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Short communication

Avian Influenza in wild birds from Chile, 2007–2009

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ABSTRACT

Aquatic and migratory birds, the main reservoir hosts of avian influenza viruses including those with high pathogenic potential, are the wildlife species with the highest risk for viral dissemination across countries and continents. In 2002, the Chilean poultry industry was affected with a highly pathogenic avian influenza strain, which created economic loss and triggered the establishment of a surveillance program in wild birds. This effort consisted of periodic samplings of sick or suspicious animals found along the coast and analyses with standardized techniques for detection of influenza A virus. The aim of this work is to report the detection of three avian influenza strains (H13N2, H5N9, H13N9) in gulls from Chile between 2007–2009, which nucleotide sequences showed highest similitudes to viruses detected in wild birds from North America. These results suggest a dissemination route for influenza viruses along the coasts of Americas. Migratory and synanthropic behaviors of birds included in this study support continued monitoring of avian influenza viruses isolated from wild birds in The Americas and the establishment of biosecurity practices in farms.

Wild birds deserve attention from Veterinary and Public Health services because of both their association with several highly transmissible zoonotic pathogens and their ability to disseminate agents over wide geographical areas in short periods of time (Coman et al., 2014; Fuller et al., 2012). Among these pathogens, influenza A viruses represent a great concern due to their changing viral genome that allows for their frequent transmission between wild reservoirs, domestic animals and humans. Aquatic birds are the most common reservoirs of influenza A viruses in wildlife, particularly dabbling ducks and gulls, which through long-distance movements may spread low (LPAI) and high (HPAI) pathogenicity AI viruses (Beato and Capua, 2011).

In Chile, two influenza outbreaks have generated substantial concern and economic losses in the poultry industry. In 2002, a H7N3 LPAI evolved to HPAI in poultry with an unprecedented recombination at the hemagglutinin (HA) cleavage site (Rojas et al., 2002; Suarez et al., 2004). Later in 2009, the pandemic AH1N1 strain was detected in turkeys, with symptoms resembling a LPAI outbreak (Mathieu et al., 2010). The 2002 episode was also the first report of HPAI in South America, which instigated the Chilean Agricultural and Livestock Service (SAG) and industry to establish an AI surveillance program targeting poultry and wild birds. Since then, the presence of sick or dead wild birds is notified to SAG, which conducts routine sampling through blood collection and cloacal and tracheal swabs for viral detection and isolation.

During 2007–2009, dead birds were reported along the coastline of northern (Arica and Atacama) and central (Valparaíso) regions of Chile, which were sampled as outlined in the SAG surveillance program and tested for the detection of influenza A viruses, according to standard OIE protocols (OIE, 2013). Serums as well as cloacal and tracheal swab samples were collected. The swab specimens were immediately submerged in viral transport media and refrigerated for up to 4 h during shipment to the laboratory. Samples were stored at −80 °C until analysis. Viral RNA was extracted using MagMax® 96 Viral RNA Isolation Kit (Life Technologies) according to manufacturer's instructions.

The influenza A matrix and HA genes were detected by real time reverse-transcription PCR (rRT-PCR) (Spackman et al., 2008; Spackman et al., 2003; Spackman and Suarez, 2008). Viral isolation was performed by inoculating swab supernatant into specific pathogen free (SPF) embryonated chicken eggs (OIE, 2013). Viral isolates were subsequently submitted to an OIE Reference

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Table 1
Laboratory results of influenza A viruses detection in samples from wild birds in Chile, 2007-2009.

Location	Sampling date	rRT-PCR		Strain	Host
		No. tested[a]	No. positive		
Atacama 28°32′S 70°53′W	12-2007	5	2	A/seagull/Chile/234/2008 (H13N2)	Franklin's gull
Valparaíso 32°54′S 71°30′W	01-2008	29	1	A/wild bird/Chile/1805/2008 (H5N9)	Kelp gull
Arica 18°24′S 70°19′W	11-2009	45	1	A/seagull/Chile/5775/2009 (H13N9)	Franklin's gull

[a] Number of animals studied in the surveillance procedure that resulted in influenza detection.

laboratory (USDA, NVSL and SEPRL) for molecular and *in vivo* pathogenicity testing. Serological sub typing was conducted by the HA and neuraminidase (NA) inhibition tests as outlined by OIE.

