Perpetuation of Avian Influenza in the Americas: Examining the Role of Shorebirds in Patagonia

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Perpetuation of avian influenza in the Americas: Examining the role of shorebirds in Patagonia.—Aquatic birds are considered the natural reservoir of all influenza viruses (Webster et al. 1992). Low-pathogenic avian influenza (AI) viruses have been isolated from Anseriformes (at least 36 species of ducks and 8 species of geese), Charadriiformes (10 species of shorebirds, 9 species of terns), and a few additional waterfowl species (Olsen et al. 2006).

Prevalence of AI virus is higher in fall than in spring in all studies of ducks (Anatidae) and gulls (Laridae) from Europe and North America (Hanson et al. 2003, 2005; Olsen et al. 2006; Munster et al. 2007; Wallensten et al. 2007). This temporal pattern of prevalence was attributed to the higher proportion of immunologically naive juveniles during fall migration. By contrast, the seasonal prevalence of AI viruses in shorebirds of North America showed a different picture. Krauss et al. (2004) found that prevalence of AI in shorebirds in the Delaware Bay area was 14.2% during northward spring migration but only 0.9% (15× lower) during southward fall migration. To explain this pattern, it has been proposed that an undetermined reservoir of AI viruses exists in South America where shorebirds may be exposed during the nonbreeding season and, subsequently, carry virus back north during spring migration (Krauss et al. 2004, 2007; Olsen et al. 2006; Wallensten et al. 2007).

Different hypotheses for the perpetuation of AI viruses have been proposed. Viruses could survive in frozen lakes, with reinfection of birds the following spring (Ito et al. 1995), or the AI-virus gene pool in aquatic birds may be perpetuated by low-level transmission among waterfowl throughout the year (Kawaoka et al. 1988, Krauss et al. 2004). Cold-temperate coastal and inland wetlands in southern South America that support large numbers of breeding and nonbreeding birds are, therefore, prime candidates to contribute to the South American virus reservoir hypothesized by Kraus et al. (2004, 2007).

We present results from 165 shorebirds sampled in freshwater and coastal wetlands in Chubut and Santa Cruz provinces in southern Argentinean Patagonia that were tested for all AI virus subtypes (see Fig. 1 for sampling locations). In this region, breeding
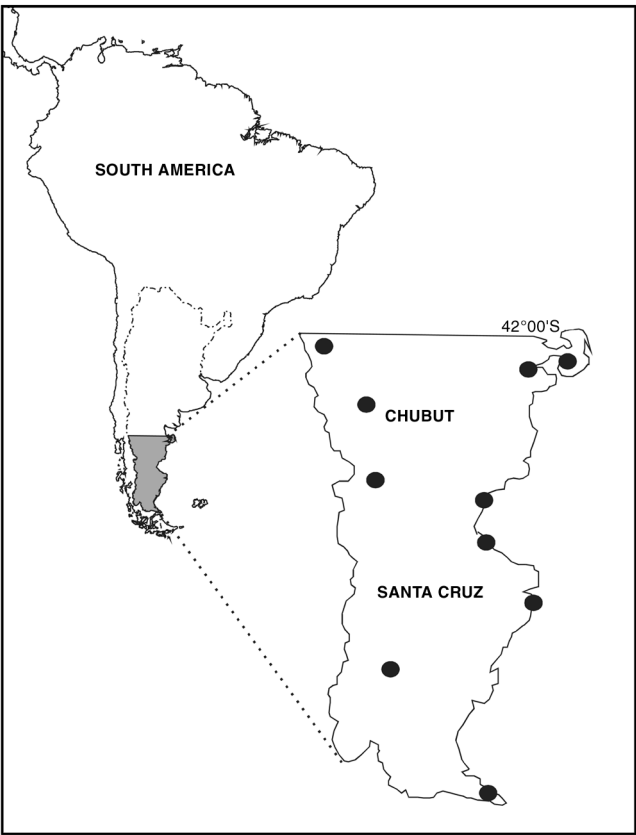

Fig. 1. Sample locations in the Argentinean provinces Chubut and Santa Cruz.

and postbreeding Neotropical shorebirds and waterfowl are found in mixed flocks with nonbreeding Nearctic shorebirds. Five Neotropical and six Nearctic shorebird species with different migratory strategies were sampled between August 2004 and December 2005 (Table 1). Birds were captured using cannon nets, mist nests, and nest traps, or shot for other projects.

