protein cleavage site and by standart ICPI. The ICPI (pathogenicity index) of our strains varies from of 1.85, 2 and 1.75, respectively which according **[19,7]** are typical for velogenic strains of NDV. We found that two NDV strain has a most close genetic relationship with the Serbia 2007 NDV, having 98% similarity at nucleotide level.Velogenic viscerotropic strains are considered endemic in our country.

Keywords: ND, ICPI, protein cleavage site, F protein, genetic relationship.

1. Introduction

According [26],free-range rural chickens (FRCs) populations dominate the poultry industry in developing countries. The management of FRCs, allows interactions between chickens and ducks, pigeons, wild birds and other animals. This predisposes chickens to multi-host infections, including Newcastle disease virus (NDV). Moreover, FRCs flocks and populations are composed of multi-age chickens, which are usually not vaccinated. Over 250 species of birds are reported to be susceptible to NDV infection **[3]**. Newcastle disease (ND) is a highly contagious and widespread disease, which causes severe economic losses in domestic poultry, especially in chickens **[2,29]**. The World Organization for Animal Health (OIE) lists it as a notifiable disease and imposes restrictions and trade embargoeson countries and areas where outbreaks occur **[22]**. The causative agent of the disease is Newcastle disease virus(NDV) or avian paramyxovirus serotype 1 (APMV-1), which belongs to the genus Avulavirus within the subfamily Paramyxovirinae and family Paramyxoviridae **[17]**.The NDV genome comprises a single-stranded, negative-sense RNA genome of ~ 15,200 nucleotides (nt) that contains six genes, which encode seven proteins [32], the matrix (M gene), the fusion (F gene), the haemagglutinin–neuraminidase (HN gene) and the RNA-dependent RNA polymerase (L gene) proteins etc **[28,5]**. Based on the analysis of the nucleotide sequence of the F gene, 19 different genotypes of NDV have been identified and classified into two classes. Class I, genotype I and class II, I–XVIII genotypes. Class II virus are typically found circulating within wild-bird and poultry species and have been divided into 18 genotypes (I–XVIII), with genotypes V–VIII being the predominant genotypes circulating in the world **[21,10,11,8,30]**.Class II viruses were responsible for all the four panzootics of ND from the 1920s to the present **[20]**.Since ND was

*Corresponding author: Marsel Borakaj; E-mail: m_borakaj@yahoo.com
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first described in 1926, three worldwide panzootics have occurred. The first panzootic (1926–1960) was caused by viruses belonging to genotypes II–IV, and the second (1960–1973) and the third (1970–1980) by genotypes V and VI **[4]** Virulent NDV (vNDV) strains are enzootic in several countries and have been responsible for outbreaks in at least six of the seven continents of the world **[21]**. Intracerebral pathogenicity index and F protein cleavage site motif are the two major parameters of virulence of NDVs. Newcastle disease virus strains with an ICPI below 0.7 are referred as lentogenic, above 1.5 as velogenic, and strains with intermediate ICPI values are classified as mesogenic **[1]**.Virulent NDV strains contain a polybasic F protein cleavage site with a consensus sequence of 112(R/K)-R-Q-(R/K)-R-F117, which can be recognized by ubiquitous host proteases and thus making it possible to spread systemically to produce fatal infection **[14,25,12,24]**. Virulent isolates from pigeon may have low ICPI in chicks **[9]**.

2. Materials and Methods

We have study three strains with number 28, 29 and 31, that were isolated in another farm in Tirana environs,which were diagnosed clinically with the ND during the years 2011-2014. The NDV strain isolate were propagated by inoculation in nine-day-old embryonated SPF chicken eggs. The embryos were incubated at 37 C^0 ; subsequently, a sample of allantoic fluid was extracted, clarified by centrifugation and stored at -70 C^0 until using **[31]**. The strains were characterized with the HA test. Recultivation was made at the SPF embryonated eggs in the Friedrich-Loefler-Institute (Insel Riems). Determination of Intracerebral Pathogenicity Index (ICPI) is carry out at the one day old SPF chicken.. Phylogenetic analysis was performed using the MEGA5 software (MEGA, version 5.2.2) **[34]**. The evolutionary history was inferred by the Maximum Likelihood method based on the Tamura-Nei model **[33]**, Estimation of the evolutionary analyses strains of NDV wer conducted method in MEGA5. The ICPI, Phylogenetic analysis and patho typing was carry out at the National Reference Laboratory for OIE and FAO and National Reference Laboratory for the Newcastle Disease (ND) at the Institute Diagnostic Virology, Friedrich-Loefler Institute- Insel Riems-Germany.

