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SHORT NOTE

Investigation of blood parasites of pygoscelid penguins at the King George and Elephant Islands, South Shetlands Archipelago, Antarctica

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Abstract Parasites may adversely affect the breeding success and survival of penguins, potentially hampering the viability of their populations. We examined 161 pygoscelid penguins (3 *Pygoscelis adeliae*, 98 *Pygoscelis antarcticus*, and 60 *Pygoscelis papua*) at the South Shetlands Archipelago during the 2010–2011 summer; blood smears were examined for 64 penguins (2 *P. adeliae*, 18 *P. antarcticus*, and 44 *P. papua*), and a PCR test targeting *Haemoproteus* sp. and *Plasmodium* sp. was applied for 37 penguins (2 *P. adeliae*, 17 *P. antarcticus*, 19 *P. papua*). No blood parasites were observed, and all PCR tests were negative, leukocyte profiles were similar to those reported in other studies for wild pygoscelid penguins, and all penguins were in good body condition and had no external signs of disease. One specimen of chewing lice (*Austrogoniodes* sp.) was recorded in one *P. antarcticus* at King George Island. Ticks (*Ixodes uriae*) were not observed on the penguins,

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but were found on the ground near *P. antarcticus* nests at King George Island. The absence of avian blood parasites in Antarctic penguins is thought to result from the absence of competent invertebrate hosts in the climatic conditions. Predicted climate changes may redefine the geographic distribution of vector-borne pathogens, and therefore, the occurrence of blood parasites and their invertebrate hosts should be monitored regularly in Antarctic birds, particularly in the northernmost Antarctic Peninsula.

Keywords Antarctic Peninsula · Hematology · Health · Sphenisciformes · Vector-borne pathogen

Introduction

Diseases and parasites may adversely affect breeding success and lead to the mortality of penguins, potentially hampering the viability of their populations (Barbosa and Palacios 2009). In particular, blood parasites are considered a potential threat to the conservation of penguins (Jones and Shellam 1999). Blood parasites known to infect penguins include *Babesia peircei* (Earlé et al. 1993), *Borrelia* sp. (Yabsley et al. 2012), *Haemoproteus* sp. (Levin et al. 2009), *Leucocytozoon tawaki* (Argilla et al. 2013), *Plasmodium* spp. (Rodhain and Adrianne 1952), *Trypanosoma eudyptulae* (Jones and Woehler 1989), and microfilariae (Merkel et al. 2007), all of which have been demonstrated or are thought to be transmitted by arthropods. Avian malaria (*Plasmodium* sp.) is considered most relevant due to its history of causing high-mortality outbreaks in captive penguins and episodic mortalities in wild penguins (Jones and Shellam 1999). Mortalities of wild penguins associated with *Leucocytozoon* sp. (Argilla et al. 2013) have also been reported, raising concerns on the potential significance of these parasites to penguin conservation.

While blood parasites have been reported in wild penguins in temperate and tropical regions, all studies to date have failed to detect blood parasites in penguins or other birds in the Antarctic (Quillfeldt et al. 2011). There are very few records of blood parasites in sub-Antarctic birds, and these were obtained in migratory seabirds that most likely acquired the infection elsewhere, such as *Haemoproteus*-infected brown skuas (*Stercorarius antarcticus*) at South Africa (Parsons et al. 2010), or involve tick-transmitted parasites such as *Hepatozoon* sp. in albatrosses (*Diomedea* spp.) at Bird Island (Peirce and Prince 1980). The most southern record of blood parasites in penguins was provided by Laird (1950), who reported *Plasmodium relictum* in a yellow-eyed penguin (*Megadyptes antipodes*) at Campbell Island (52°32′S 169°9′E).

