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Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features

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ABSTRACT

Virulent Newcastle disease virus (NDV) isolates from new sub-genotypes within genotype VII are rapidly spreading through Asia and the Middle East causing outbreaks of Newcastle disease (ND) characterized by significant illness and mortality in poultry, suggesting the existence of a fifth panzootic. These viruses, which belong to the new sub-genotypes VIIh and VIIi, have epizootic characteristics and do not appear to have originated directly from other genotype VII NDV isolates that are currently circulating elsewhere, but are related to the present and past Indonesian NDV viruses isolated from wild birds since the 80s. Viruses from sub-genotype VIIh were isolated in Indonesia (2009–2010), Malaysia (2011), China (2011), and Cambodia (2011–2012) and are closely related to the Indonesian NDV isolated in 2007, APMV1/Chicken/Karangasem, Indonesia (Bali-01)/2007. Since 2011 and during 2012 highly related NDV isolates from sub-genotype VIIi have been isolated from poultry production facilities and occasionally from pet birds, throughout Indonesia, Pakistan and Israel. In Pakistan, the viruses of sub-genotype VIIi have replaced NDV isolates of genotype XIII, which were commonly isolated in 2009–2011, and they have become the predominant sub-genotype causing ND outbreaks since 2012. In a similar fashion, the numbers of viruses of sub-genotype VIIi isolated in Israel increased in 2012, and isolates from this sub-genotype are now found more frequently than viruses from the previously predominant sub-genotypes VIId and VIIb, from 2009 to 2012. All NDV isolates of sub-genotype VIIi are approximately 99% identical to each other and are more closely related to Indonesian viruses isolated from 1983 through 1990 than to those of genotype VII, still circulating in the region. Similarly, in addition to the Pakistani NDV isolates of the original genotype XIII (now called sub-genotype XIIIa), there is an additional sub-genotype (XIIIb) that was initially detected in India and Iran. This sub-genotype also appears to have as an ancestor a NDV strain from an Indian cockatoo isolated in1982. These data suggest the existence of a new panzootic composed of viruses of subgenotype VIIi and support our previous findings of co-evolution of multiple virulent NDV genotypes in unknown reservoirs, e.g. as recorded with the virulent NDV identified in Dominican Republic in 2008. The co-evolution of at least three different sub-genotypes reported here and the apparent close relationship of some of those genotypes from ND viruses isolated from wild birds, suggests that identifying wild life reservoirs may help predict new panzootics.

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1. Introduction

Newcastle disease virus (NDV) is distributed worldwide and its continual presence in multiple avian species presents a constant threat to all poultry industries and other activities that involve the raising or keeping of birds (Anonymous, 2011). The etiological agent of ND, virulent NDV, belongs to the genus Avulavirus of the family Paramyxoviridae (Mayo, 2002). The virus was originally detected in Java, Indonesia and Newcastle-'on-Tyne, England (Doyle, 1927), and since then various genotypes have been responsible for different ND panzootics. The virus is enveloped, with a single-stranded, non-segmented, negative sense RNA genome.

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Multiple genotypes of NDV have been circulating worldwide (Miller et al., 2010). NDV isolates may be classified into genotypes based on either the complete genome sequences or the full fusion protein sequences from NDV isolates (Diel et al., 2012a). At this time, ND viruses are grouped into one genotype for class I NDV isolates, and in eighteen genotypes for class II NDV isolates, some with sub-genotypes (Courtney et al., 2013; de Almeida et al., 2013; Diel et al., 2012a; Snoeck et al., 2013). The 2012 a classification system of NDV was proposed based on the utilization of the complete sequence of the fusion (F) protein gene (Diel et al., 2012a). The system was based on the mean inter-population evolutionary distance between previous existing NDV genetic groups, and differences of 10% (at the nucleotide level) were proposed as the cutoff value to assign new genotypes. This system grouped NDV isolates of class I into a single genotype comprised of mainly viruses that have been isolated from waterfowl and shorebirds, and occasionally from samples collected in live bird markets worldwide and captured wild birds (Kim et al., 2007a,b; Miller et al., 2010). Class II viruses were initially grouped into 15 genotypes; however, four additional genotypes have been added since 2012 (Courtney et al., 2013; Diel et al., 2012a; Snoeck et al., 2013).

