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Characterisation of a type 1 Avian Paramyxovirus belonging to a divergent group

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ABSTRACT

Newcastle disease, induced by a type 1 Avian Paramyxovirus (APMV-1), is one of the most serious poultry diseases. APMV-1 are divided into two classes based on genetic analysis: class II strains have been recovered from wild or domestic birds and include virulent and avirulent isolates whereas class I strains have been mainly isolated from wild birds and are avirulent. Within class I, a new proposed genotype has recently been reported. The only full genome strain of this group is presently characterised from the point of view of codon usage with reference to class I and class II specificities. Class-specific residues were identified on HN and F proteins that are the two major proteins involved in cell attachment and pathogenicity. Comparison of protein patterns and codon usage for this newly identified APMV-1 strain indicates it is similar to class I viruses but contains a few characteristics close to the viruses of class II. Transmission of viruses from this recently identified divergent group from wild birds to domestic birds could have a major impact on the domestic poultry industry.

1. Introduction

Newcastle disease, induced by a type 1 Avian Paramyxovirus (APMV-1), is one of the most serious avian diseases and causes severe economic losses in the poultry production. APMV-1 viruses belong to the *Avulavirus* genus, *Paramyxoviridae* family and *Mononegavirales* order and possess a negative single-stranded RNA genome coding for 6 major structural proteins: nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutinin–neuraminidase (HN), and an RNA-dependant RNA-polymerase (L) (de Leeuw and Peeters, 1999). APMV-1 viruses are divided into two classes based on genetic analysis. Class II strains have been recovered from wild and domestic birds, include virulent and avirulent isolates and are divided into various genotypes (Czegledi et al., 2006; Diel et al., 2012; Maminiaina et al., 2010; Miller et al., 2009). Class I strains have mainly been isolated from wild birds and are avirulent (except for one isolate (Alexander et al., 1992)). Class I viruses were divided into nine genotypes (Kim et al., 2007). These were however condensed into a single genotype in a recent study (Diel et al., 2012), but since then, divergent virus sequences were reported in Finland and in France (Briand et al., 2013; Lindh et al., 2012). APMV-1 are also classified into three main pathotypes depending on the pathogenicity induced by the isolate in chickens. Viruses from the lentogenic pathotype usually do not cause disease in adult poultry and are defined as avirulent in a regulatory context.

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Viruses from the mesogenic pathotype induce moderate virulence and cause respiratory disease. The most virulent viruses that cause high mortality are classified into the velogenic pathotype (Alexander, 2000). APMV-1 had two envelope glycoproteins: Fusion (F) protein and haemagglutinine–neuramidase (HN) protein. F protein is a type I glycoprotein which is 553 amino acids long and composed of a C-terminal transmembrane domain and four heptad repeat motifs (Morrison, 2003). The activation of paramyxovirus F proteins requires cleavage of F0 into F1 and F2. The motif of the cleavage site is principally correlated with the pathogenicity of APMV-1. In addition, F mediates fusion of the viral envelope with the cellular plasma membrane (Nagai et al., 1989) and plays a major role in viral immunogenicity (Morrison, 2003; Samal et al., 2012). The second major protein implicated in viral immunogenicity corresponds to the type II transmembrane glycopreotein HN that is from 571 to 616 amino acids long depending on the strain. The HN N-terminal transmembrane domain is followed by a stalk region and a large C-terminal globular head domain (Lamb et al., 2006; Yuan et al., 2012). HN is involved in the virus's entry into the host cell by attachment to sialic acid-containing receptors, and also plays additional roles in the virus life cycle, including F activation and receptor-destroying (neuraminidase) activity to facilitate virus budding. It has been shown that F and HN induce protective immunity to APMV-1 in chickens due to characterised epitopes (Meulemans et al., 1988; Nishino et al., 1991; Umino et al., 1987).

