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SHORT NOTE

A rare case of *Plasmodium* (*Haemamoeba*) *relictum* infection in a free-living Red Knot (*Calidris canutus rufa*, Scolopacidae)

Verónica L. D'Amico · Allan J. Baker

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Abstract The Red Knot (*Calidris canutus rufa*) is a Nearctic migrant shorebird that breeds in the Canadian Arctic and spends the winter season in coastal sites in South America. A rare case of a blood protozoan was found by molecular analyses from an adult bird captured during spring migration at the last refuelling stopover in Delaware Bay USA in 2006. The parasite was identified as *Plasmodium relictum* belonging to subgenus *Haemamoeba* based on the shape of meronts, roundish gametocytes, and its position in the erythrocytes from the blood smears examination. A partial cytochrome *b* sequence was a 100% match to a sequence of *Plasmodium relictum*, sequence Genbank accession number: id DQ659543.1 (lineage code haplotype P5). This is the first report of avian malaria in a wild individual of *C. c. rufa*.

Keywords Avian malaria · *Plasmodim relictum* · Red Knot

Introduction

Some species and lineages of avian malaria are globally distributed (Atkinson and van Riper 1991; Valkiūnas 2005; Valkiūnas et al. 2007). However, some members of avian families such as ducks, geese and swans appear to be more susceptible than others, such as migratory shorebirds (Friend and Franson 1999). Differences in the prevalences, geographic distributions, and host ranges of most parasites are associated with habitat preferences, seasonal movements, and feeding habits of bird hosts (Figuerola 1999; Friend 2002; Piersma 2003).

The Red Knot (*Calidris canutus rufa*, Scolopacidae) is a long-distance migrant shorebird that uses extreme cold–temperate habitats (high Canadian Arctic tundra to breed and southern tip of South America to winter), and has a strong preference for coastal habitats (Piersma 2003), thereby largely avoiding exposure to malaria owing to lack of suitable vectors (Piersma 1997, 2003; Figuerola 1999; Mendes et al. 2005). Three populations spend the non-breeding season in Florida, Northern Brazil or Tierra del Fuego. Even though an epizootic caused by a *Besnoitia* like-organism and susceptibility to *Plasmodium hermani* have been reported in Red Knots in Florida (Woodard et al. 1977; Forrester and Humphrey 1981), there have not been any subsequent reports of protozoan parasites in *C. c. rufa*.

The largest population of *C. c. rufa* winters in Tierra del Fuego, and has declined from 51,000 in 2000 to around 14,900 in 2008 (Niles et al. 2008). The decline has been linked mainly to late arrival and a decline in the food supply at the last stopover site in Delaware Bay (United States) before departure to the Arctic breeding grounds (Baker et al. 2004). Therefore, the conservation of the population along the flyway has become a top priority. As part of a project to monitor the northwards migration, bird catches are organized annually by international research teams. Expeditions are carried out in several sites used by Red Knots along the migratory flyway from Tierra del Fuego to Canada. Here we report a blood parasite found in

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V. L. D'Amico (✉)
Centro Nacional Patagónico, Bvard. Brown 3500,
Puerto Madryn, 9120 Chubut, Argentina
e-mail: damico@cenpat.edu.ar

A. J. Baker
Department of Natural History, Royal Ontario Museum,
Toronto, ON, Canada

a Red Knot captured at the last refuelling site in Delaware Bay, United States.

Methods

Birds were captured with cannon nets, sampled and released at Delaware Bay in New Jersey, United States (075°14′W, 39°11′N) during May 2006. Blood samples were taken from the brachial vein using capillary tubes. Tubes were centrifuged at 13,700×*g* for 2 min (CritSpin Haematocrit Centrifuge model M961) to separate cellular and plasma components. Hematocrit was measured with a microhematocrit ruler. Blood was placed in EDTA and transported to the Department of Natural History, Royal Ontario Museum, Canada, where polymerase chain reaction (PCR) assays were performed. DNA was isolated in SDS-EDTA lysis buffer followed by a phenol–chloroform precipitation. The DNA concentration was checked by running the isolated samples in a 1.8% agarose gel. Samples were screened for the presence of haemosporidian parasites (*Haemoproteus* spp. and *Plasmodium* spp.) using a PCR protocol to amplify a region of the parasite mitochondrial cytochrome *b* gene, as suggested by Valkiūnas et al. (2008): F2/R2 (expected size = 91 bp) and 850F/1024R (expected size = 167 bp) (Beadell and Fleischer 2005); FIFI/4292RW2 (expected size = 351 bp) (Ishtiaq et al. 2006) and F2/4292RW2 (expected size = 256 bp), HAEMF/HAEMR2 (expected size = 525 bp) (Waldenström et al. 2004), L15183/H15725 (expected size = 157 bp) (Fallon et al. 2003). The amplicon produced with the primers FIFI/4292RW2 was sequenced on an ABI 3100 using the amplification primers, and sequence similarity was assessed with a BLAST search of avian malaria sequences in Genbank.

