Veterinary Parasitology 233 (2017) 52–61

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

# Occurrence of blood parasites in seabirds admitted for rehabilitation in the Western Cape, South Africa, 2001–2013

N.J. Parsons[a,b,*], N.M. Voogt[a], A.M. Schaefer[c], M.A. Peirce[d,e], R.E.T. Vanstreels[f,g]

[a] Southern African Foundation for the Conservation of Coastal Birds (SANCCOB), P.O. Box 11116, Bloubergrant, 7443, South Africa
[b] Bayworld Centre for Research and Education, Port Elizabeth, South Africa
[c] Harbor Branch Oceanographic Institution, Florida Atlantic University, 5600U.S. 1 North, Fort Pierce, FL 34946, USA
[d] MP International Consultancy, 6 Normandale House, Normandale, Bexhill-on-Sea, East Sussex, TN39 3NZ, UK
[e] International Reference Centre for Avian Hematozoa, Queensland Museum, South Brisbane, Queensland, Australia
[f] Laboratory of Wildlife Comparative Pathology (LAPCOM), University of São Paulo, Avenida Professor Orlando Marques de Paiva, 87, São Paulo, SP, 05508-270, Brazil
[g] Marine Apex Predator Research Unit (MAPRU), Department of Zoology, Nelson Mandela Metropolitan University, Port Elizabeth, 6031, South Africa

ARTICLE INFO

*Article history:*
Received 21 August 2016
Received in revised form
15 November 2016
Accepted 1 December 2016

*Keywords:*
Cape cormorant
Cape gannet
Epidemiology
Haemosporida
Marine fauna
Pathology

ABSTRACT

Blood parasites are generally uncommon in seabirds, and knowledge on their epidemiology is further limited by the fact that they often inhabit remote locations that are logistically difficult or expensive to study. We present a long term data set of blood smear examinations of 1909 seabirds belonging to 27 species that were admitted to a rehabilitation centre in Cape Town (Western Cape, South Africa) between 2001 and 2013. Blood parasites were detected in 59% of species (16/27) and 29% of individuals examined (551/1909). The following blood parasites were recorded: *Babesia ugwidiensis*, *Babesia peircei*, *Babesia* sp., *Plasmodium* sp., *Leucocytozoon ugwidi*, *Hepatozoon albatrossi*, *Haemoproteus skuae* and Spirochaetales. Several of the records are novel host-parasite associations, demonstrating the potential of rehabilitation centres for parasite and disease surveillance, particularly for species infrequently sampled from which no host-specific parasites have been described.



## 1. Introduction

Global seabird populations are under threat and have been declining rapidly in recent decades (Croxall et al., 2012), with disease becoming more important as a threat to species with an increase in the International Union for Conservation of Nature (IUCN) status category (Heard et al., 2013). Diseases are infrequently the single threat leading to the decline of seabird populations, but instead act in synergy with other ecological factors (Heard et al., 2013). Blood parasites can affect survival, reproductive success, plumage colouration and changes in community structure (Valkiūnas, 2005; Quillfeldt et al., 2011), and even though most studies on blood parasites in wild bird populations have found little to no evidence of these parasites as the cause of mortality, they were not designed to test for pathogenicity (Bennett et al., 1993). Furthermore, most studies on Antarctic seabirds are limited to the description of diseases and parasites rather than on their prevalence and pathogenicity (Barbosa and Palacios, 2009).

A number of parasites have been described in the blood of seabirds, including intracellular (*Haemoproteus*, *Leucocytozoon*, *Plasmodium*, *Hepatozoon* and *Babesia*) and extracellular protozoans (*Trypanosoma*) (Jones and Shellam, 1999; Peirce, 2005; Quillfeldt et al., 2011), spirochaete bacteria (*Borrelia*) (Lobato et al., 2011) and microfilariae of nematode worms (Onchorcercidae) (Hoberg, 1986; Siers et al., 2010). In comparison to terrestrial birds, however, blood parasites are remarkably uncommon in seabirds. The main hypothesis proposed to explain this pattern is that coastal and marine environments inhabited by these species are not favourable to the arthropod vectors responsible for their transmission (Jones and Shellam, 1999; Quillfeldt et al., 2011). However, the causes may be more complex and include other factors such as the lack of the correct host-parasite assemblages or the immunological capabilities of the hosts (Martínez-Abraín et al., 2004).

Another factor limiting our understanding of the blood parasites of seabirds is the fact that they often inhabit remote locations that are logistically difficult or expensive to reach, limiting the sampling effort for disease surveillance. With the characteristically low

* Corresponding author at: Southern African Foundation for the Conservation of Coastal Birds (SANCCOB), P.O. Box 11116, Bloubergrant, 7443, South Africa.
*E-mail address:* nolaparsons@yahoo.co.uk (N.J. Parsons).

http://dx.doi.org/10.1016/j.vetpar.2016.12.001
0304-4017/

prevalence of blood parasites in seabirds (Quillfeldt et al., 2011) and the endangered status of a large fraction of seabird species regularly handled (Croxall et al., 2012), which limits the sample sizes that can be obtained, it is not surprising that data on the blood parasites of these species is scarce. In this context, rehabilitation centres may provide a unique opportunity for disease surveillance among multiple seabird species, performing structured non-random surveillance (OIE 2010, 2014). In fact, because birds admitted to such facilities often present signs of illness or debilitation, they may serve as valuable sentinels for pathogens that are otherwise uncommon in the species' general population.

In this study we evaluate a 13-year dataset on the presence of blood parasites from 27 seabird species admitted for rehabilitation at the Cape Town facility of the South African Foundation for the Conservation of Coastal Birds (SANCCOB).

