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Exposure of Red Knots (*Calidris canutus rufa*) to Select Avian Pathogens; Patagonia, Argentina

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ABSTRACT: As part of the shorebird surveillance, Red Knots (*Calidris canutus rufa*) were sampled in two Patagonian sites in Argentina, Río Grande and San Antonio Oeste, during 2005–2006. Cloacal swabs and serum samples were collected from 156 birds and tested by virus isolation (Newcastle disease virus), polymerase chain reaction (PCR; avian influenza virus and *Plasmodium/Hemoproteus*), and for antibodies to St. Louis encephalitis virus. All test results were negative.

Key words: Argentina, avian influenza virus, *Hemoproteus*, Newcastle disease virus, Patagonia, *Plasmodium*, Red Knot, St. Louis encephalitis virus.

Migratory birds are the natural reservoirs of several viruses that can infect a diversity of wild and domestic animals and humans (Cooper, 1990; Webster et al., 1992; Suss et al., 1994; Reed et al, 2003; Heeney, 2006), and because of this, surveillance is justified. At present, shorebird surveillance in Argentina includes testing for the agents responsible for avian influenza, Newcastle disease, viral encephalitis, and avian malaria. Avian influenza viruses (AIV), including both highly pathogenic and low pathogenic strains, have never been isolated in Argentina (SENASA, 2006). Newcastle disease virus (NDV) was first diagnosed in Argentina in 1961 with an additional occurrence in Entre Ríos province in 1987. Currently, there is a nationwide systematic vaccination program in poultry that is implemented by Servicio Nacional de Sanidad y Calidad Agropecuaria (SENASA). St. Louis encephalitis virus (SLEV) has been detected in Argentina, and in 2005, an outbreak of SLEV encephalitis in human occurred in Córdoba, Argentina (Diaz et al., 2006). Although, the blood parasites *Plasmodium*, *Hemoproteus*, and *Leucocytozoon* are distributed worldwide, avian malaria has never been detected in migratory birds in Argentina.

Systematic surveillance of migratory birds for diseases is carried out in several countries of the western Atlantic flyway, and isolations of AIV and NDV have been reported from shorebirds sampled on the coast of Brazil. Isolations include AIV (H3) from Red Knot (*Calidris canutus*), Sanderling (*Calidris alba*), Least Sandpiper (*Calidris minutilla*), White-rumped Sandpiper (*Calidris fuscicollis*), Ruddy Turnstone (*Arenaria interpres*), Semipalmated Sandpiper (*Calidris pusilla*), and NDV (APMV-1) from both Least Sandpiper and Ruddy Turnstone (Secretaria de Vigilância em Saúde, 2004). These results are significant because migratory birds passing through the Brazilian coast also reach Argentina during the migratory season.

Wetlands in the Patagonian region of Argentina are used by the Red Knots as both wintering and stopover sites. Red Knots are long-distance migrants that breed in the Canadian Artic and winter in coastal areas in South America (Hayman et al., 1986). During annual migrations, stopovers are in different flyways along the coast of North and South America, such as Delaware Bay and Florida (USA) and Maranhão and Lagoa do Peixe (Brazil). In Argentina, a program to monitor diseases in shorebirds is carried out by Centro Nacional Patagónico (CENPAT-CONICET) and SENASA, and in this manuscript, we report virus isolation (NDV), polymerase chain reaction (PCR; AIV and *Plasmodium/Hemoproteus*), and

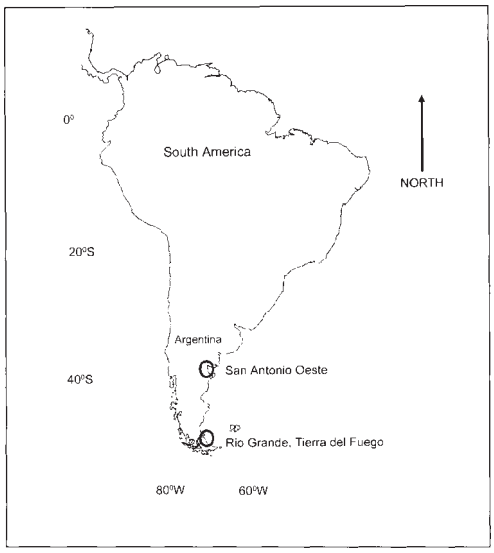

FIGURE 1. Patagonian sites of Argentina where Red Knots were captured: Río Grande (Tierra del Fuego) and San Antonio Oeste (Río Negro).

serologic (SLEV) results for Red Knots sampled from wetlands of Patagonia, Argentina.

Surveillance was conducted at the wintering site of Río Grande, Tierra del Fuego, Argentina (53°44,983′S, 67°44,354′W) in November 2005 (Fig. 1). This site was established by the Western Hemisphere Shorebird Reserve Network (WHSRN) as the "Hemispheric Reserve of the Atlantic Coast of Tierra del Fuego." During the northward migration, Red Knots were captured in the stopover site San Antonio Oeste, Río Negro, Argentina (64°32′60″W, 40°26′60″S) in March 2006 (Fig. 1). This site is also recognized as an International Site by WHSRN. Red knots were sampled as part of international banding expeditions organized annually by the Royal Ontario Museum of Canada, the Inalafquen Foundation, and the Virginia Choquintel Museum, Argentina.