Thin blood smears were prepared from fresh non-heparinized blood on individual slides, air-dried, fixed with methanol for 3 min, and stained with Giemsa to be examined to distinguish *Plasmodium* spp. from *Haemoproteus* spp. Smears were analyzed under 1,000× microscope magnification with oil immersion for intra-erythrocytic parasites (Valkiūnas 2005).

Results

Of the 371 birds tested for blood parasites, just one had protozoan parasites detected molecularly with PCR and by analysis of the associated blood smear. The sample was strongly positive for the six different pairs of primers for avian malaria. Based on examination of the smear, the parasite was identified as a *Plasmodium* spp. and the intensity was 22.6% (451 infected erythrocytes from 2000) (Fig. 1). The parasite belongs to the subgenus *Haemamoeba* because of the round shape of mature erythrocytic meronts and roundish gametocytes that exceeded the size of the erythrocyte nucleus, and also because they markedly deform host cells. Based on the morphology of blood stages and the centrally located roundish pigment granules, this parasite is similar to *Plasmodium relictum* (Fig. 1). The cytochrome *b* fragment we sequenced was also a 100% match to a *P. relictum* with a sequence in Genbank

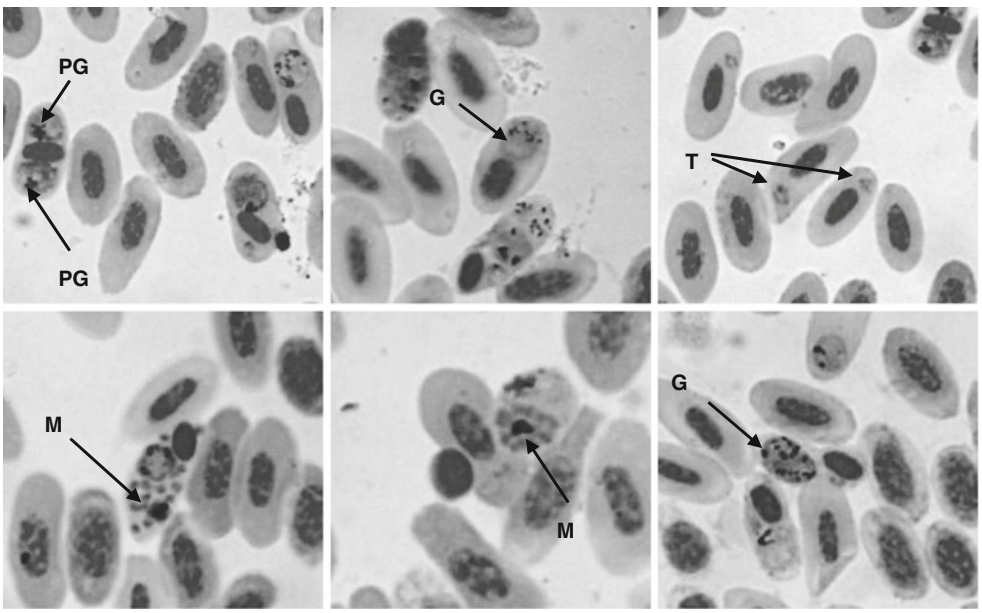

Fig. 1 Blood stages of *Plasmodium* (*Haemamoeba*) *relictum* from the Red Knot *Calidris canutus rufa*: *T*, trophozoites; *M*, meronts; *G*, gametocytes; *PG*, pigment granules. *Arrows* indicate parasites. Giemsa-stained thin blood film

(accession number: id DQ659543.1, lineage code: haplotype P5).

