*Multi-year surveillance of selected avian pathogens in the migrant shorebird Red Knot (*Calidris canutus rufa*) at its main stopover site in Patagonia, Argentina*

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SHORT NOTE

Multi-year surveillance of selected avian pathogens in the migrant shorebird Red Knot (*Calidris canutus rufa*) at its main stopover site in Patagonia, Argentina

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Abstract To investigate possible reasons for recent declines in Red Knot (*Calidris canutus rufa*) populations we surveyed for selected pathogens in Red Knots captured in San Antonio Bay, Argentina, on their northward migration during the period 2006–2011. Blood, cloacal swabs and faeces were analysed for bacteria [*Salmonella* sp., *Shigella* sp., enteropathogenic *Escherichia coli* ($N = 42$) and other coliforms ($N = 35$)], viral agents [responsible for avian influenza ($N = 239$), St. Louis encephalitis ($N = 51$) and Newcastle disease ($N = 239$)] and avian malaria parasites [*Plasmodium* sp. and *Haemoproteus* sp. ($N = 284$)]. All 698 samples taken from 303 individuals were negative, providing no evidence that Red Knots sampled at this stopover site were infected with these pathogens at the time of sampling.

Keywords Patagonia · Pathogens · Red Knot · Surveillance

Zusammenfassung

Mehrjährige Kontrolle ausgewählter aviärer Krankheitserreger beim Knutt (*Calidris canutus rufa*) im Hauptrastgebiet in Patagonien, Argentinien

Zur Erforschung möglicher Gründe für die aktuellen Bestandrückgänge von Knuttpopulationen (*Calidris canutus rufa*), wurden Knutts auf ihrem Heimzug in San Antonio Bay, Argentinien in den Jahren 2006 bis 2011 gefangen und auf ausgewählte Krankheitserreger hin untersucht. Blut, Kloakenabstriche und Kot wurden auf Bakterien [*Salmonella* sp., *Shigella* sp., enteropathogene *Escherichia coli* ($N = 42$) und andere Kolibakterien ($N = 35$)], virale Erreger [verantwortlich für aviäre Influenza ($N = 239$), St. Louis Enzephalitis ($N = 51$), Newcastle Krankheit ($N = 239$)] and sowie Vogelmalaria übertragende Parasiten [*Plasmodium* sp. und *Haemoproteus* sp. ($N = 284$)] hin analysiert. Alle 698 Proben von insgesamt 303 Individuen waren negativ. Es gibt damit keinen Hinweis darauf, dass Knutts in diesem Rastgebiet zur Zeit der Probennahme mit den untersuchten Krankheitserregern infiziert waren.

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Introduction

Pathogenic agents may cause declines in the host populations, as seen in Hawaiian land birds infected with avian malaria. Therefore, from a conservation perspective, systematic monitoring for pathogens is crucial. Migratory shorebirds are a focus of concern in terms of conservation, and the occurrences of pathogenic agents in shorebird species have been well described in some cases. For