Birds were captured with a cannon net, sampled, banded, and released. Cloacal samples were obtained using small swabs (170KS01, Copan Diagnostic, Corona, California, USA) and placed in transport medium (Hanks' balanced salt solution: 10% glycerol 200 U/ml, penicillin 200 mg/ml, streptomycin 100 U/ml, polymyxin B 250 mg/ml, gentamycin 50 U/ml, nystatin, Sigma-Aldrich, St. Louis, Missouri, USA). Blood samples were collected from the brachial vein using capillary tubes (ML0067 40 mm SafeCrit®, MarketLab, Caledonia, Michigan, USA) and kept in a cooler until analysis. Capillaries were spun for 120 sec at 13,700×G (CritSpin hematocrit centrifuge model M961), and cellular and serum components were separated. Cloacal samples were divided for AIV and NDV testing. One aliquot was transported to the laboratory of SENASA in Buenos Aires. These samples were vortex and centrifuged for 10 min at 1,000×G. The supernatant was inoculated (0.20 ml/egg) via the allantoic route into four 10-day-old specific pathogen-free embryonated chicken eggs. Eggs were incubated at 37 C, and hemagglutination testing was completed following standard procedures (SENASA, 2003; de la Sota and Espinoza, 2004). The second set was transported to the Department of Virology, Rotterdam University, where samples were tested using a one-step reverse-transcriptase-polymerase chain reaction (RT-PCR) as described (Fouchier et al., 2000). Serum samples were transported to Laboratorio de Arbovirus, Universidad Nacional de Córdoba, to be tested for SLEV-neutralizing antibodies using a plaque neutralization assay (Early et al., 1967). Blood samples were sent to the Department of Natural History, Royal Ontario Museum, Canada, where PCR assays were performed. Samples were screened for presence of avian malaria (AM) by attempting to amplify a 157-bp section of ribosomal RNA sequence from the 6-kb mitochondrial DNA genome of the *Plasmodium* sp. and *Hemoproteus* sp. (Fallon et al., 2003).

Samples taken in both Río Grande (n=101) and San Antonio Oeste (n=55) were negative for AIV and NDV. No SLEV-neutralizing antibodies were detected in the serum samples from Río

Grande ($n=58$) or San Antonio Oeste ($n=51$). *Plasmodium* and *Hemoproteus* PCR results were negative for all samples from Río Grande ($n=84$) and San Antonio Oeste ($n=47$). Results provided no indication of current infection with AIV, NDV, or *Plasmodium/Hemoproteus*, and no indication of previous infection with SLEV.

Very little is known about viruses circulating within and among shorebirds species, and most of published accounts involve AIV (Kawaoka et al., 1988; Hanson, 2001; Krauss et al., 2004). In North America, AIV have been isolated from migrating shorebirds, including some species that migrate from the southern tip of South America, such as Red Knot, Ruddy Turnstone, Sanderling, and Semipalmated Sandpiper. All of these viruses were low-pathogenic strains, and although subtypes were diverse, the predominant subtypes included H3N8 and H11N9 (Krauss et al., 2004). Migrating shorebirds from the coast of Brazil were serologically tested for 20 different arboviruses (*Alphavirus*, *Phebovirus*, *Orthobunyavirus*, and others); antibodies to Cacicapore (*Flavivirus*) and Mayaro (*Alphavirus*) viruses were detected in samples from Ruddy Turnstone and Semipalmated Sandpiper (Secretaria de Vigilância em Saúde, 2004). During migration, these species share habitats in Brazil with Red Knots, and some continue their migration to the coast of Argentina. Although not detected in this study, antibodies against SLEV have been reported from aquatic and inland birds in Argentina, including two migrating shorebirds, White-rumped Sandpiper and Pectoral Sandpiper (*Calidris melanotos*), suggesting that shorebirds could potentially disseminate this virus (Diaz et al., 2005). The prevalence of *Plasmodium* and *Hemoproteus* is very low in migrating shorebirds (Mendes et al., 2005); thus, it was not surprising that Red Knots were negative for the disease.

Although not included in this study, it is also important to emphasize that West Nile virus was introduced to North America in 1999, and since, there has been concern about its dispersal and potential impacts on wild bird populations (Nash et al., 2001). In March 2006, three horses died because of West Nile virus (Morales et al, 2006), and between February and May 2006, four nonfatal human cases were registered in two provinces (Córdoba and Chaco). Although West Nile virus has never been detected in birds in Argentina, it may be important to include this pathogen in future testing.

In summary, there is no evidence that Red Knots migrating through, and wintering in, Patagonia are infected with AIV, NDV, SLEV, or *Plasmodium/Hemoproteus*. However, it is very important to continue surveillance because they share habitats in their hemispheric flyway with several species of shore and marine birds that are potentially at risk of infection.

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