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Research paper

Limited evidence of intercontinental dispersal of avian paramyxovirus serotype 4 by migratory birds

Andrew B. Reeves [a,*], Rebecca L. Poulson [b], Denys Muzyka [c], Haruko Ogawa [d], Kunitoshi Imai [d], Vuong Nghia Bui [d], Jeffrey S. Hall [e], Mary Pantin-Jackwood [f], David E. Stallknecht [b], Andrew M. Ramey [a,*]

[a] US Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, Alaska 99508, USA
[b] Southeastern Cooperative Wildlife Disease Study, The University of Georgia, 589 D. W. Brooks Drive, Athens, Georgia 30602, USA
[c] National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Pushkinska Street 83, 61023, Kharkiv, Ukraine
[d] Diagnostic Center for Animal Health and Food Safety, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Inada, Obihiro, Hokkaido 080-8555, Japan
[e] US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA
[f] Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, U.S. Department of Agriculture, Agricultural Research Service, Athens, Georgia 30677, USA

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ABSTRACT

Avian paramyxovirus serotype 4 (APMV-4) is a single stranded RNA virus that has most often been isolated from waterfowl. Limited information has been reported regarding the prevalence, pathogenicity, and genetic diversity of AMPV-4. To assess the intercontinental dispersal of this viral agent, we sequenced the fusion gene of 58 APMV-4 isolates collected in the United States, Japan and the Ukraine and compared them to all available sequences on GenBank. With only a single exception the phylogenetic clades of APMV-4 sequences were monophyletic with respect to their continents of origin (North America, Asia and Europe). Thus, we detected limited evidence for recent intercontinental dispersal of APMV-4 in this study.

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1. Introduction

Evidence supports the intercontinental dispersal of bacterial, viral, and parasitic agents by migratory birds. For example, seabirds may play a role in the transhemispheric exchange of the bacteria *Borrelia garinii* (Olsen et al., 1995). Avian influenza viruses (AIV), avian paramyxovirus serotype 1 (APMV-1), eastern equine encephalitis virus, Sindbis virus, and West Nile virus may be dispersed between continents by a variety of avian taxa (Calisher et al., 1971; Lundstrom and Pfeffer, 2010; Malkinson et al., 2002; Ramey et al., 2013; Ramey et al., 2015a). Similarly, migratory birds may facilitate the intercontinental exchange of ticks, trematodes, and blood parasites (Hoogstraal et al., 1963; Levin et al., 2013; Ramey et al., 2015b; Smith and Ramey, 2015; Tallman et al., 1985; Wallmenius et al., 2014). Thus, there is a growing body of literature supporting a routine exchange of infectious agents between regions by migratory birds and the potential for identifying patterns of dispersal that may be useful to predict the spread of emerging avian pathogens.

Relative to many other viral agents infecting avian species (e.g., APMV-1 and AIV), relatively little research has been conducted regarding the epidemiology and genetics of avian paramyxovirus serotype 4 (APMV-4). APMV-4 is one of 12 serotypes of avian paramyxoviruses (Gogoi et al., 2015), all of which are classified in the genus Avulavirus, characterized as having negative-sense single-stranded RNA that upon transcription encodes for six proteins: a nucleocapsid protein (N), a phosphoprotein (P), a matrix protein (M), a fusion glycoprotein (F), a hemagglutinin-neuraminidase glycoprotein (HM), and a large polymerase protein (L) (Gogoi et al., 2015; Zohari, 2013). APMV infections primarily concentrate in epithelial cells of the avian host's respiratory and gastrointestinal tracts (Zohari, 2013). Although the full range of hosts for APMV-4 is unknown, these viruses are isolated most often from wild waterfowl hosts with infections also detected in domestic ducks, geese and occasionally in chickens (Zohari, 2013). The pathogenic impact of APMV-4 infection on avian hosts is largely unknown; however, disease has not been reported for birds from which viruses have been isolated and experimental inoculation produced no clinical signs in specific pathogen free chickens despite the presence of microscopic lesions in the trachea, lungs, and viscera (Warke et al., 2008). For APMV-1, virulence is linked to specific mutations in the F protein sequence (de Leeuw et al., 2003, 2005; Panda et al., 2004; Peeters et al., 1999), a characteristic that makes F sequences a commonly used marker in phylogenetic studies for this and other serotypes (Diel et al., 2012; Miller et al., 2009; Ramey et al., 2013). While genetic diversity of APMV-4 has been explored using field isolate F gene sequences from South Korea and Italy (Choi et al., 2013; Nayak et al., 2013), genetic information for viruses originating from North America (Parthiban et al., 2013) and Africa (Abolnik et al., 2012) has been limited to