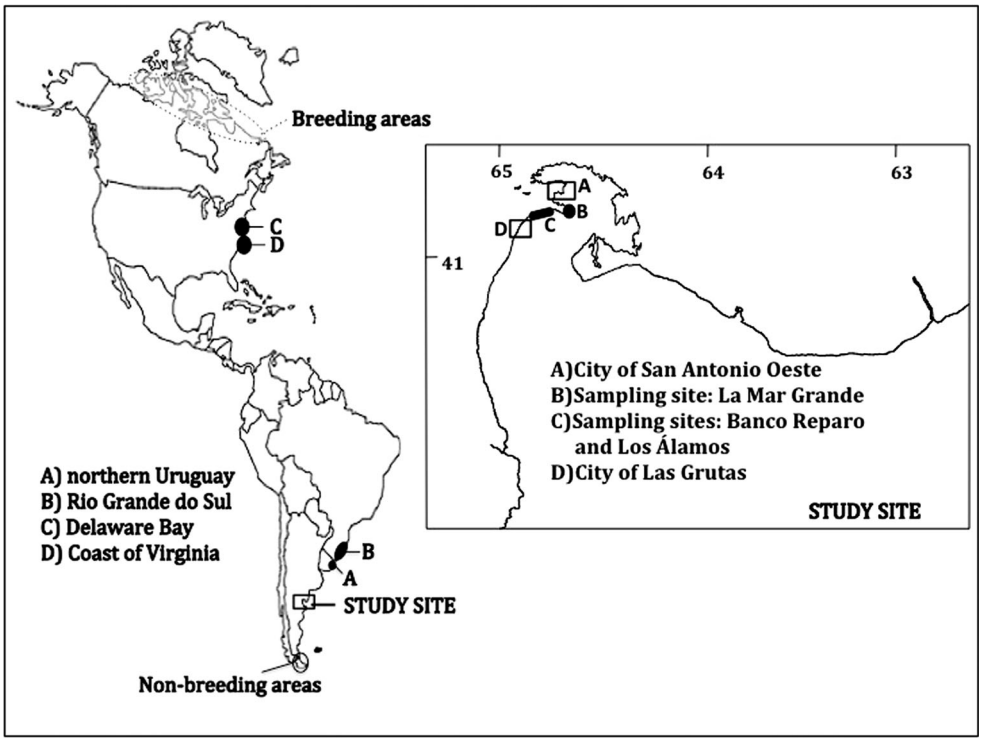

Fig. 1 Northward migration of Red Knots (*Calidris canutus rufa*) from non-breeding sites in Tierra del Fuego to breeding sites in the Canadian Arctic. The map on the *left* shows other important migratory sites used by Red Knots as stopovers: northern Uruguay, Río Grande do Sul, the coast of Virginia and Delaware Bay, USA. The map on the *right* shows an enlargement of the stopover study site in San Antonio Bay, Río Negro, Argentina. The area includes the sites where Red Knots were captured and sampled in La Mar Grande, Banco Reparo and Los Álamos. These sites are close to the cities of San Antonio Oeste and Las Grutas

example, shorebirds play a major role as reservoirs for low pathogenic strains of avian influenza virus (AIV) (Klaassen et al. 2012), and 64 species have been diagnosed with avian botulism (Rocke and Bollinger 2007). Pathogenic strains of bacteria, such as *Helicobacter*, *Staphylococcus*, *Clostridium*, *Mycobacterium*, *Rhodococcus*, *Legionella* and *Corynebacterium*, have also been reported in shorebirds (Santos et al. 2012), and some shorebirds harbor protozoan parasites in low prevalence (D'Amico and Baker 2010).

The Red Knot (hereafter Knots) *Calidris canutus rufa* is a long-distance migrant that travels approximately 16,000 km each way between its Arctic breeding sites and its Patagonian wintering areas. Knot populations are in drastic decline (Dey et al. 2011), and the subspecies has been listed as endangered by the Convention on Migratory Species (Bonn), the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), and the government of Argentina. During their migrations, Knots may become infected with pathogens contracted from conspecifics or individuals of different species at staging areas (Jourdain et al. 2007). Pathogens could be acting as a source of mortality for Knots, and disease pressures might be limiting knot conservation and population recovery.

During their northward migration, Knots use San Antonio Bay, Río Negro, Argentina as one of their main stopover sites from February to late April/early May. Here we report on our systematic monitoring for pathogens in Knots, carried out as part of annual catching and ringing expeditions led by the Inalafquen Foundation in conjunction with the Royal Ontario Museum (ROM) in Toronto, Ontario, Canada. We monitored the bacteria *Salmonella* sp., *Shigella* sp. and enteropathogenic *Escherichia coli*, the viruses AIV, Newcastle disease virus (NDV) and Saint Louis encephalitis virus (SLEV) and the malarial blood parasites *Plasmodium* sp. and *Haemoproteus* sp. These pathogens were chosen because their assessment is required by government health programs for wildlife conservation in Argentina. West Nile Virus (WNV) is not included in Argentina's National Surveillance Program, and due to logistical challenges we were unable to send samples out of Argentina for WNV testing.