The aim of this study was to better characterise viruses belonging to the newly identified divergent class I group. So, HN and F sequences of these strains were compared with those of class I and class II viruses. Protein patterns of the viruses from the two classes were identified and were then compared with the protein patterns of the virus studied here. In addition, the particular codon usage characteristics of the strain belonging to this putative new genotype were compared to those observed in class II and other class I strains.

2. Materials and methods

2.1. Isolation and pathogenicity assessment

During avian influenza surveillance in France in 2009, 1972 cloacal swabs were collected from wild birds in wetlands. One of these swabs was collected from an apparently healthy common teal trapped in the "Vigueirat" marshes in the Bouches-du-Rhône *département* in the south of France (Briand et al., 2013). Virus isolation was performed on 9-day-old specific pathogen free (SPF) eggs. The virus obtained was called teal/France/100011/2010 (referred to as "APMV1/100011" for the remainder of this paper).The intra-cerebral pathogenicity index (ICPI) was determined according to international standards approved by the ANSES/ENVA/UPEC Ethics Committee (no.11-0039-14/06/2011-4). At the same time, the mean death time (MDT) value was established. MDT values under 60 h correspond to velogenic viruses, between 60 h and 90 h correspond to mesogenic viruses and higher than 90 h correspond to lentogenic viruses. Isolation and the two techniques used to determine viral pathogenicity were conducted according to the manual of standards for diagnostic tests and vaccines (OIE, 2012).

2.2. Antigenic reactions

Positive allantoic fluid in HA assays was tested by a haemagglutination inhibition (HI) assay using a reference panel of 35 specific avian *Influenzavirus* (AIV) (H1–H15) and APMV (1–4, 6–11) sera according to international standards (OIE, 2012). This panel was completed with monoclonal pigeon-specific APMV-1 (pPMV-1) antibodies (Collins et al., 1989) and 3115-57-45b antibodies specific to many class II APMV-1 except pPMV1 (Jestin et al., 1989).

2.3. Phylogenetic analyses

For phylogenetic analysis, all the APMV-1 genome sequences available in the Genbank database were downloaded. The complete genome sequence of APMV1/100011 (JQ013039) (Briand et al., 2013; Brown et al., 2013) studied here was aligned with 109 other APMV-1's genome sequences. Alignment was performed using MEGA 5.05 software using the ClustalW method (Kumar et al., 2008). In this analysis, 95 sequences belonging to the class II viruses and 14 sequences belonging to the class I viruses were used. Neighbor-Joining (NJ) and maximum likelihood (ML) methods with 1000 bootstrap replicates were performed using the Kimura 2-parameter nucleotide substitution model. The same phylogenetic analysis was performed independently for each of the 6 APMV-1 genes.

2.4. Analysis of HN and F protein patterns

To compare amino acid sequence specificity between class I and class II viruses, all available amino acid sequences of APMV-1's HN and F proteins were downloaded from the Genbank database and aligned using the clustalW algorithm from MEGA 5.05 (Kumar et al., 2008). For each protein, two sets of viruses were constituted, the first set corresponding to aligned amino acid sequences of class I viruses ($n = 114$ for F and $n = 58$ for HN) and the second set was made up of the aligned class II sequences ($n = 604$ for F and $n = 558$ for HN). For both sets, the frequency of each amino acid was determined for every position. The means of the absolute values (ABS) of the difference between the frequencies of the two sets was calculated (indice P_k) according to the following equation:

$$P_k = \frac{\left(\sum_{j=1,20} ABS(f1_{jk} - f2_{jk})\right)}{2}$$

where f_{jk} corresponds to the frequency for an amino acid j at sequence position k, and $f1$ and $f2$ correspond to the frequency of class I and class II sets respectively. For a given amino acid position, a value of 0 for P_k indicated no difference between the frequencies of the two sets of sequences (i.e. same amino acids with same frequencies), whereas a value of 100 indicated no common amino acids between class I and class II. To be more stringent and to