3. Results and Discussion

All data are presented at the Table 1 and 2, and diagram as following:

Table 1. Homology and identity of the three isolates of NDV from Tirana environs

Nr of strains	**Isolated from:**	**F-cleavage site**	**Homologous Virus (NCBI-BLAST)**	**IDENTITY**
28	Tirana environs	116-RRQKR*?-117	Dpuble infection, phylogeny not possible	Not possible
29	Tirana environs	116-RRQKR*F-117	NDV SERBIA/749/2007	445/455 (98%)
33	Tirana environs	116-RRQKR*F-117	NDV SERBIA/749/2007	442/452 (98%)

Table 2. Genotype and pathotye of three isolated from Tirana environs

Nr. of strains	Isolated from:	Genotype	ICPI	Diagnose
28	Tirana environs	?	1.85	NDV: Velogen
29	Tirana environs	2 VII	2	NDV: Velogen
33	Tirana environs	2 VII	1.75	NDV: Velogen

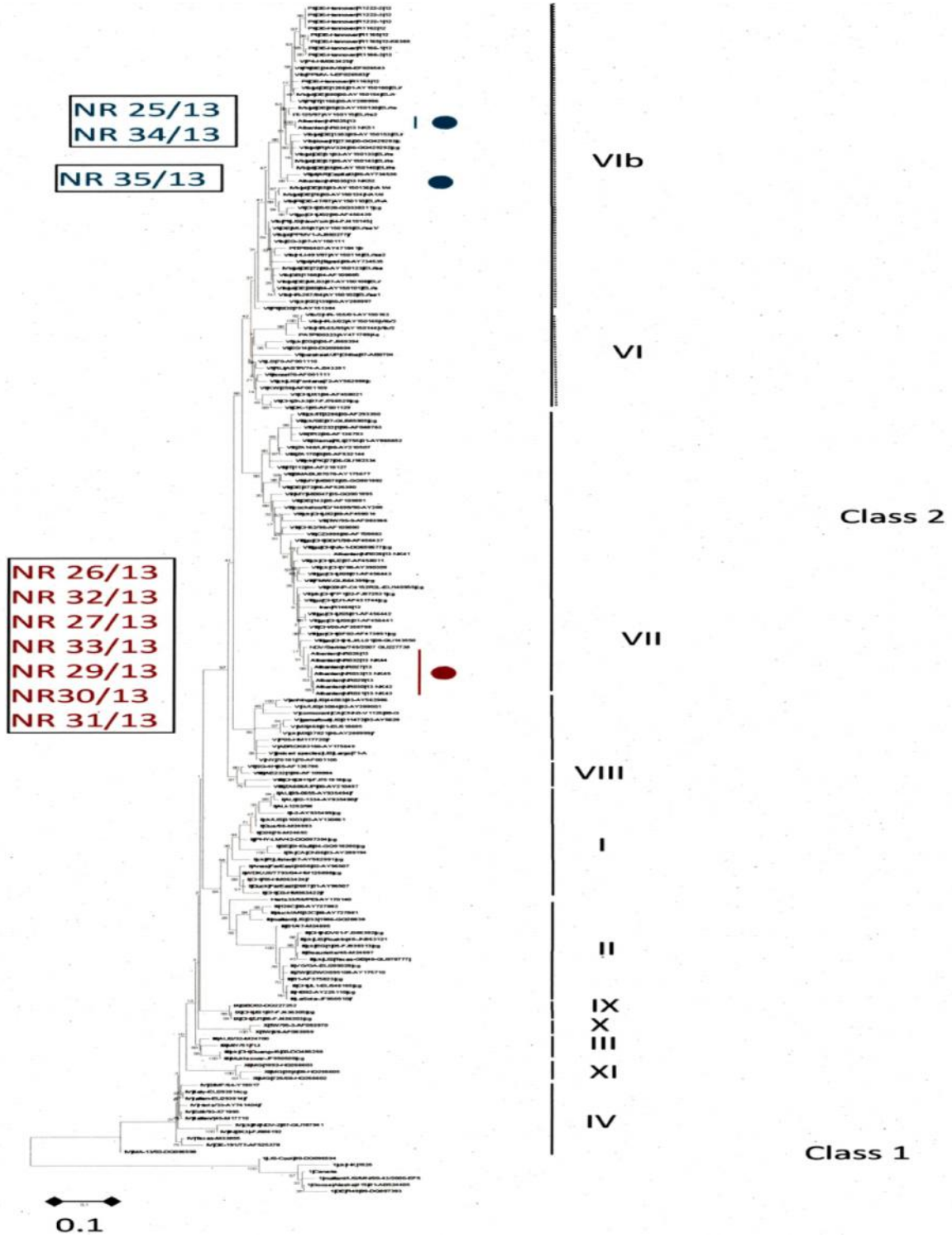

Figure 1. Molecular phylogenetic analysis by Maximum Likelihood method(MLm).

The evolutionary history was inferred by using MLm on the Tamura-Nei model.. The our strain position on the tree is shown in a red diamond and the virulent strains (vNDV) are highlighted with an asteristic ( ) The evolutionary history was inferred by using the Maximum Likelihood method based on **[33]** model. The bootstrap consensus tree inferred from 100 replicates **[13]** is taken to represent the evolutionary history of the taxa analyzed.**[13]** . Initial tree for the heuristic search were obtained automatically as follow. When the number of common site was < 100 or less one fourth of the total number of site, the maximum parsimony method was used; otherwise BIONJ method with Maximum Composite Likelihood (MCL) distance matrix was used. The tree is drawn to scale , with branch lengths measured in the number of substitutions per site. The analysis involved 179 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were total of 341 positions in the

final dataset. Evolutionary analyses were conducted in MEGA5 **[34]**

As a result of the study done in SPF 1 day old birds from Tirana district, we evaluate the citopathogenicity in these birds and also we realise the proteolitic sequencing of the cleavage site (sequencing proteolytic cleavage site), and was made the deduction of amino acid motif at proteolytic cleavage site. More over performed BLAST search and phylogenetic analysis of obtained RNA sequences. All strains replicated well in the SPF – chicken emryo eggs. The isolates displayed an aminoacid motif at the proteolitic cleavage site at the Fusion (F) protein with multiple basic amino acids as a well a Phenylalanine on position 117. For one isolate (28) numerous nucleotide positions had signals for at last two nucleotides, making it imposible to conclude on a specific sequence. For this isolate (28), no definite aminoacid sequence could be deduced. This high proportion of multiple amino acids and the presence of a phenylalanine at position 117 (^{116}RRQKRF117F^{117}) are characteristic of highly virulent strains, as previous described in other vNDV strains **[18,25,23,15,16]**. Motif sequence 116R-R-Q-K-R;F117, is the major determinant of virulence for NDV strains **[27]**. The pathogenicyti of all three isolates (28, 29 and 33) **(see table 2)**, was assessed by the analysis of the F protein cleavage site and by standart ICPI. The ICPI (pathogenicity index) of our strains varies from of 1.85, 2 and 1.75, respectively which according **[19,7]** are typical for velogenic strains of NDV too.

We found that two NDV strain has a most close genetic relationship with the Serbia/2007/ NDV, having 98% similarity at nucleotide level **(Table 1)**. Those velogenic viscerotropic strains are considered endemic in our country. Based on genothype analysis, all strains formed a specific cluster within cllas II, genotype VII viruses **(Fig-diagram 1)**. According **[20]** , class II viruses were responsible for all the four panzootics of ND from the 1920s to the present. We think that also that epizootis that happened in our country from 1953 year till now-days, must have been caused from the strains of this group.

4. Conclusions

From the above mentioned data we can draw conclusions that in our country are circulating strains with high pathogenic qualities and this has been confermed not only by evaluating the pathogenicity index in SPF birds aged 1 day, but also with genitico-molecular studies, by clearly identifying the aminoacide positions (ex.fenilalanin). This positioning of aminoacides is characteristic for strains of high pathogenicity. Based on these data we can draw conclusions that for the management of this infection in our country, we should use vaccines with high antigenic qualities.

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