AVIAN DISEASES 51:273–274, 2007

An Avian Influenza Virus from Waterfowl in South America Contains Genes from North American Avian and Equine Lineages

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Received 21 March 2006; Accepted 20 June 2006

SUMMARY. Apart from an outbreak in commercial poultry in Chile in 2002, there have been few reports of avian influenza in South America. However, surveillance in free-flying birds has been limited. An avian influenza virus was isolated from a Cinnamon Teal (*Anas cyanoptera*) in Bolivia in 2001 from samples collected for an avian influenza virus and avian paramyxovirus surveillance study. This isolate was determined to be an H7N3 virus by gene sequencing. Analysis of all eight genes revealed that five genes were most closely related to the H7N3 in Chile in 2002. Two genes were most closely related to North American wild aquatic bird virus lineages and one gene was most closely related to an equine influenza virus from South America.

RESUMEN. Un virus de influenza aviar aislado de aves acuáticas en América del Sur contiene genes de linajes aviares provenientes de América del Norte y de equinos.

Fuera de un brote en aves comerciales ocurrido en Chile durante el año 2002, pocos casos de influenza aviar han sido reportados en América del sur. Sin embargo, la vigilancia epidemiológica en aves silvestres ha sido muy limitada. En Bolivia durante el año 2001, se aisló un virus de influenza aviar de una Cerceta colorada (*Anas cyanoptera*) en muestras tomadas como parte de un estudio de vigilancia para influenza y paramixovirus aviares. Mediante secuenciación genética se determinó que este aislamiento corresponde a un virus H7N3. El análisis de todos los ocho genes reveló que 5 de los genes estaban relacionados con el virus H7N3 aislado en Chile en el año 2002. Dos genes estaban más relacionados con virus de aves acuáticas silvestres de América del Norte y un gen estaba más relacionado con un virus de influenza equina proveniente de América del Sur.

Key words: avian influenza virus, wild waterfowl, H7 hemagglutinin, influenza phylogenetics, influenza pathogenesis

Abbreviations: AIV = avian influenza virus; HA = hemagglutinin; LPAIV = low-pathogenicity AIV; M = matrix; NA = neuraminidase; NP = nucleoprotein; NS = nonstructural; nt = nucleotide; PA = polymerase acidic protein; PB1 = polymerase basic protein 1; PB2 = polymerase basic protein 2; RRT-PCR = real-time reverse-transcription polymerase chain reaction

Prior to an outbreak of avian influenza virus (AIV) in commercial chickens in Chile in 2002 (6), detection of AIV had not been reported in South America. However, surveillance of wild aquatic birds for AIV in South America has historically been minimal, although commercial poultry is routinely monitored for AIV antibody due to export regulations. Here the initial genetic characterization of an AIV collected from Cinnamon Teal (*Anas cyanoptera*) during a survey of wild birds in Bolivia in 2001 is presented.

MATERIALS AND METHODS

Specimen collection. During an ornithological study in Bolivia in 2001, free-flying birds were captured and cloacal swabs were collected, placed in brain heart infusion broth, frozen immediately in liquid nitrogen, and shipped frozen to the Southeast Poultry Reaserch Laboratory for processing. A total of 93 samples (24 form Cinnamon Teal) were collected from 11 species which included ducks, sheldgeese, and doves.

Avian influenza virus detection and isolation. RNA was extracted from cloacal swab material with Trizol reagent (Invitrogen, Inc., Carlsbad, CA) in accordance with manufacturers instructions. RNA was tested for avian influenza virus (AIV) by real-time reverse-transcription polymerase chain reaction (RRT-PCR) with primers and a probe directed to the matrix (M) gene that detect all type A influenza viruses as previously reported (4). Virus isolation was performed in embryonated chickens eggs as per standard procedures (8) with swab material from RRT-PCR positive samples. One AIV was isolated from a Cinnamon Teal (*Anas cyanoptera*), collected on October 27, 2001 (University of Alaska Museum 19,003: Bolivia, Departmento La Paz, Lake Titicaca; lat 16°11′45″S, long 68°37′28″W, elev. 3808 m) (3).

Genetic analysis. The entire coding sequences of all eight viral gene segments were amplified by RT-PCR as previously reported (7), directly sequenced with the BigDye terminator kit (Applied Biosystems, Foster City, CA) on an ABI 3730 DNA analyzer (Applied Biosystems). Multiple alignments for each gene were performed with ClustalV (Lasergene V.6, DNAStar, Madison, WI). Phylogenetic trees were generated using maximum parsimony by heuristic search with 500 bootstrap replicates (PAUP*4.0b10, Sinauer Associates, Sunderland, MA).

