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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

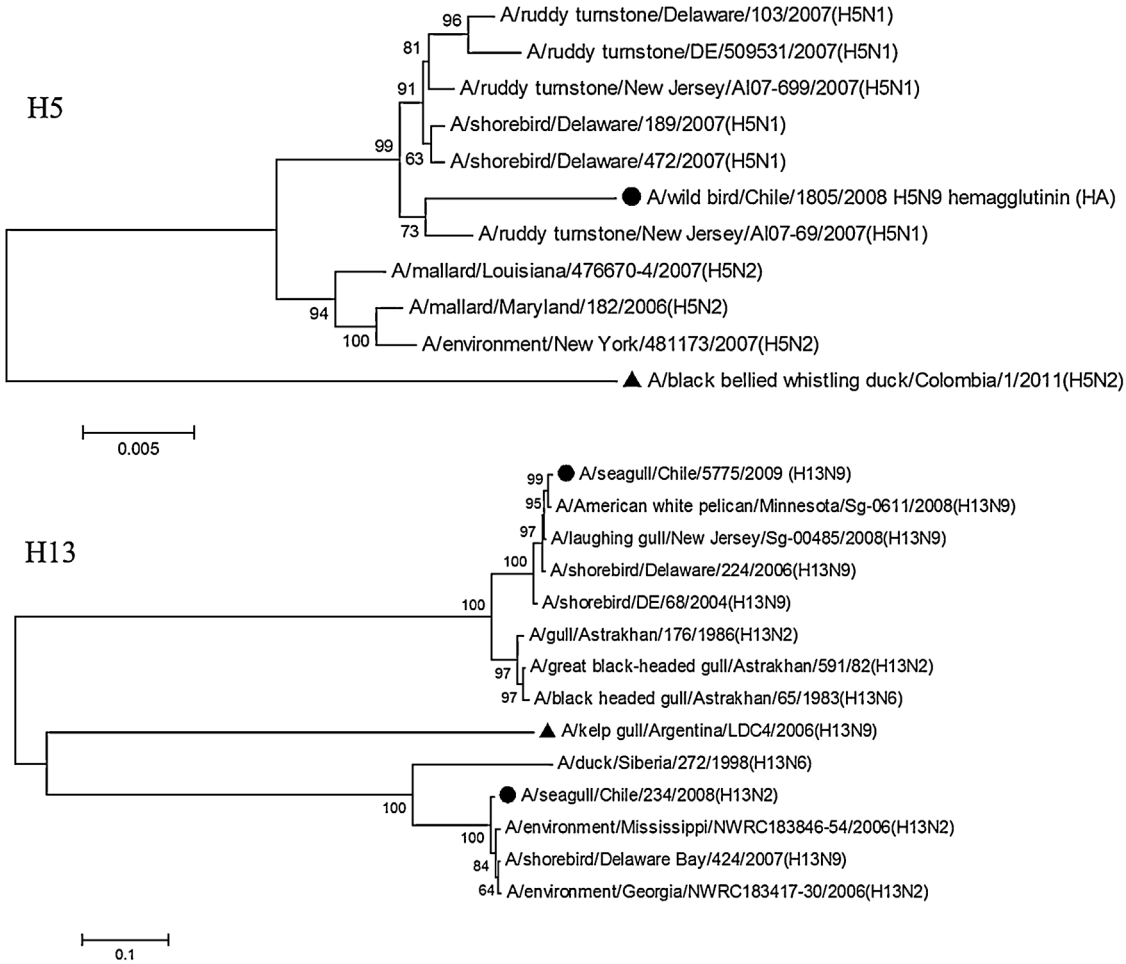

Fig. 1. Phylogenetic trees representing relationships of H5 and H13 sequences obtained by the Neighbor-joining method. The tree was derived from alignment of sequences from Chilean avian influenza strains (filled circles) and their closely related strains, which were determined through a BLAST analysis. 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.

Table 2
Results of standard nucleotide Basic Local Alignment Search Tool (BLAST) analysis using GenBank for gene segments of Influenza strains detected in Chile, 2007–2009. The top most similar result is shown for each segment.