Cloacal samples were collected using small sterile cotton swabs with a metal stem. The swabs were stored in transport media (Munster et al. 2007) and transported from the field in liquid

TABLE 1. Overview of the species sampled for AI virus, their migratory status, the environment in which the samples were taken, sample size per species, and 95% confidence interval (CI) for virus prevalence.

Species	Migratory status	Environment	Sample size	95% CI
Magellanic Oystercatcher (*Haematopus leucopodus*)	Neotropical migrant	Coast	20	0.00–11.7
American Oystercatcher (*H. palliatus*)	Neotropical resident	Coast	1	0.00–95.0
Least Seedsnipe (*Thinocorus rumicivorus*)	Neotropical migrant	Freshwater	2	0.00–77.6
Southern Lapwing (*Vanellus chilensis*)	Neotropical partial migrant	Freshwater	4	0.00–52.7
Two-banded Plover (*Charadrius falklandicus*)	Neotropical migrant	Coast	54	0.00–5.39
White-rumped Sandpiper (*Calidris fuscicollis*)	Nearctic migrant	Coast–freshwater	48	0.00–6.05
Pectoral Sandpiper (*C. melanotos*)	Nearctic migrant	Freshwater	2	0.00–77.6
Red Knot (*C. canutus*)	Nearctic migrant	Coast	1	0.00–95.0
Baird´s Sandpiper (*C. bairdii*)	Nearctic migrant	Freshwater–coast	24	0.00–11.7
Sanderling (*C. alba*)	Nearctic migrant	Coast	3	0.00–63.1
Lesser Yellowlegs (*Tringa flavipes*)	Nearctic migrant	Freshwater	6	0.00–39.3

nitrogen to Centro Nacional Patagónico (Pto. Madryn, Argentina), where they were stored in liquid nitrogen or at −20°C. All samples were shipped (at −20°C) to The Netherlands, arriving within 24 h (World Courier). RNA isolation and reverse-transcriptase polymerase chain reaction (RT-PCR) were performed at the Department of Virology of the Erasmus Medical Centre, Rotterdam, as described in Munster et al. (2005).

In contrast to predictions by Krauss et al. (2004) and Ito et al. (1995), all samples were negative for AI types, even though the technique for virus isolation used in the present study is more sensitive than techniques previously used for isolation in embryonated hens' eggs (*Gallus gallus domesticus*; Munster et al. 2005). The probability of obtaining zero positive virus samples among 165 samples is 1.06×10^{-11} at the population infection rate of 14.2% found by Krauss et al. (2004) in migrating Nearctic shorebirds in spring (two-tailed test probability taken from binomial distribution; Sokal and Rohlf 1995). Using the binomial distribution to calculate the probability of finding zero positive samples when assuming a particular prevalence, we simulated the 95% confidence interval (CI) for AI prevalence across all species as 0.00–1.79% (CIs for each species—necessarily wider because of smaller sample sizes—are presented in Table 1). In support of our findings, AI virus was also not detected in another study that sampled 156 Red Knots (*Calidris canutus rufa*) in southern Argentina (D´Amico et al. 2007).

Our results show that neither Neotropical nor Nearctic shorebirds in southern Patagonia were shedding virus at the time of sampling. Hence, although the sample size is relatively small, we found no support for the hypothesis that Nearctic shorebirds become infected with AI at these high-latitude South American nonbreeding grounds.

The absence of AI viruses in shorebirds of southern Patagonia is an important result because of the potential role of this group of birds as reservoirs of AI viruses. More extensive surveillance of wild South American aquatic birds is required before making definitive statements, but our results may help narrow the search for AI viruses to other shorebird species that reach the area or to other stopover sites farther north.

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