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Comparison of the full-length genome sequence of *Avian metapneumovirus* subtype C with other paramyxoviruses

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Abstract

We determined the nucleotide (nt) sequence of the small hydrophobic (SH), attachment glycoprotein (G), and RNA polymerase (L) genes, plus the leader and trailer regions of the Colorado strain of *Avian metapneumovirus* subtype C (aMPV/C) in order to complete the genome sequencing. The complete genome comprised of 13,134 nucleotides, with a 40 nt leader at its 3′ end and a 45 nt trailer at its 5′ end. The aMPV/C L gene was the largest with 6173 nt and consisting of a single open reading frame encoding a 2005 amino acids (aa) protein. Comparison of the aMPV/C SH, G, and L nt and predicted aa sequences with those of Human metapneumoviruses (hMPV) revealed higher nt and aa sequence identities than the sequence identities between the aMPV subtypes A, B, C, and D, supporting earlier finding that aMPV/C was closer evolutionary to hMPV than the other aMPV subtypes.

Keywords: *Paramyxovirus*; *Metapneumovirus*; Subtype; Intergenic region

1. Introduction

Avian metapneumovirus (aMPV) is an enveloped, non-segmented, negative-strand RNA virus that is the primary causal agent of severe rhinotracheitis in turkeys. The virus was first isolated in 1978 in South Africa; however, major outbreaks of the disease were later reported in Europe (Anon., 1985), United States (US), and other parts of the world (Buys and Du Preez, 1980; Kleven, 1997; Cook and Cavanagh, 2002). Apart from infecting turkeys, aMPV has been associated with swollen head syndrome of chickens, aMPV antibodies detected in pheasants and aMPV RNA and infectious particles have been isolated from asymptomatic wild birds (Shin et al., 2000; Bennett et al., 2002; Welchman et al., 2002). The aMPV genome consists of eight viral genes arranged in the order: nucleocapsid–phosphoprotein–matrix–fusion–second matrix–small hydrophobic–glycoprotein–large polymerase (3′–N–P–M–F–M2–SH–G–L–5′), flanked by a leader and trailer at the 3′ and 5′ ends, respectively. The linear genomic RNA molecule is tightly encapsidated by the N protein that associates with the P and L proteins to form a helical ribonucleoprotein complex, which is the biologically functional unit common to all members of the Mononegavirales order. The ribonucleoprotein complex serves as template for transcription of mRNAs as well as genome replication (Conzelmann, 1998). Early biochemical and sequence analyses of European isolates of aMPV led to its classification into Pneumovirinae subfamily, which exhibited shared characteristics including a high sequence homology and possession of the SH and M2 genes (Cavanagh and Barrett, 1988; Yu et al., 1991, 1992a; Ling et al., 1992). However, aMPV differs from members of

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the *Pneumovirus* genus, including Human respiratory syncytial virus and Bovine respiratory syncytial virus (hRSV and bRSV) in that it lacks two non-structural proteins located upstream of the N gene (Randhawa et al., 1997; Marriot et al., 2001), and differences in gene order (Yu et al., 1992b). In addition, the SH and G genes of pneumoviruses are located upstream of the F gene, whereas they are located downstream of F gene for aMPV (Ling et al., 1992). As a result, aMPV was classified into a new genus called *Metapneumovirus* (Pringle, 1998).

The avian viruses have been classified into four subtypes, A, B, C, and D based on nucleotide (nt) and predicted amino acid (aa) sequence analyses. European viruses have 97–99% nt and predicted aa sequence identities within a subtype, whereas strains across subtypes have 56–61% nt and 33.2–38% predicted aa sequence identities within the highly variable attachment G gene (Juhasz and Easton, 1994). More detailed analyses of nucleotide sequence of N, P, M, F, and M2 revealed that these genes could also be used to classify aMPV strains into the subtypes (Seal, 1998; Shin et al., 2002). For example, US viruses classified as subtype C (aMPV/C) had over 90% nt sequence identity within the five genes, whereas comparison with subtypes A and B revealed 40–70% nt sequence identity (Shin et al., 2002). Phylogenetic analyses of subtypes A, B, and C demonstrated that A and B viruses were more closely related to each other, than either subtype A or B were to C viruses (Seal, 1998; Shin et al., 2002). Viruses isolated in France in 1985 were analyzed in 2000 and demonstrated to be different from aMPV subtypes A, B, and C based on the G gene, and subsequently classified as subtype D (aMPV/D).