Methods

Knots were captured and sampled in San Antonio Bay at the end of March or early April 2006, 2007, 2008, 2010 and 2011 (fieldwork was not possible in 2009). We used cannon nets to capture Knots at three sites—La Mar Grande, Banco Reparo and Los Álamos—near to the cities San Antonio Oeste and Las Grutas (Fig. 1). A total of 303 individuals were sampled, ringed and released; however logistical constraints meant that we could not perform all tests on all birds. Table 1 presents data on the distribution of the 698 samples across specific tests and years).

Fresh faeces ($N = 35$) were collected and spread on microscope slides to detect coliform bacteria. Cloacal samples were obtained using small swabs (170KS01; Copan Diagnostics, Brescia, Italy) and placed in transport medium

Table 1 Selected pathogenic agents tested for in Red Knots (*Calidris canutus rufa*) sampled in San Antonio Bay

Date of capture	No. of sampled birds	Saint Louis encephalitis virus	Avian influenza virus	Newcastle disease virus	Avian malaria	*Salmonella* sp.	*Shigella* sp.	*Escherichia coli*	Coliforms
2006 (28 March)	55	0 (51)	0 (55)	0 (55)	0 (55)[a]				
					0 (47)[b]				
2007 (22 March)	60		0 (60)	0 (60)	0 (41)[a]				
2008 (26 March)	82		0 (82)	0 (82)	0 (82)[a]				
2010 (30 March)	64				0 (64)[a]	0 (42)	0 (42)	0 (42)	NI (20)
2011 (07 April)	42		0 (42)	0 (42)	0 (42)[a]	0 (42)	0 (42)		NI (15)

All samples were negative. The number in parenthesis is the number of samples analysed for each pathogen and year. Fieldwork was not possible in 2009. Data and results from 2006 include data reported by D'Amico et al. (2007)

[a] Analyses of blood smears

[b] Molecular analyses of blood samples (see D'Amico et al. 2007)

for the detection of bacteria ($N = 42$, Stuart's medium) and viruses ($N = 239$, Hanks' balanced salt solution) at a later date. Blood samples were collected from the brachial vein using capillary tubes (ML0067; 40 mm SafeCritH), which were spun for 2 min at 13,700*g* (CritSpin centrifuge model M961; Beckman Coulter, Brea, CA) to separate cellular and serum components. Cellular components were collected in Eppendorf tubes for molecular analyses of avian malaria (AM; $N = 47$), and serum was sent to specialized laboratories for analyses of viral agents ($N = 51$). Smears were prepared from a drop of fresh blood, fixed with alcohol and stained (Tinción 15; Biopur, Rosario, Argentina) for later analyses of blood protozoa ($N = 284$). Appropriate positive and negative controls were used for all tests.

The bacteria *Salmonella* sp., *Shigella* sp. and enteropathogenic *Escherichia coli* were detected through specific techniques of selective and differential culture. To isolate *Salmonella* sp. and *Shigella* sp., samples were incubated for 18–24 h in selenite broth and then cultured on *Salmonella–Shigella* agar (agar SS). To isolate enteropathogenic *E. coli*, we used a differential medium agar that was cysteine-lactose deficient in electrolytes (agar BD-CLDE). We found no evidence for colony formation despite expected growth on positive controls; therefore, no further biochemical or molecular tests were performed. The faecal smears were Gram-stained and observed under an optical microscope (400×) to detect the presence/absence of Gram-negative *Bacillus*.

Assays for the viral agents AIV and NVD were performed by SENASA (the Argentinean National Service of Animal Health, Food and Agriculture, Argentina). Samples were centrifuged and the supernatant inoculated into specific pathogen-free embryonated chicken eggs (allantoic route). The eggs were incubated at 37 °C, and hemagglutination assays were performed following standard procedures (SENASA 2003). Surveillance for SLEV was performed using a standard hemagglutination inhibition and neutralization assay at the Laboratorio de Arbovirus, Universidad Nacional de Córdoba (D'Amico et al. 2007). Specificity and sensitivity for all assays were ≥95 %, and positive samples were used in all runs and also to test stock solutions.