The hematocrit and body weight of the infected bird were both low (36.76% and 114 g, respectively) compared to average values of 51.3% and 158.9 g, respectively, for the other captured Red Knots. The bird was an adult that showed no external signs of illness based on the brightness of breeding plumage, good flight capacity, and absence of feather damage from ectoparasites.

Discussion

The low prevalence of avian malaria could mean that it is rare in Red Knots, or that it is rare in the blood at the time and place where the Red Knots were sampled. Red Knots pass through Delaware Bay during their northwards migration each spring. During this season most bird species get infected with hematozoa because conditions for transmission become optimal in North America (Friend and Franson 1999). The infection reported here is likely an occasional case, as migratory coastal birds of Scolopacidae appear to be infected only rarely with hematozoa because they usually occupy relatively haemosporidian parasite-free salt water habitats as a migratory strategy (Forrester and Humphrey 1981; Piersma 2003; Mendes et al. 2005). The intensity of infection together with the low values of weight and hematocrit displayed by the bird could mean that either the bird was in poor physical condition or it was suffering from the infection, even if did not show other external signs of illness (Remple 2004). In the latter case it could have fitness consequences.

Identification of malaria parasites in blood films from naturally infected birds is difficult, even for experts, because parasitemia is usually light, and simultaneous infections are common (Valkiūnas et al. 2006). In studies of blood parasites, the use of optical microscopy in parallel with the now widely employed molecular methods is strongly encouraged, as microscopy is unlikely to result in false positives, which is a major concern in large-scale PCR studies (Valkiūnas et al. 2006, 2008). Based on its morphological characteristics and partial cytochrome *b* sequence, the parasite found in the smear belongs to the subgenus *Haemamoeba*, and is undoubtedly *Plasmodium relictum* (Valkiūnas 2005; Atkinson 2008).

Some diseases of bacterial and viral origin among shorebird migrants have been recorded along the flyway of Red Knots, for example in Brazil (Araújo et al. 2004; Niles et al. 2005), Uruguay (Niles et al. 2005), and the United States (Southeastern Cooperative Wildlife Disease Study 2002; Niles et al. 2005). However, recent monitoring of avian malaria in the Patagonian wintering and stopover areas used by Red Knots did not return any positives (D'Amico et al. 2007). This is the first report of avian malaria in a wild individual of *C. c. rufa*.

The world's climate is changing rapidly due to global warming (IPCC 2007). Increases in precipitation and temperature could possibly make more breeding sites available for avian malaria vectors like Culicidae mosquitoes. Malaria transmission is exponentially related to temperature (Becker et al. 2003), and an increase in malaria prevalence and intensity in individual Red Knots could have fitness consequences if their immune system is not adapted to parasites and infections (Nordling et al. 1998; Marzal et al. 2005). With global warming and consequently increasing vector populations, the parasite avoidance strategy of Red Knots could be rendered ineffective and possibly increase mortality in this threatened subspecies.

Zusammenfassung

Ein seltener Fall einer Infektion eines wildlebenden Amerikanischen Knutts (*Calidris canutus rufa*, Scolopacidae) mit *Plasmodium* (*Haemamoeba*) *relictum*

Der Amerikanische Knutt (*Calidris canutus rufa*, Scolopacidae) ist ein nearktischer ziehender Watvogel, der in der kanadischen Arktis brütet und den Winter in südamerikanischen Küstengebieten verbringt. Ein seltener Fall von Protozoen im Blut wurde durch molekulare Analysen eines Altvogels entdeckt, der während des Frühjahrszuges am letzten Rastplatz in der Delaware-Bucht, USA, im Jahr 2006 gefangen wurde. Der Parasit wurde als *Plasmodium relictum* in der Untergattung *Haemamoeba* identifiziert, auf der Grundlage der Form der Meronten, rundlichen Gametocyten, und seiner Position in den Erythrozyten bei der Untersuchung der Blutausstriche. Eine unvollständige Cytochrom b-Sequenz stimmte zu 100% mit einer Sequenz von *Plasmodium relictum* überein, Sequenz-Genbank-Zugangsnummer id DQ659543.1 (lineage code haplotype P5). Dies ist der erste Nachweis von Vogelmalaria bei einem wildlebenden Individuum von *C. c. rufa*.

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