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Avian metapneumovirus (AMPV) attachment protein involvement in probable virus evolution concurrent with mass live vaccine introduction

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ABSTRACT

Avian metapneumoviruses detected in Northern Italy between 1987 and 2007 were sequenced in their fusion (F) and attachment (G) genes together with the same genes from isolates collected throughout western European prior to 1994. Fusion protein genes sequences were highly conserved while G protein sequences showed much greater heterogeneity. Phylogenetic studies based on both genes clearly showed that later Italian viruses were significantly different to all earlier virus detections, including early detections from Italy. Furthermore a serine residue in the G proteins and lysine residue in the fusion protein were exclusive to Italian viruses, indicating that later viruses probably arose within the country and the notion that these later viruses evolved from earlier Italian progenitors cannot be discounted. Biocomputing analysis applied to F and G proteins of later Italian viruses predicted that only G contained altered T cell epitopes. It appears likely that Italian field viruses evolved in response to selection pressure from vaccine induced immunity.

1. Introduction

Avian metapneumovirus (AMPV) causes an upper respiratory tract infection in turkeys, leading to turkey rhinotracheitis (TRT), and in some other avian species is involved in the etiology of multi-factorial diseases such as swollen head syndrome of chickens. In the late-1970s AMPV infection was first reported in South Africa. During the mid-1980s the disease was observed in Europe where the etiological agent was first isolated and characterized. From that time, the disease has been reported in most regions of the world and more recently in the United States (Gough and Jones, 2008).

AMPV is classified as the type species of the genus *Metapneumovirus*, family Paramyxoviridae of which 4 subtypes (A, B, C and D) have been recognized based on the nucleotide sequence differences (Juhasz and Easton, 1994; Seal, 1998; Bayon-Auboyer et al., 2000). The AMPV genome is a non-segmented, negative-stranded RNA approximately 13 kb in length containing eight genes (3′-N-P-M-F-M2-SH-G-L-5′) which encode nine proteins (Easton et al., 2004). The fusion (F) and attachment (G) proteins both occur on the virus surface and have been assumed to be responsible for virus fusion and attachment, respectively.

When comparing between the subtypes, the F protein genes sequenced to date have been shown to be more highly conserved than the G protein (Lwamba et al., 2005; Shin et al., 2005). Within each subtype, the G protein has been found to be more variable than F and this has made it useful for some

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previous epidemiological studies (Juhasz and Easton, 1994; Bayon-Auboyer et al., 1999; Cavanagh et al., 1999; Alvarez et al., 2003; Banet-Noach et al., 2005; Chacon et al., 2007; De Graaf et al., 2008; Owoade et al., 2008). The G protein has also been shown to be superfluous to virus viability in cell culture (Naylor et al., 2004) and to some degree in turkeys (Ling et al., 2008). As it may be unnecessary for attachment, it is possible that the G protein has an additional role. Whatever its role, its continued presence makes it a potential target for a host immune response. This continued presence on the viral surface is therefore likely to inevitably subject the virus to immune interaction, as has been demonstrated for subtype C AMPV (De Graaf et al., 2008).

The vast majority of AMPVs detected in Italy have proved to be of subtype B (Catelli et al., 2004). The first G sequence study in 1994 (Juhasz and Easton, 1994) showed that genes from Italy, Hungary and Spain were very similar and a further study of a UK virus in 1994 confirmed this similarity (Cavanagh et al., 1999). No later complete subtype B European G gene sequences have been reported.

The current study investigates the genetic relatedness of F and G genes of subtype B AMPVs. The study focuses on viruses in circulation in the Veneto region of northern Italy from 1987 to 2007. The area has an unusually high density of poultry farms and the majority of these have been operated by a single commercial company. Disease typical of AMPV infection started to become a problem in turkeys in the 1980s and this led to the use of a live subtype B vaccine in an experimental manner on perceived problem farms. The vaccine adopted was the only subtype B live vaccine available for turkeys at that time and this remained the case for the duration of the investigation. During the 1990s a range of vaccine doses were applied to turkeys by either eye drop or spray so as to determine best practise for the particular environment. In late 2001, a single 1-day-old live spray vaccination became the standard for all meat turkeys in the region.

The study determines and compares the sequences of the G and F proteins present in viruses over the period and in discussing the findings, considers possible effects of live vaccination. It includes analysis of 2 Italian AMPVs collected at two time periods which in an earlier study had been shown to cause different levels of disease in the face of immunity induced by the subtype B vaccine (Catelli et al., 2010). Comparisons were achieved using the neighbour-joining approach to distance-based phylogeny reconstruction.

Computational analysis was applied to F and G protein sequences of selected strains to predict impact of observed mutations on the protein properties of viruses isolated in different years. In addition the vaccine commonly used in Italy was included in the analysis. In particular, secondary structure, order–disordered regions, O-glycosylation and amphipathic regions of proteins were predicted using suitable software.

2. DNA sequence and phylogenetic analysis

2.1. Material and methods

2.1.1. Viruses

Fourteen AMPVs collected in Europe between 1986 and 2007 and characterized as subtype B, were included in the study. Eight strains were isolated between 1986 and 1994 (strains pre-1994) and six between 2001 and 2007 (strains post-2000). All the strains of the last group were isolated in the Veneto region of Italy. The commercial subtype B vaccine widely used in Italy was also included in the study. Country, species of origin and year of collection of AMPVs analyzed are reported in Table 1.

Most AMPVs were isolated in tracheal organ cultures then given a maximum of 2 further passages. In one case (strain IT/Ck/1348-01/07) the virus was characterized using RNA extracted from dry swabs.

2.1.2. RNA extraction, reverse transcription-polymerase chain reaction

RNA was extracted using a commercial kit (QIAamp viral mini kit, Qiagen).

Sequencing of each F and G gene was performed following one reverse transcription and 3 overlapping

Table 1
Year of detection, country and species of origin of the AMPV strains analyzed.

AMPV strains	Year of detection	Country of isolation	Species	Reference—source
Strains pre-1994				
Vaccine B subtype[a]	1986[b]	France	Turkey	Eterradossi et al. (1995)
France 38/86	1986	France	Turkey	Naylor et al. (1998)
IT/Ty/Vr240/87[a]	1987	Italy	Turkey	Sperati Ruffoni (Laboratory "Tre Valli")
NL 6726/90	1990	Holland	Turkey	Cook et al. (1995)
Italy 16-91	1991	Italy	Turkey	Naylor (University of Liverpool)
France 147	Pre-1994	France	Turkey	Naylor (University of Liverpool)
Spain 149	Pre-1994	France	Turkey	Naylor (University of Liverpool)
UK/8/94	1994	United Kingdom	Turkey	Naylor et al. (1998)
UK/11/94	1994	United Kingdom	Chicken	Cook et al. (1995)
Strains post-2000				
IT/Ty/2a/01	2001	Italy	Turkey	Catelli et al. (2004)
IT/Ck/33a/02	2002	Italy	Chicken	Catelli et al. (2004)
IT/Ck/34a/02	2002	Italy	Chicken	Catelli et al. (2004)
IT/Ty/129-18/04	2004	Italy	Turkey	Catelli et al. (2010)
IT/Ty/205-16/04[a]	2004	Italy	Turkey	Catelli et al. (2010)
IT/Ck/1348-01/07	2007	Italy	Chicken	Catelli (University of Bologna)

[a] F and G genes of these strains were examined by computational analysis.
[b] Year, country of isolation and species are referred to the progenitor strain.