		GenBank BLAST results						
Chilean strain	Segment	Description	Max score	Total score	Query cover	*E* value	Identity	Accession
A/wild bird/Chile/1805/2008(H5N9)	PB2	A/mallard/Alberta/128/2006(H1N1)	4056	4056	92%	0.0	98%	CY137665.1
A/seagull/Chile/5775/2009 H13N9	PB2	A/shorebird/Delaware Bay/424/2007(H13N9)	4006	4006	100%	0.0	98%	CY127806.1
A/wild bird/Chile/1805/2008(H5N9)	PB1	A/ruddy turnstone/New Jersey/AI07-839/2007(H12N1)	4183	4183	100%	0.0	99%	CY144713.1
A/seagull/Chile/5775/2009 H13N9	PB1	A/laughing gull/New Jersey/Sg-00485/2008(H13N9)	4056	4056	100%	0.0	99%	CY144987.1
A/wild bird/Chile/1805/2008(H5N9)	PA	A/ruddy turnstone/New Jersey/AI07-699/2007(H5N1)	4004	4004	90%	0.0	99%	CY144752.1
A/seagull/Chile/5775/2009 H13N9	PA	A/shorebird/Delaware/168/06(H16N3)	3845	3845	100%	0.0	99%	EU030975.1
A/wild bird/Chile/1805/2008(H5N9)	HA	A/ruddy turnstone/New Jersey/AI07-69/2007(H5N1)	3094	3094	100%	0.0	99%	CY144739.1
A/seagull/Chile/5775/2009 H13N9	HA	A/American white pelican/Minnesota/Sg-0611/2008(H13N9)	3064	3064	100%	0.0	99%	CY054302.1
A/seagull/Chile/234/2008(H13N2)	HA	A/shorebird/Delaware Bay/424/2007(H13N9)	2996	2996	99%	0.0	98%	CY127799.1
A/wild bird/Chile/1805/2008(H5N9)	NP	A/mallard/Washington/44338-195/2007(H12N5)	2776	2776	99%	0.0	99%	CY076064.1
A/seagull/Chile/5775/2009 H13N9	NP	A/laughing gull/New Jersey/Sg-00559/2008(H13N9)	2726	2726	100%	0.0	99%	CY145456.1
A/wild bird/Chile/1805/2008(H5N9)	NA	A/green winged teal/California/AKS1305/2008(H11N9)	2490	2490	99%	0.0	98%	CY039534.1
A/seagull/Chile/5775/2009 (H13N9)	NA	A/American white pelican/Minnesota/Sg-0611/2008(H13N9)	2573	2573	100%	0.0	99%	CY054303.1
A/seagull/Chile/234/2008(H13N2)	NA	A/great black-backed gull/Newfoundland/296/2008(H13N2)	2440	2440	99%	0.0	98%	GU724155.1
A/wild bird/Chile/1805/2008(H5N9)	MP	A/blue-winged teal/Louisiana/Sg-00163/2007 (mixed)	1840	1840	98%	0.0	99%	CY078099.1
A/seagull/Chile/5775/2009 H13N9	MP	A/ring-billed gull/Quebec/02622-1/2009(mixed)	1659	1659	100%	0.0	99%	CY125318.1
A/wild bird/Chile/1805/2008(H5N9)	NS	A/shorebird/Delaware/554/2007(H9N1)	1574	1574	99%	0.0	99%	CY077178.1
A/seagull/Chile/5775/2009 H13N9	NS	A/shorebird/Delaware Bay/424/2007(H13N9)	1517	1517	94%	0.0	99%	CY127803.1

(Edgar, 2004), and phylogenetic trees were computed with the Neighbor-joining method using the software MEGA6 (Tamura et al., 2013).

No significant findings were reported upon necropsy of affected birds and samples collected from poultry and wild birds near detection sites were negative for influenza viruses. Isolated strains were classified as LPAI viruses by chicken inoculation and analysis of the amino acid sequence at the HA cleavage site.

Sequence analysis of gene segments from all Chilean strains determined a closer similarity to influenza strains identified in aquatic birds from the western, central and eastern regions of North America (Table 2), than to other South American strains reported both in the Pacific and Atlantic coast (Figs. 1 and 2, Fig S1). On one hand, the data suggests that geographical distribution of Chilean strains can be associated with latitudinal migratory flyways, including all of those described in North America (http://www.birdnature.com/flyways.html), and on the other hand, that this has occurred during independent events both in the Chilean and South American context.

This homogeneity and similarity to North American lineages, which can also be seen with the A/black bellied whistling duck/Colombia/1/2011(H5N2) strain (GenBank accession nos. KC703328 to KC703335), is in contrast with other viruses detected on Argentinian coasts, which have a diversity of segment similarities to other South American, North American and even Eurasian lineages (Pereda et al., 2008; Xu et al., 2012). These apparent differences among Pacific and Atlantic South American reports require more isolates for establishing distribution patterns, such as those described in North America where rapid temporal and geographical movements of AI viruses and virus gene segments have been seen across migratory flyways (Chen and Holmes, 2009; Dugan et al., 2011).

A unique finding with the A/wild bird/Chile/1805/2008 (H5N9) strain is a tandem duplication of 24 nucleotides at position 130-174 of the polymerase B2 (PB2) coding sequence, the effects of which in the protein function or viral replication are unknown.

