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Virulence in Newcastle disease virus: A genotyping and molecular evolution spectrum perspective

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ABSTRACT

In our research, the molecular evolutions of NDV F and HN genes were analyzed. The phylogenetic analyses of NDV sequences indicated that NDV could be divided into two genotypes: Class I (lentogenic strains) and Class II (velogenic or mesogenic strains). Each genotype possesses high gene homology. Furthermore, the selected pressure analysis showed that the dN/dS of velogenic, mesogenic NDV strains F gene was significantly high and the ω(dN/dS) is 1.1725 > 1. These results imply that mutations in velogenic, mesogenic NDV F gene are favored by positive natural selection and it has acted to diversify NDV virulence at the nucleotide and amino acid level. We estimated that the subsequent rapid adaptation of the Newcastle disease virus to chickens were likely dependent on a high rate of mutation and the positive selection of mutations in the major F gene.

1. Introduction

Newcastle disease (ND) is one of the most severe avian diseases and usually causes economic catastrophe in poultry industry in the world (Zhu et al., 2010). Newcastle disease virus (NDV) is the member of Avulavirus genus in the Paramyxoviridae family (Yue et al., 2009). The paramyxoviruses have been classified into twelve subtypes that designated APMV-1 to APMV-12 (Terregino et al., 2013), and NDV belongs to APMV-1 subtypes (Alexander, 2000). The NDV has been continually evolving and caused genetic diversity in the world (Ebrahimi et al., 2012), since the ND first outbreak in 1926. NDV contains six main structure proteins: nucleocapsidprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin–neuraminidase (HN), and largeprotein (L) (De and Peeters, 1999; Mayo, 2002; Czeglédi et al., 2006). The envelope of NDV is comprised of two interactive surface glycoproteins, the HN and the F proteins. The two important genes make vital sense in virus infection process and determinant of NDV virulence (Zhu et al., 2010). It has been identified that the potential pathogenicity of NDV is related to the F_0 cleavage site motif (residues112–117) of the NDV F protein ('standard' method) (de Leeuw et al., 2003). In our study, we consulted the Genbank information and referenced the paper of selected strains to identify the genotype of used sequences. The phylogenetic method was used to analyze the molecular evolution process of NDV.

Recent studies state that evolution of viruses primary related to the evolution of functional proteins in the gene duplication process (Nei et al., 2008). The most extensively referred model for the protein function evolution supposed that the selectively neutral mutations provide opportunities to the protein to get a new function, adventitiously (Horvath, 2005). A variety of methods about detecting a high rate of evolution, relative to selectively neutral sites (Nielsen, 2005) claim that selection is revealed by comparing the patterns of synonymous and non-synonymous (amino acid-altering) nucleotide substitution. If the number of synonymous substitutions per site (dS) exceeds the number of non-synonymous substitutions per site (dN), in another word, dN/dS < 1, this is so-called "purifying" selection. Another opposite pattern is natural selection, which is the major factor of favoring amino acid changes (Kryazhimskiy and Plotkin, 2008). In this pattern, the number of non-synonymous substitutions per site (dN) exceeds the number of synonymous substitutions per site (dS), that is to say, dN/dS > 1. The strong positive selection usually led to an accumulation of additional beneficial mutations, resulting in the emergence of the highly successful virus variants. Another factor of functional protein evolution is a priori to these episodes of positive selection locations on the phylogenetic tree (Travers et al., 2005) or the pattern of substitutions on the tree (Guindon et al., 2004).

Currently, the existence of NDV in world chicken population has been widely reported, and several genotyping and genetic diversity studies have been studied on NDV based on published complete genome sequences (Courtney et al., 2013; Fernandes et al., 2014), but no

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extensive molecular evolution studies have been conducted on NDV. Furthermore, although there is a consensus that NDV can be divided into two major genotypes, no research on relationship about genotyping, evolution and virulence based on F and the HN genes. Our data provide important new information to study NDV virulence based on F gene evolution.