The aMPV was the only recognized member of *Metapneumovirus* genus, until the isolation of *Human metapneumovirus* (hMPV) in Netherlands during 2001 (van den Hoogen et al., 2001). Subsequently, hMPV strains were isolated in Australia (Nissen et al., 2002), Canada (Peret et al., 2002), Israel (Wolf et al., 2003), Norway (Christensen et al., 2003), Thailand (Thanasugam et al., 2003), France (Bach et al., 2004), Hong Kong (Peiris et al., 2003), and Japan (Ebihara et al., 2004). Analysis of the genomic sequences from hMPV demonstrated a close resemblance to aMPV subtype C than to aMPV/A, B, or D, based on nucleotide sequence comparisons (van den Hoogen et al., 2002; Wise et al., 2004). The nucleotide sequence identity between aMPV and hMPV utilizing N, P, M, F, M2, and L ranged from 56% to 88% (van den Hoogen et al., 2002). As is typical for paramyxoviruses, the upstream five genes (N, P, M, F, and M2) of aMPV/C were easily sequenced due to the abundance of mRNA transcripts, whereas SH, G, and L genes were more difficult due to the paucity of RNA. In the present study, we completed sequencing the SH, G, and L genes, as well as the leader, trailer, and the intergenic (IG) regions, in order to obtain the full-length genome of aMPV/C (Colorado (CO) strain). Subsequently, the aMPV/C genome was compared with the other aMPV subtypes, and with other paramyxoviruses, including six newly sequenced virulent strains of Newcastle disease virus.

2. Material and methods

2.1. Virus purification and RNA isolation

The aMPV/C isolates (one Colorado isolate and 10 Minnesota (MN 1-10) isolates were propagated in Vero cells and purified as discussed previously (Shin et al., 2002; Mbiguino and Menezes, 1991; Ueba, 1978; Trapanier et al., 1983). Vero cells were cultured to 95% confluence and infected with virus. Infected Vero cells showing extensive CPE were freeze-thawed three times followed by centrifugation at 3200 $\times$ *g* for 20 min to remove cellular debris. Polyethylene glycol (50%) was added to the supernatant to a final concentration of 10% (v/v) and incubated at 4 °C for 1.5 h with gentle shaking to precipitate virus particles, and then centrifuged for 30 min. The precipitated virus pellet was diluted 1:20 with NTE buffer (150 mM Tris–HCl, 1 mM EDTA, pH 7.5) and layered onto a discontinuous 60%, 45%, and 30% sucrose gradient and centrifuged in SW-41 at 35,000 $\times$ *g* for 1.5 h at 4 °C. The layer located between the 30% and 45% interface was aspirated and diluted 1:2 with NTE followed by a final purification step on a continuous 30–60% sucrose gradient. Purified aMPV recovered was dialysed in NTE and then stored at −80 °C. Total RNA from infected Vero cells or from purified virus was isolated using QIAamp Viral RNA Mini Spin total RNA isolation kit or RNAeasy total RNA isolation kit (Qiagen, Valencia, CA) as per the manufacturer's protocol.

2.2. cDNA synthesis

Total cellular RNA from infected Vero cells was reverse-transcribed using gene specific primers at 60 °C utilizing

Table 1
Comparison of SH, G, and L proteins of aMPV/C with subtypes A, B, and hMPV

Protein	Size of aMPV/C		Percentage predicted amino acid identity				
	Nucleotides	Amino acids	Subtype C isolates	Subtypes C and A	Subtypes C and B	Subtypes A and B	Subtype C and hMPVs
Small hydrophobic (SH)	623	175	90.3–100	17.7	18.2	47.0	28.0
Attachment glycoprotein (G)	783	252	93.9–99	14.7	15.9	33.0	27.2
Large polymerase (L)	6176	2005	–	64.0	–	–	80.0

The table shows the size of the aMPV/C (Colorado) proteins SH, G, and L, and the percentage predicted amino acid identities among aMPV/C isolates, and comparison between aMPV/C and aMPV/A, B, A and B, and hMPVs. Dashes denote that the sequence has not been determined.