Avian malaria was surveyed on blood smears examined at 1,000× magnification with oil immersion to distinguish *Plasmodium* sp. and *Haemoproteus* sp. (see protocols in D'Amico and Baker 2010). Molecular detection, by the PCR was also performed on samples from 2006 using a positive control from common myna *Acridotheres tristis* (D'Amico et al. 2007).

Results and discussion

All 698 fecal, cloacal and blood samples from 303 knot individuals were negative for the pathogens analysed (see

Table 1 for the distribution of the samples across specific tests and years).

There are several possible explanations for these negative results. The negative results for *Salmonella* sp., *Shigella* sp. and enteropathogenic *E. coli* may be due to contamination levels that were too low to cause the transmission and spread of the bacteria. Faecal samples collected from eight shorebirds at another major stopover site along the Knot flyway (Delaware Bay, USA) also contained no culturable *Yersina*, *Shigella*, *Salmonella*, *Camplyobacter* or *E. coli* (DM Buehler, unpublished data). However, because the majority of microbial organisms are not culturable (Rappé and Giovannoni 2003) molecular methods should be incorporated into pathogen surveillance schemes for shorebirds in the future. For example, pathogenic strains of bacteria in shorebirds in Europe have been detected by denaturing gradient gel electrophoresis (DGGE) and16S rRNA gene amplification (Santos et al. 2012).

Our finding of no viral agents (AIV, NDV and SLEV) or blood parasites is consistent with other studies in shorebirds. Escudero et al. (2008) found that the prevalence of disease-causing viruses was very low in shorebirds in Patagonia, and D'Amico and Baker (2010) reported a very low prevalence of blood parasites using blood smear and molecular analyses in Knots in the Americas. This low or no prevalence of viral and blood parasites in migratory shorebirds is most frequently explained in the context of the scarcity of vectors in the environment (Blanco et al. 1998). Cold-temperate zones and marine environments, such as those used by Knots throughout their flyway, correlate with a low density and low species diversity of viral and blood pathogens, as well as with the vectors that carry them (Blanco et al. 1998). A low prevalence of viral and blood parasites may also result if the parasite is not be able to infect specific hosts due to a lack of host/parasite specificity (Blanco et al. 1998). Alternatively, infected individuals could have died before we had a chance to sample them. Finally, it is possible that our assays did not detect all infections; however, we suggest that this is unlikely is slim since the sensitivities of our assays were >95 % and we used positive controls to limit false negatives.

Although we did not detect evidence for presence of those pathogens under surveillance in Knots using the San Antonio Bay at a stopover site, pathogenic agents have been reported for shorebirds in the Western Atlantic migratory corridor. For example, bacteria (genera: *Aeromonas*, *Pseudomonas*, *Shewanella*, *Enterococcus*, *Escherichia*, *Proteus*, *Citrobacter*) have been isolated in a subset of shorebirds [Knots, White-rumped sandpiper (*Calidris fusciollis*) and Sanderling (*Calidris alba*)] found dead during a mortality event in April 1997 in southern Brazil (Buehler et al. 2010). Knots had the highest prevalence of bacterial infection among these shorebird species during this event and also showed the most severe lesions caused by Acanthocephala and Trematoda endoparasites. Furthermore, WNV has been isolated from migratory shorebirds (*Calidris alba* and *Arenaria interpres*) that use the coast of Brazil (Petry et al. 2006). In Delaware Bay (USA), avian *Paramixovirus*, responsible for Newcastle disease, has been isolated in *Arenaria interpres* (Coffee et al. 2010), a shorebird species that also experiences annual infections with low pathogenicity AIV (Maxted et al. 2012). Finally, a case of avian malaria in a Knot has also been reported from Delaware Bay (D'Amico and Baker 2010).