* Corresponding authors.
E-mail addresses: areeves@usgs.gov (A.B. Reeves), aramey@usgs.gov (A.M. Ramey).

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only a single strain from each continent in the GenBank database as of the time of manuscript preparation. Consequently, information on global phylogeography and dispersal of APMV-4 between continents is limited.

In this study, we obtained genetic sequences for the complete coding region of the fusion gene of APMV-4 isolates obtained from wild birds in Japan, Ukraine, and the United States and compared them to publically available information on GenBank to assess global phylogeny and evidence for the intercontinental dispersal of this viral agent. We predicted that there would be extensive evidence for viral exchange among regions within the Old and New Worlds, with lesser but detectable evidence for interhemispheric viral dispersal between Eurasia and North America, patterns consistent with generalized migratory tendencies of wild birds (Boere and Stroud, 2006) and data for other avian infectious agents such as AIV (Krauss et al., 2007). Results of this study build upon the existing body of literature on the potential of migratory birds to disseminate infectious agents across the landscape.

2. Materials and methods

During 2005–2014, cloacal, oropharyngeal, and fecal samples were collected from migratory birds in Japan, Ukraine, and the United States as part of multiple independent AIV, and in some instances APMV, surveillance efforts. Swab samples were subjected to virus isolation in embryonated chicken eggs using standard techniques as described in Senne (2008). Twenty isolates originating from samples collected in Japan from 2008 to 2010, 13 isolates originating from samples collected in Ukraine in 2010–2014, and 25 isolates originating from samples collected in the United States in 2005–2013, were identified as possible APMV-4 strains through serological screening methods (Japan, Ukraine and 2005–2007 USA samples) or molecular (RT-PCR) testing of non-influenza hemagglutinating allantoic fluid (2008–2013 USA samples). Confirmation of APMV-4 was based on genetic sequencing of a partial fragment of the F gene prior to genetic characterization. Complete protocols and results for isolation and diagnostic tests from specific laboratories are available on request.

Viral RNA was extracted from allantoic fluid using the High Pure Viral RNA Kit (Japan samples; Roche, Indianapolis, IN), QIAamp Viral RNA Mini kit (continental United States samples; Qiagen Inc., Valencia, CA), MagMAX AI/NDV RNA extraction kit (Alaska United States samples; Ambion Inc., Austin, TX) and innuSolv RNA reagent (Ukraine samples; Analytik Jena AG, Jena, Germany). The complete coding region for the fusion gene was sequenced for all 58 APMV-4 isolates using the one-step RT-PCR kit (Qiagen Inc., Valencia, CA). Primers used and the RT-PCR conditions are provided in Table 1. Partial fusion genes were previously sequenced for two of the Ukraine (GenBank accession numbers: KF851266–KF851267; Muzyka et al., 2014) and 18 Japan (GenBank accession numbers: KT867035–KT867036, KT867037–KT867053; Bui et al., unpublished) isolates. All 20 of these isolates were further sequenced as a part of this study to complete the coding region sequences of the fusion gene. PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA) or with ExoSap-IT (USB Inc., Cleveland, OH) without additional purification prior to sequencing. Cycle sequencing was performed with identical primers used for PCR along with BigDye Terminator version 3.1 mix (Applied Biosystems, Foster City, CA). Samples were analyzed on an Applied Biosystems 3730xl automated DNA sequencer (Applied Biosystems, Foster City, CA). Sequences were assembled, edited and translated to study the amino acid motifs of the fusion gene cleavage site with Sequencher version 4.7 (Gene Codes Corp., Ann Arbor, MI). GenBank accession numbers for the complete coding region of fusion genes for APMV-4 isolates sequenced as part of this study are: KT732290–KT732345.