With the exception of these rare reports, the lack of blood parasites in the sub-Antarctic and Antarctic avifauna is considered to be due to an inability of their arthropod hosts to survive under such relatively cold and harsh environmental conditions (Merino et al. 1997; Jovani et al. 2001), although competition among competent and non-competent ectoparasites could also play a role (Martínez-Abraín et al. 2004). Temperature may also be a limiting factor for the development of the protozoan parasites within the arthropod host (Benning et al. 2002). Predicted climate changes are expected to produce poleward changes in the geographic distribution of arthropods and arthropod-transmitted pathogens (Harvell et al. 2002), and regions such as sub-Antarctic islands and the Antarctic Peninsula can be predicted to be at higher risks of the emergence of conservation-threatening parasites such as avian malaria. In particular, the Antarctic Peninsula has been one of the regions with the most pronounced warming trends in the recent past (Vaughan et al. 2003) and is inhabited by large populations of *Pygoscelis* sp. penguins (Woehler 1993). This is of particular concern as pygoscelid penguins are known to be highly susceptible to avian malaria (Rodhain and Adrianne 1952; Griner and Sheridan 1967).

Blood smear examination is the standard method for the detection of hemoparasites, while molecular methods such as polymerase chain reaction (PCR) are highly valuable to detect cases of chronic infections and low parasitemia (Garamszegi 2010). Blood smear examination also allows for differential erythrocyte and leukocyte counts, conveying indirect information on the general health status and the physiological and immune system responses to stress, reproduction, and pathogens (Vleck et al. 2000; Clark et al. 2009). In this study, we present integrated results from physical examinations, blood smear examinations, and PCR to obtain information on the health status and parasites of pygoscelid penguins at the King George and Elephant Islands, South Shetlands Archipelago.

Materials and methods

A total 161 randomly selected adult penguins—3 Adelie penguins (*Pygoscelis adeliae*), 98 Chinstrap penguins (*Pygoscelis antarcticus*), and 60 Gentoo penguins (*Pygoscelis papua*)—were examined between December 2010 and March 2011 at breeding colonies in five sites at the South Shetlands Archipelago: Keller Peninsula (62°4′55″S 58°24′32″W), Chabrier Rock (62°11′13″S 58°17′50″W), Demay Point (62°12′25″S 58°27′7″W), and Thomas Point (62°10′23″S 58°28′31″W) at Admiralty Bay, King George Island, and Stinker Point, Elephant Island (61°13′20″S 55°21′35″W). Penguins were subjected to a rapid clinical examination for lesions and external signs of disease (general external aspect, respiratory frequency, oral mucosae, eyes and conjunctivae, limb and abdominal palpation, external lesions, cloaca). Ectoparasites were

carefully visually inspected for and collected in ethanol 70 %; lice were mounted and identified (Clay 1967; Palma 1978). Blood samples were collected from the metatarsal or jugular veins of 64 of these penguins from January 31 to February 8, 2011 (late chick-rearing and early molting): 2 Adelie penguins (Thomas Point), 18 Chinstrap penguins (7 at Keller Peninsula, 10 at Demay Point, and 1 at Thomas Point), and 44 Gentoo penguins (24 at Stinker Point, 18 at Keller Peninsula, and 2 at Thomas Point).

For each penguin, two thin blood smears were freshly prepared, then fixed in methanol and stained with Diff-Quick and Wright-Rosenfeld (Rosenfeld 1947). Minimum 3×10^4 erythrocytes per animal were examined for blood parasites under 1,000× magnification light microscopy. Differential leukocyte counts were conducted for 200 leukocytes, and pro-erythroblasts and erythroblasts were quantified as a percentage of the cells from the erythrocytic lineage (Clark et al. 2009); the heterophil-to-lymphocyte ratio was calculated (Vleck et al. 2000). Additionally, for 37 of these penguins a small aliquot of blood was frozen for molecular testing: 2 Adelie penguins (Thomas Point), 17 Chinstrap penguins (6 at Keller Peninsula, 10 at Demay Point, and 1 at Thomas Point), and 19 Gentoo penguins (17 at Keller Peninsula, 2 at Thomas Point). DNA was extracted from the blood samples in the laboratory using a standard phenol/chloroform and proteinase K extraction followed by ethanol precipitation (Sambrook et al. 2001) and non-phenolic extraction (Carvalho 2010); DNA extraction was verified and quantified through Nanodrop 2000 spectrophotometry (ThermoScientific, Wilmington, USA). A nested PCR targeting sequences of the cytochrome *b* gene of *Haemoproteus* sp. and *Plasmodium* sp. was used, as described by Hellgren et al. (2004) (each reaction had 25 μL, with 75 ng of sample DNA; first reaction with primers HaemNFI and HaemNR3, and second reaction with primers HaemF and HaemR2). Blood samples from chicken experimentally infected with *Plasmodium gallinaceum* or raised in arthropod-free environments were used as positive and negative controls, respectively. Gel electrophoresis was conducted to visualize amplification products, using 3 % agarose gel, SYBR Safe (Invitrogen, Carlsbad, USA), and a high-resolution imaging system (Gel Doc EZ System—Bio-Rad, Hercules, USA).