RESULTS

The virus isolate (A/Cinnamon Teal/Bolivia/4537/01) was determined to be the H7 HA, N3 neuraminidase (NA), and nonstructural (NS) type A subtypes by gene sequencing (Table 1). Based on phylogenetic analysis, the hemagglutinin (HA), nucleoprotein (NP), polymerase acidic protein (PA), polymerase basic protein 1 (PB1), and polymerase basic protein 2 (PB2) genes were most closely related to the AIV isolates collected from chickens and turkeys during an outbreak in commercial poultry in Chile in 2002. The HA protein cleavage site was consistent with a low-pathogenicity AIV (LPAIV) and identical to the cleavage site of the LPAIV from commercial poultry in Chile in 2002.

The NA and M genes were most closely related to genes from North American wild bird lineages. The NA had 96.7% nucleotide (nt) identity with A/Emu/TX/25412-1/95, and the M gene had 97.4% nt identity with A/Blue-wingedTeal/LA/B182/86 (Table 1).

Table 1. Type A influenza isolates with the highest nt identity to A/CinnamonTeal/Bolivia/4537/01 by individual gene segment.

Gene segment	Subtype	Isolate with the highest sequence identity	Percent identity
HA	H7	Chicken/Chile/176822/02	96.6
NA	N3	Emu/TX/25414-1/95	96.7
M		Blue-winged Teal/LA/B182/86	97.4
NS	A	Equine/LaPlata/1/88	93.6
NP		Chicken/Chile/176822/02	95.8
PA		Chicken/Chile/176822/02	90.1
PB1		Chicken/Chile/176822/02	95.2
PB2		Chicken/Chile/176822/02	95.5

The NS gene was most closely related to the NS gene of an equine virus, with 93.6% nt identity to A/Equine/LaPlata(Argentina)/1/88.

DISCUSSION

Because surveillance of free-flying birds for avian influenza in South America has been minimal, a lack of reported isolations is likely due to poor sampling. Importantly, commercial poultry in South America is routinely screened for AIV to comply with trade standards, showing that commercial birds are free of the virus. From an ecological standpoint there is no clear reason why AIV would not be present in South America as there are numerous species of waterfowl, which are the natural reservoir for AIV (2), that migrate between North and South America, thus providing a mechanism. However, further work is necessary to determine the prevalence of AIV in wild birds in South America. Importantly, the timing of migration in regard to seasonal patterns of AIV in wild birds will affect how efficiently the virus is disseminated among different regions. Interestingly, the subspecies of teal, *A. cyanoptera orinmous*, from which this virus was isolated, is a resident, nonmigratory species.

An outbreak of H7N3 AIV in commercial poultry in Chile in 2002 is the only other reported isolation of AIV in South America (6). It was speculated that the source of the virus was wild waterfowl; however, there were no isolates available for genetic comparison at that time (the sample used in this study had been collected, but not processed). The viruses from Chile were genetically different from other reported AIVs and were proposed to represent a separate South American AIV lineage (6), indicating that AIV has been circulating long enough in South America to diverge enough to be distinct from any common ancestors with North American or Eurasian viruses.

This virus from Bolivia, collected in 2001, about 9 mo prior to the isolation of the H7N3 in commercial poultry in Chile, contains five genes (HA, NP, PA, PB1, and PB2) that are most closely related to a the viruses from Chile (3), suggesting a common relative and supporting the theory that the virus in Chile was introduced by a wild bird. The HA from this virus had a cleavage site consistent with the LPAIV H7N3 initially isolated in Chile. However, the three remaining genes appear to be from diverse sources indicating a reassortant virus. The NA and M were most closely related to North American wild aquatic bird virus lineages (5) providing evidence that there is some exchange of AIV genes between North and South America. Finally, the NS gene was most closely related to NS genes from equine viruses; however, it is not clearly an equine-type gene, because the nt divergence is greater than that among the other equine virus genes and phylogenetic analysis of the NS genes of the most closely related equine viruses (H3N8 lineage) classified them as being most similar to North American wild bird viruses (1). The NS gene from CT/Bolivia/4537/01 had only 1%–2% more identity with the equine viruses than with the North American wild bird viruses at the nt level and is probably a derivative of the wild bird virus and not the equine viruses.

Although the frequency of AIV infection in wild aquatic birds in South America is not known, the detection and molecular characterization of AIV from 2001 from a Cinnamon Teal demonstrates that the virus is present in these species in South America and further reinforces the ability of free-flying birds to disseminate AIV worldwide.

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ACKNOWLEDGMENTS

We thank Scott Lee, Joan Beck, James Doster, Kira Moresco and Dawn Williams-Coplin and the SAA sequencing facility for technical assistance. Victor Hugo Eztellan, Gordon Jarrell, Pamela McCracken, Carmen Quiroga, Marolyn Vidaurre, and Colección Boliviana de Fauna aided with field work. This work was supported by USDA-ARS CRIS project #6612-32000-039, the Institute of Arctic Biology, and the National Science Foundation (NSF EPS-0092040, DEB-0444748).

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