## 2. Materials and methods

### 2.1. Admission and bleeding

Seabirds brought to the SANCCOB Cape Town rehabilitation centre from 1 January 2001–31 December 2013 were admitted and treated using the methods described by Parsons and Underhill (2005). African penguins (*Spheniscus demersus*), the most frequently received species at this facility, were not included in this study. All species examined are included in Table 1. The vast majority of these seabirds were found along the coast of the Western Cape, South Africa, however occasionally some individuals were transferred from the Eastern Cape, South Africa. All birds were dusted with an insecticide powder (Karbadust (carbaryl (carbamate) 50 g/kg, Efekto™, Agro-Serve (Pty) Ltd, Benmore, South Africa) on admission to remove all ectoparasites present; this was repeated if necessary. Birds were subjected to varying blood collection schedules depending on veterinary assessment of their clinical condition and logistical constraints; most individuals were bled on admission and then weekly until they were released. Blood samples were obtained from the dorsal metatarsal vein (penguins) or from the medial tibiotarsal vein (other birds). Depending on the size of the bird, a 0.50 or 0.65 mm-thick needle was used to collect a drop of blood that was directly transferred into a heparinised capillary tube. If a bird died or was admitted as dead on arrival, a necropsy was performed and blood was collected from the subcutaneous veins.

### 2.2. Blood smear preparation and analysis

A thin blood smear was prepared immediately after blood collection. The slides were fixed with methanol and stained with a modified Wright-Giemsa stain (Kyro-Quick stain set, Kyron Laboratories (Pty) Ltd, Benrose, South Africa). Smears were examined by light microscopy for approximately 10 min each, using 50X oil immersion objective lens (*c.* 600 erythrocytes per field). Experienced veterinary and laboratory personnel examined the smears and the presence of any blood parasites was recorded. Parasites were identified on the basis of morphology alone. Blood smears of poor quality (too thick, dirty or incorrectly stained) were excluded from the dataset due to a low confidence in their negative results.

### 2.3. Statistical analysis

Individual bird information and blood smear results were recorded and then entered into a centralised database. 'Age class' was classified as chick (C), juvenile (J) or adult (A) on the basis of plumage. 'Time to first positive' was calculated as the number of days from the admission date to the first date in which a blood smear was found to be positive. 'Duration of stay' was calculated as the number of days from admission to outcome (release or death). 'Release rate' was calculated as the percentage of individuals admitted to the facility that survived until release back into the wild.

Additional analyses were conducted for the most prevalent parasite (*Babesia*) in the two seabird species with more than a hundred individuals sampled (Cape cormorants and Cape gannets). Fisher's exact tests were used to compare *Babesia* infections (present or absent) in relation to the rehabilitation outcome (released or died) and by age class. When data was not normally distributed (according to an Anderson-Darling test), the first quartile ($Q_1$), median ($Q_2$), and third quartile ($Q_3$) were used to describe the distribution. Mann-Whitney tests were used to compare time to first positive and duration of stay between *Babesia*-positive and *Babesia*-negative individuals in each age class. Only juveniles or adults were included in these analyses, due to small sample size for chicks. Additionally, Chi-square tests were used to compare the release rate between individuals that were positive or negative to *Plasmodium*.

## 3. Results

### 3.1. Detection and characterisation of blood parasites

A total of 4825 blood smears from 1909 individuals belonging to 27 species of seabirds were examined (Table 1), with an average 2.5 blood smears examined per individual. Five groups of blood parasites were detected (Figs. 1 and 2, Tables 2 and 3), with 551 infected individuals (28.7%). Multiple infections were recorded in 25 cases: *Babesia* and *Plasmodium* (16 Cape cormorants, one crowned cormorant, one rockhopper penguin), *Babesia* and *Leucocytozoon* (six Cape cormorants), *Plasmodium* and Spirochaetales (one rockhopper penguin).

Three parasite species were described in previous publications based on the same dataset: *Haemoproteus skua* in a Subantarctic skua, *Leucocytozoon ugwidi* in a Cape cormorant (Parsons et al., 2010), and *Babesia ugwidiensis* in four species of cormorants (Peirce and Parsons, 2012). All leucocytozooids in the blood smears of Cape, crowned and reed cormorants were considered morphologically consistent with *L. ugwidi* as described in Parsons et al. (2010). Even though not all blood smears of *Babesia* in cormorants were thoroughly morphologically characterized, they were found to be generally consistent with *B. ugwidiensis*. *Babesia peircei* was identified in a king penguin and details will be provided in a future publication. Spirochaetes were not morphologically or genetically characterized, but their morphology was generally consistent with the Relapsing Fever *Borrelia* previously reported in African penguins at the same facility (Yabsley et al., 2012). The several cases of *Plasmodium* spp. were not morphologically characterized, but the occasional presence of large round gametocytes displacing the nucleus (characteristic to the subgenus *Haemamoeba*) and elongated gametocytes that did or did not displace the nucleus (compatible with the subgenera *Novyella*, *Huffia*, *Giovannolaia*) indicates that more than one species were present.

The only case of *Hepatozoon* was morphologically consistent with *Hepatozoon albatrossi* as described by Peirce and Prince (1980). A colour plate was prepared to assist its identification in future studies (Fig. 1). Measurements of 50 gametocytes averaged $10.71 \pm 0.59$ μm in length (range: 8.52–11.23 μm) and $4.97 \pm 0.43$ μm in width (range: 3.87–5.74 μm).

### 3.2. Epidemiology of blood parasites of cape cormorants and cape gannets

For Cape cormorants, *B. ugwidiensis* infections were more frequent in juveniles (59.1%) than in adults (50.6%) ($p = 0.05$). The release rate of *Babesia*-positive juveniles was significantly lower