Mann–Whitney tests were used to compare relative leukocyte counts between Gentoo penguins sampled at Stinker Point and Keller Peninsula, and between Chinstrap and Gentoo penguins sampled at Keller Peninsula. Significance level was 0.05 for all tests. All research procedures were conducted under the Brazilian Antarctic Project (PROANTAR) and complying with the Scientific Committee on Antarctic Research (SCAR).

Results

All penguins had no clinical signs of disease. One male chewing louse was present in a Gentoo penguin at Keller Peninsula, King George Island. We determined that louse belonged to the genus *Austrogoniodes*. No other ectoparasites were observed on the penguins. Even though not specifically searched for, one adult female tick *Ixodes uriae* was found amidst guano and rocks at Chabrier Rock, an islet with a breeding colony of approximately 800 Chinstrap penguins, 31 of which were examined and presented no ticks.

No blood parasites were observed on the blood smears, and all PCR tests were negative. Differential leukocyte counts are presented in Table 1; results from Thomas Point are omitted due to the low sample sizes (2 *P. adeliae*, 1 *P. antarcticus*, and 2 *P. papua*). All animals had a few circulating polychromatic erythrocytes (<10 %), which were abundant (approx. 20 %) only in two Gentoo penguins. Erythroblasts and pro-erythroblasts were seen sporadically regardless of the abundance of polychromatic erythrocytes, having occurred in one Chinstrap penguin (6 %) and in nine Gentoo penguins (20 %). Significant differences between Gentoo penguins from Keller Peninsula and Stinker Point occurred in monocyte ($P = 0.007$), heterophil ($P = 0.039$), and lymphocyte counts ($P = 0.024$), but

Table 1 Differential leukocyte counts (Mean ± SD) for penguins at Demay Point (DP), Keller Peninsula (KP), and Stinker Point (SP), January–February 2011

	Chinstrap penguins		Gentoo penguins	
	DP ($n = 10$)	KP ($n = 7$)	KP ($n = 18$)	SP ($n = 24$)
Heterophils (%)	47.6 ± 11.0	37.2 ± 3.8	52.3 ± 8.1	46.7 ± 9.0
Lymphocytes (%)	48.7 ± 11.3	59.8 ± 4.2	41.3 ± 7.6	47.0 ± 9.6
Eosinophils (%)	2.9 ± 2.7	1.1 ± 0.7	5.1 ± 2.7	5.7 ± 4.0
Basophils (%)	0.2 ± 0.5	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.5
Monophils (%)	0.7 ± 0.6	1.8 ± 0.7	1.2 ± 1.4	0.3 ± 0.5
Heterophil-to-lymphocyte ratio	1.09 ± 0.59	0.63 ± 0.11	1.33 ± 0.40	1.08 ± 0.46

not in eosinophil ($P = 0.980$) and basophil counts ($P = 0.919$). Significant differences between Chinstrap and Gentoo penguins from Keller Peninsula occurred in heterophil ($P = 0.001$), lymphocyte ($P = 0.001$), eosinophil ($P = 0.001$), and monocyte counts ($P = 0.036$), but not in basophil counts ($P = 0.759$).