From the four infected animals detected by rRT-PCR (Table 1), influenza A(H13N2), A(H5N9) and A(H13N9) strains were isolated. Partial (A[H13N2] strain, GenBank accession nos. KP003921 and KP003922) and complete genomic sequencing (A[H5N9] and A[H13N9] strains, GenBank accession nos. KF772945-KF772960) were performed using the Ion Sequencing Kit v2.0 (Life Technologies). Closely related influenza viruses were determined through a Basic Local Alignment Search Tool (BLAST) analysis. Multiple-sequence alignments were performed with MUSCLE

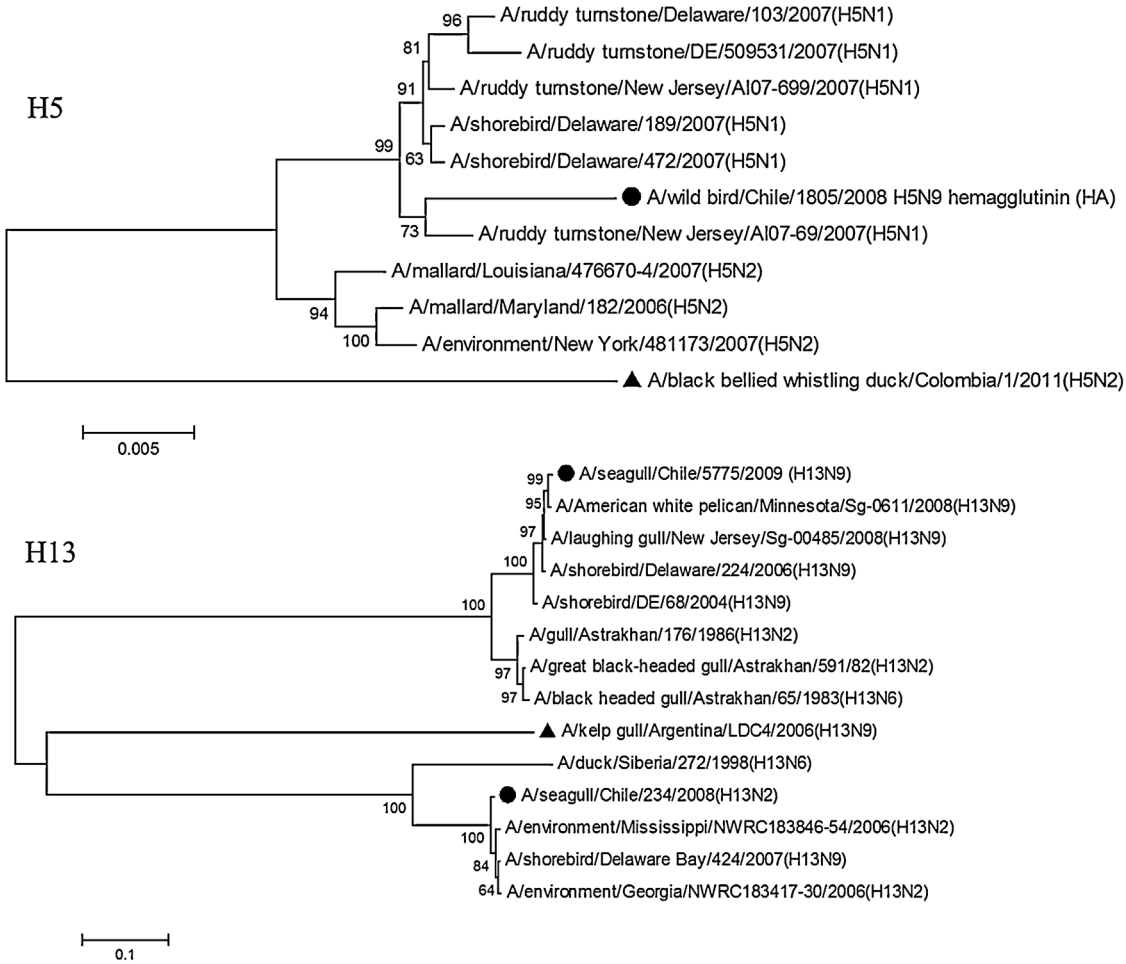

Fig. 1. Phylogenetic trees representing relationships of H5 and H13 sequences obtained by the Neighbor-joining method. The tree was derived from alignment of sequences from Chilean avian influenza strains (filled circles) and their closely related strains, which were determined through a BLAST analysis. Other South American sequences were also included (filled triangles). It was used a bootstrap 1000, and the strength of each branch is indicated in the respective node (percentage). The evolutionary distances were computed using the Tamura-Nei method and are in the units of the number of base substitutions per site. These analyses were conducted in MEGA6.