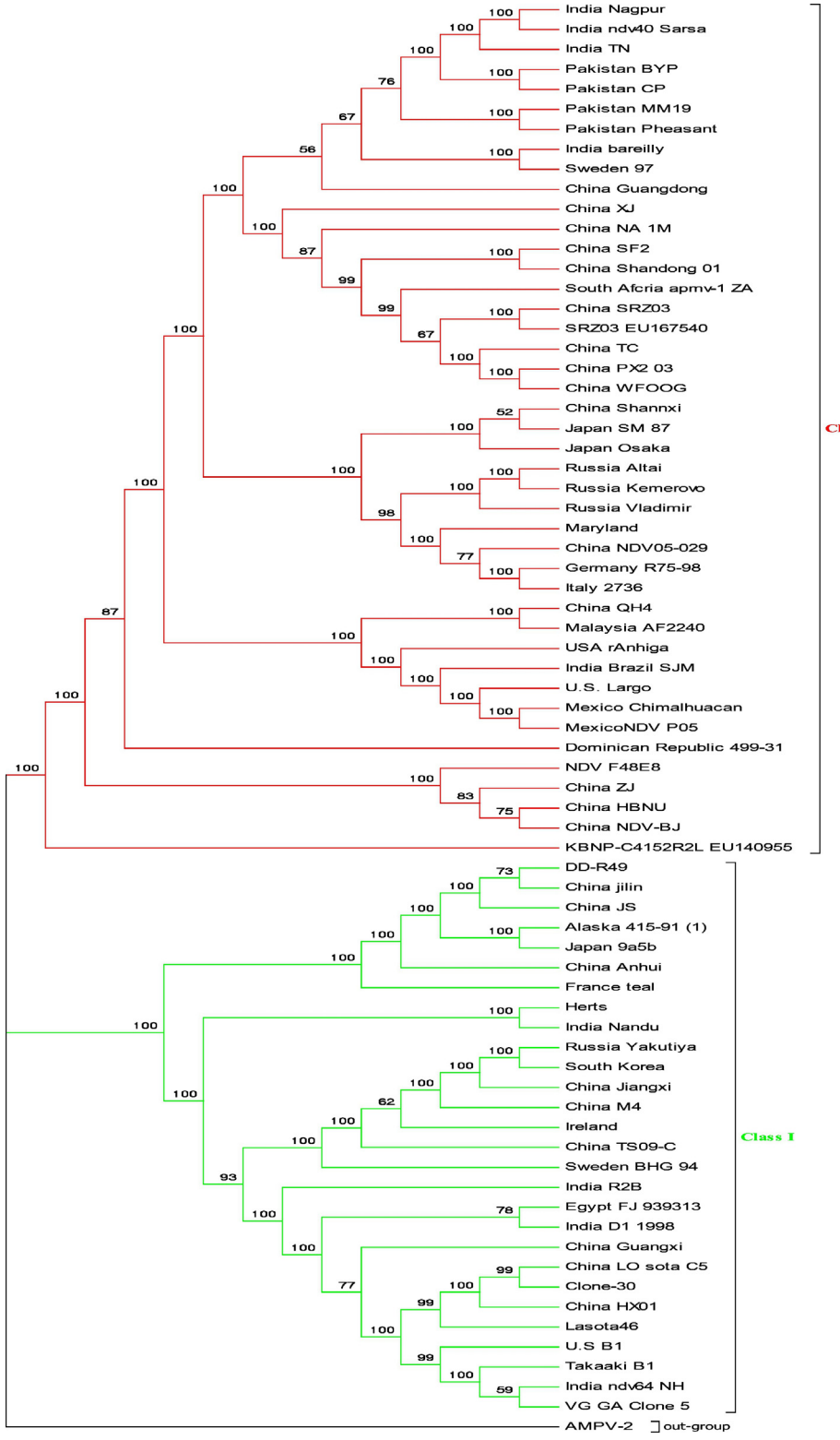

Fig. 1. Evolutive relationships among NDV groups. A: Phylogenetic tree based on the nucleotide sequences of complete genome and the NJ method for the 72 NDV sequences. Numbers along the branches refer to the percentages of confidence in the NJ analyses by using MEGA6. Only bootstrap support values of >50% are indicated. The regions of red color indicated the velogen or mesogen isolates, the region of green color indicated the lentogenic NDV strains. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)