Discussion

Austrogoniodes spp. chewing lice and *I. uriae* ticks are common parasites of sub-Antarctic and Antarctic penguins, including at the South Shetlands Archipelago (Barbosa et al. 2011). We were not able to identify the species of the recovered *Austrogoniodes* louse because its reproductive tract was damaged; however, it is most likely to be *Austrogoniodes gressitti* (see Clay 1967). While it is unknown whether *Austrogoniodes* spp. are competent hosts or vectors to any of the blood parasites recorded so far on penguins, *I. uriae* is thought to play a key role in the transmission of *Babesia peircei* and *Borrelia* sp. to penguins (Earlé et al. 1993; Yabsley et al. 2012) and between penguins and long-distance migrating seabirds such as skuas and storm petrels (Olsén et al. 1995). Our results suggest an overall low prevalence and intensity of infection by *Austrogoniodes* sp. and *I. uriae* in pygoscelid penguins at the sampling sites; however, this may be an underestimate associated with visual inspection (Clayton and Drown 2001). In some instances, extreme parasitism by *I. uriae* has been shown to hamper reproduction or lead to mortality (Gauthier-Clerc et al. 1998; Mangin et al. 2003). We observed no apparent signs of disease or negative effects from ectoparasitism (which does not exclude the possibility of subclinical disease); however, it is also possible that sick individuals skipped breeding and/or were distributed elsewhere within the breeding colonies and could therefore have been less likely to be sampled in the study.

Low but statistically significant differences were observed in the leukocyte profiles among localities and penguin species, particularly in the heterophil, lymphocyte, and monocyte counts. Low-level changes in leukocyte differential counts could potentially reflect differences in exposure to and resultant physiological changes associated with microbial infections, parasites, stress, and breeding (Vleck et al. 2000; Clark et al. 2009). However, as all relative leukocyte counts were similar to those observed in other studies in wild pygoscelid penguins (e.g., Hawkey et al. 1989; Vleck et al. 2000) and both erythrocyte and leukocyte results were well within the reference values for healthy individuals of other avian species (see Clark et al. 2009), it is difficult to determine the biological significance, if any, of the observed differences. The heterophil-to-lymphocyte ratios observed in this study for Chinstrap and Gentoo penguins (mean 0.63–1.33) were considerably lower than those reported by other authors for captive penguins (mean > 1.8) (Hawkey et al. 1985; ISIS 2002), and this may reflect stress—as occurs in captivity—is known to significantly increase absolute and relative heterophil counts (Vleck et al. 2000).

The absence of avian blood parasites in this and in previous studies should not dissuade future studies in the sub-Antarctic and Antarctic region. The emergence of pathogens in regions where they had been historically absent can have profound ecological and conservation consequences, as exemplified by the population decreases and extinctions of native Hawaiian birds during the twentieth century following the introduction of avian malaria and poxvirus (Atkinson and LaPointe 2009). More recently, the introduction of avian malaria to the Galápagos Islands has raised great concern on the potential implications for the conservation of the Galápagos penguin (*Spheniscus mendiculus*) and other endemic birds (Levin et al. 2009). Because penguins are remarkably susceptible to avian malaria (Jones and Shellam 1999), a climate change-induced emergence of this disease in sub-Antarctic and Antarctic regions could have dramatic consequences. Furthermore, tick-transmitted pathogens such as *Babesia* sp. or *Hepatozoon* sp. can also be expected to better thrive in these regions if they became warmer. It is therefore important to investigate and monitor these pathogens in the sub-Antarctic and Antarctic region to assure their early detection if ever they are to occur. The South Shetlands Archipelago, because of their relatively mild climate and geographic positioning close to the northern tip of the Antarctic Peninsula, is a strategic location for monitoring these parasites. In this context, it will be important for the Antarctic programs, including that of Brazil (PROANTAR), to develop and maintain consistent and long-term monitoring of the wildlife health in the region.

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