A phylogeny of the complete coding region of the fusion gene was constructed by comparing sequence data for these APMV-4 isolates to all available full length (1701 nts) sample sequences ($n = 11$) obtained from the GenBank database at the National Center for Biotechnology Information. GenBank acquired sequences were aligned with those generated in this study and cropped using Sequencher version 4.7 (Gene Codes Corp., Ann Arbor, MI). A Maximum Likelihood (ML) tree with 1000 bootstrap replicates was generated with MEGA version 6.0 (Tamura et al., 2013) using models that best fit the sequence data under the Akaike Information Criterion (AIC; Akaike, 1974). Strongly supported clades comprised of APMV-4 fusion gene sequences from multiple continents were interpreted as providing evidence for intercontinental viral dispersal. Conversely, a lack of evidence for the movement of APMV-4 viruses between continents was inferred if and where continental monophyly was identified in the phylogeny. The mean nucleotide (NT) and translated amino acid (AA) distances between clades were calculated in MEGA6 (Tamura et al., 2013), as the average number of base and amino acid differences per site over all sequence pairs between clades.

Table 1
Primers used in the study.

	Name	Sequence	Designer/source
A	APMV4_F_fwd	AGTTGATTGGGTGTCTAAAC	This study
B	p5490 (antisense)	CCCCACTGAGACACTTGACTAACT	Nayak et al. (2013)
C	p5340 (sense)	TCAATAGGGATGGTGGACTTTA	Nayak et al. (2013)
D	p6239 (antisense) ABR	ACCTTCCCCACCCTACCATGC	Nayak et al. (2013); modified in this study
E	APMV4_HN59-81R (antisense)	ACTTCTGTTGACTTCTCTTGGTA	Nayak et al. (2013)

Primer combinations used: A:B (5′ of F gene sequence) in conjunction with C:D and/or C:E (3′ of F gene sequence). RT-PCR Parameters: 30 min at 50 °C, followed by 15 min at 95 °C and 40 cycles of 45 s at 94 °C, 30 s at 55 °C and 1 min 45 s at 72 °C, followed by a final 10 min extension at 72 °C.

3. Results

The deduced amino acid motif at the fusion protein cleavage site sequence for all APMV-4 isolates sequenced in this study was DIQPR↓F (Supplementary Table 1), consistent with all previously reported APMV-4 strains obtained from GenBank. The ML phylogeny for 69 APMV-4 fusion gene sequences was formed from five major clades (A–B; Fig. 1) that were almost exclusively monophyletic by continent of sample origin. Only sequences for two APMV-4 strains from Asia (APMV-4/Anas sp./Japan/10KI182/2010 and FJ177514 APMV-4/duck/Hong Kong/D3/75) did not fall within these major clades. Clade A was well-supported (bootstrap value of 99) and was comprised of 21 sequences for viruses isolated from ducks sampled in the Asian countries of China, Japan, and South Korea. Clade B also had fairly strong support (bootstrap value of 86) and was made up of 15 sequences of viruses isolated from ducks, geese, and a starling (family Sturnidae) sampled in the European countries of Italy and Ukraine, as well as a single sequence for an APMV-4 strain originating from an Egyptian goose (*Alopochen aegyptiaca*) sampled in South Africa. Clade C had less support as compared to others (bootstrap support value of 64) and contained four sequences of viruses isolated from mallards (*Anas platyrhynchos*) sampled in Italy and Belgium. Clades D and E were both well-supported (bootstrap values of 100) and comprised of sequences for viruses originating from waterfowl sampled from the USA. With regard to the two sequences from Asia that were not nested within these five major clades, strain APMV-4/Anas sp./Japan/10KI182/2010 was most closely related to Old World Clades A–C (bootstrap support value 99) whereas strain APMV-4/duck/Hong Kong/D3/75 was phylogenetically most closely related to North American Clade D (bootstrap support value 100; Fig. 1).

Genetic distances between all clades ranged from 0.024–0.145 and 0.012–0.049 for nucleotide and amino acid comparisons, respectively