Knowledge of the potential pathogens circulating among birds is of growing importance, both at national and international levels. Migrant shorebirds may be both reservoirs and spreaders of pathogens and infected vectors, with important consequences for species conservation and human public health (Altizier et al. 2011). Therefore, despite our negative results, it is crucial to continue to survey for pathogens in shorebirds along their migratory routes.

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References

Altizier S, Bertel R, Han BA (2011) Animal migration and infectious disease risk. Science 331:296–302

Blanco G, Gajon A, Doval G, Martínez F (1998) Absence of blood parasites in Griffon vultures from Spain. J Wildl Dis 34:640–643

Buehler DM, Bugoni L, Dorrestein GM, González PM, Pereira-Jr J, Proenca L, de L Serrano I, Baker AJ, Piersma T (2010) Local mortality events in migrating sandpipers (*Calidris*) at a staging site in southern Brazil. Wader Study Gr Bull 117:150–156

Coffee LL, Hanson BA, Luttrell PM, Swayne DE, Senne DA, Goekjian VH, Niles LJ, Stallknecht DE (2010) Avian paramyxoviruses in shorebirds and gulls. J Wildl Dis 46:481–487

D'Amico VL, Baker AJ (2010) A rare case of *Plasmodium* (Haemamoeba) *relictum* infection in a free-living red knot (*Calidris canutus rufa*, Scolopacidae). J Ornithol 151:951–954

D'Amico VL, Bertellotti M, Baker AJ, Diaz LA (2007) Exposure of red knots (*Calidris canutus rufa*) to select avian pathogen; Patagonia, Argentina. J Wildl Dis 43:794–797

Dey A, Niles L, Sitters H, Kalasz K, Morrison G (2011) Update to the red knot *Calidris canutus rufa* in the Western Hemisphere, April 2011. Western Hemisphere Shorebird Group IV Meeting, Vancouver

Escudero G, Fouchier R, Bertellotti M, Edelaar P (2008) Perpetuation of avian influenza in the Americas: a hypothesis review from Neotropical Shorebirds. Auk 125:494–495

Jourdain E, Gauthier-Clerc M, Bicout DJ, Sabatier P (2007) Bird migration routes and risk for pathogen dispersion into western Mediterranean wetlands. Emerg Infect Dis 13:3

Klaassen M, Hoye BJ, Nolet BA, Buttemer WA (2012) Ecophysiology of avian migration in the face of current global hazards. Philos Trans R Soc B 367:1719–1732

Maxted AM, Luttrell MP, Goekjian VH, Brown JD, Niles LJ, Dey AD, Kalasz KS, Swayne DE, Stallknecht DE (2012) Annual survival of Ruddy Turnstones is not affected by natural infection with low pathogenicity avian influenza virus infection dynamics in shorebirds hosts. J Wildl Dis 48:322–334

Petry R, Schmitz PA, Demétrio L, Guadagnin DL (2006) Avifauna do Rio Grande do Sul e doenças emergentes: conhecimento atual e recomendações para a vigilância ornitológica da Influenza Aviária e da Febre do Nilo Ocidental. Rev Bras Ornitol 14:269–277

Rappé MS, Giovannoni SJ (2003) The uncultured microbial majority. Annu Rev Microbiol 57:369–394

Rocke TE, Bollinger TK (2007) Avian botulism. In: Thomas NJ, Hunter DB, Atkinson CT (eds) Infectious diseases of wild birds. Wiley-Blackwell, Ames, pp 377–416

Santos SS, Pardal S, Proença DN, Lopes RJ, Ramos JA, Mendes L, Morais PV (2012) Diversity of cloacal microbial community in migratory shorebirds that use the Tagus estuary as stopover habitat and their potential to harbor and disperse pathogenic microorganisms. FEMS Microbiol Ecol 82:54–63

SENASA (2003) Manual de procedimientos: influenza aviar altamente patógena y enfermedad de Newcastle. Programa de Animales de Granja. Dirección Nacional de Sanidad Animal, Buenos Aires, pp 20–23