Viruses from class II are present in both wild bird and poultry species; however, most virulent NDV (vNDV) isolates are obtained from poultry and are responsible for significant economic losses to the poultry industry worldwide (Dundon et al., 2012). Viruses of genotypes II, III and IV of class II were responsible for the first panzootic during 1920s to 1960s (Alexander, 2001), whereas the second panzootic in Europe during the late 1960s was resulted from isolates of genotype V (Lomniczi et al., 1998). Viruses from genotypes III, IV, IX and X are related to those of genotypes of I and II, but only circulate in limited areas of the world. Viruses of sub-genotype VIb originated in the Middle East and were responsible for the third panzootic in pigeons during the 1980s (Kaleta et al., 1985). Genotypes VII and VIII were responsible for ND outbreaks in Asia, including Pakistan, and in Europe since 1984 or earlier (Diel et al., 2012a; Shabbir et al., 2013). Viruses from genotype VII are responsible for the fourth panzootic, which continues today, having spread from Asia, Africa, Europe and has even been isolated in South America (Miller and Koch, 2013; Perozo et al., 2012). The fourth panzootic of ND began around 1985 in Southeast Asia and spread to most countries of Africa and in Venezuela, South America (Herczeg et al., 1999; Perozo et al., 2012; Yu et al., 2001). Genotypes V, VI, VII, VIII and XI emerged after 1960's and are considered "late" genotypes (Czegledi et al., 2006) and only contain vNDV strains. Currently, viruses from genotype VII are most frequently associated with outbreaks of ND in the Middle East (Radwan et al., 2013), and Asia (Yi et al., 2011). These viruses are of particular concern as some have demonstrated higher mortality in vaccinated poultry (Yi et al., 2011), while others may have expanded their host range and are now able to cause disease in geese (Wang et al., 2012). In Israel the first case of genotype VII NDV was reported in 2000 (data not published). Genotype XIV contains vNDV isolates obtained in West and Central Africa between 2006 and 2008 (Snoeck et al., 2013), which are divided into three sub-genotypes XIVa, b and c (de Almeida et al., 2013). Genotype XV (Diel et al., 2012a) comprises isolates obtained from chickens and geese in China, which have been previously classified into sub-genotype VIId (isolates XJ-2/97 and FJ-2/99) or VIIe (isolate JX-2/99) (Liu et al., 2003).

The emergence and spread of new genotypes across the world represents a significant threat to poultry and suggest that vNDV is continuously evolving, leading to more diversity (Miller et al., 2009). However, little has been done to understand the mechanisms of maintenance and evolution of new genotypes (Alexander et al., 2012). Here we have characterized recent vNDV isolates and present evidence that suggest the emergence of a fifth panzootic constituted by highly related vNDV isolates from Indonesia, Israel and Pakistan. These virus strains belong to a new vNDV sub-genotype (VIIi), and together with the existence of additional sub-genotypes (VIIh and XIIIa and XIIIb) related to older strains from wild birds suggest that unknown reservoirs harbor new vNDV isolates capable of additional panzootics.

2. Material and methods

2.1. Isolation of NDV virulent viruses

All laboratories followed the same protocol to isolate NDV strains except that chickens used to produce the 9–11 day old embryonating chicken eggs needed for virus isolation were specific pathogen free (SPF) for those isolated in Israel and The United States of America (USA) and were free of NDV antibodies for those isolated in Pakistan and Indonesia (Alexander and Swayne, 1998). NDV strains cockatoo/Indonesia/87-36724-524/1988; lory/Indonesia/88-08989-523/1988 and parrot/Indonesia/C300 (19625)-520/1976 were obtained and propagated in SPF embryos from the repository of the United States Department of Agriculture (USDA) Southeast Poultry Research Laboratory (SEPRL) (Alexander and Swayne, 1998). These three historical samples were obtained during the importation and quarantine of exotic birds into the USA and it is presumed that the birds were infected at their origin rather than during the transport process. Pakistani isolates were obtained from swabs samples from poultry obtained from 16 outbreaks in different regions of the country during the winter of October 2011 through March 2012 and propagated in embryonating chicken eggs that were free from antibodies against NDV. Representative samples of each outbreak were characterized by sequencing of the full fusion (F) protein. Israeli isolates consisted of a total of 33 diagnostic swab samples obtained from dry cloacal and tracheal swabs from poultry and pet birds that were sent to the Kimron Veterinary Institute (KVI) of Israel for evaluation. All data regarding the origins of the samples, and the health and vaccination status of the birds sampled were documented. Swabs were maintained in −20 °C until processing. 300 μl of phosphate buffer solution (PBS) were added to each swab and incubated at room temperature for 30 min before extraction of RNA. Virus isolation was carried out by inoculation of embryonating SPF chicken eggs that were 11-days old, incubated at 37 °C, and monitored for 6 days (Senne, 1998). Intracerebral pathogenicity index (ICPI) assays were conducted on hemagglutination (HA) positive allantoic fluids (Alexander and Swayne, 1998) following established procedures (OIE, 2012). Fourteen Indonesia isolates were either obtained from samples from the repository at the Faculty of Veterinary Medicine, Bogor Agricultural University (IPB), or isolated from diagnostic swab samples collected from field visits to live bird markets and poultry handling facilities. Samples from commercial poultry farms were from producers willing to participate in the study. While the study area included the islands of Sumatra, Kalimantan (Indonesian Borneo), Java, Bali, and Nusa Tenggara (The Lesser Sunda Islands) representing the western and central areas of Indonesia that includes the top six most populated urban areas, NDV strains were only able to be isolated from the samples from the island of Java. Oropharyngeal and cloacal swabs were collected from all birds, and organ tissue samples from dead birds. Sample collection information for each bird was included when possible: date of sampling, host species (poultry species and breed if known), estimated age (breeder, layer, broiler, chicks), environment description (type of facilities), and the geographic location (name of city and province or GPS coordinates). The GPS coordinates, flock size data, and percent mortality were not available for the Indonesian samples.

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