Host species from which viruses were isolated have different distribution ranges and behaviors, which can explain the sequence similarities to AI strains reported in North America. For example, Franklin's gull (*Leucophaeus pipixcan*) is a migratory species with a broad distribution in The Americas, which breeds mainly in central North America and overwinters along the Pacific Coast of South America, from central Peru to central Chile.

In contrast, Kelp gull (*Larus dominicanus*) is a resident species distributed in the Southern Hemisphere, including coastal areas of South America, South Africa, Australia, New Zealand and Antarctica. During warmer months in Chile (Nov–Mar), both species can merge and establish large flocks, with thousands of birds normally found in wetlands and mouths of rivers. However, Kelp gulls also inhabits urban centers, fish markets and farms, constituting a local risk for inter-species transmission of pathogens carrying both resident and migratory species, among which influenza viruses and multidrug-resistant bacteria have already been pointed out (Clark et al., 2004; Fresno et al., 2013; Pereda et al., 2008).

During the last 10 years in the Chilean AI surveillance program, gulls have represented 20% of samples collected from wild birds. Our results and previous reports support increased efforts for sampling and detection of influenza in these animals along The Americas, and highlight the need for improving biosecurity measures of poultry farms to avoid close contacts with wild hosts, as the most effective method to avoid future outbreaks.

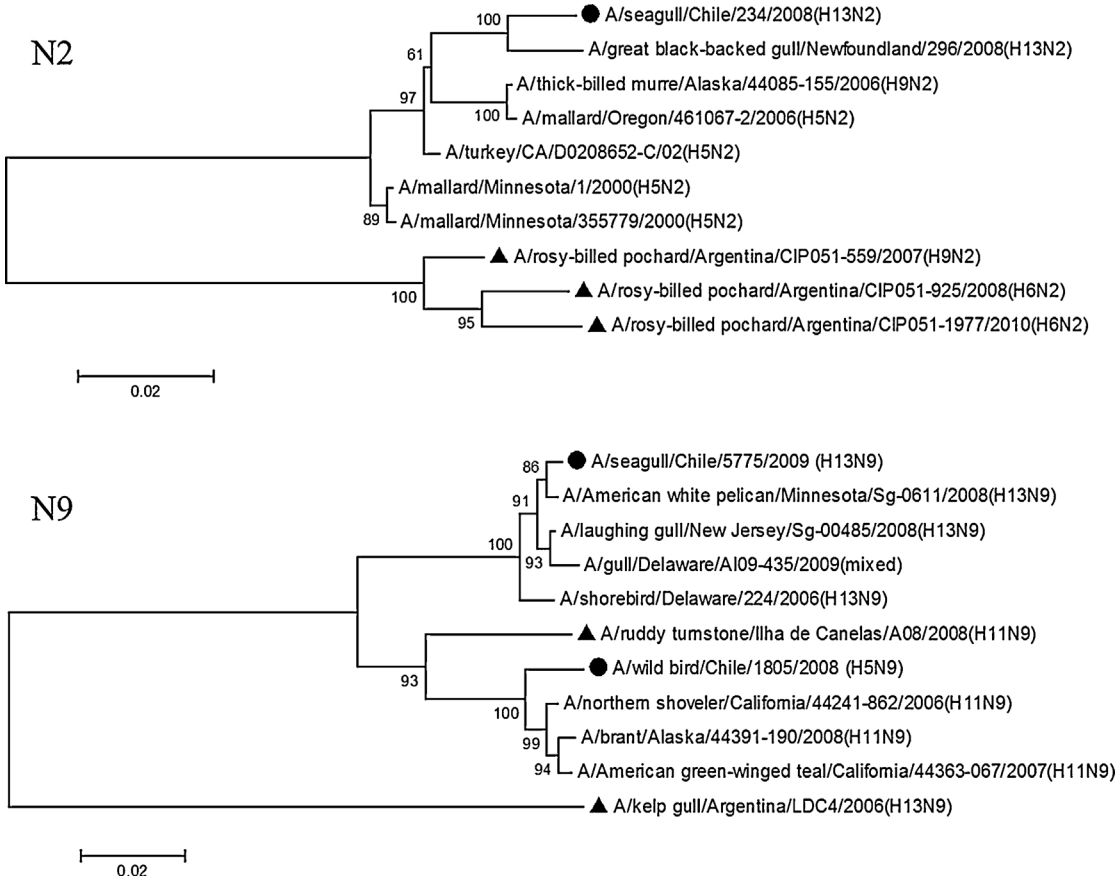

Fig. 2. Phylogenetic trees representing relationships of N2 and N9 sequences obtained by the Neighbor-joining method. The tree was derived from alignment of sequences from Chilean avian influenza strains (filled circles) and their closely related strains, which were determined through a BLAST analysis. 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.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.virusres.2015.01.008.

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