Parasitology

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Determinants of external and blood parasite load in African penguins (*Spheniscus demersus*) admitted for rehabilitation

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Abstract

We investigate the factors associated with the occurrence and abundance of external and blood parasites in African penguins (*Spheniscus demersus*), an endangered seabird that breeds exclusively on the coasts of Namibia and South Africa. External parasites were collected using the dust-ruffling method from 171 African Penguins admitted at a rehabilitation facility in the Western Cape, South Africa. Additionally, blood smears were obtained upon admission and weekly during rehabilitation and examined for blood parasites. Fleas *Parapsyllus longicornis humboldti*, ticks *Ornithodoros capensis* and lice *Austrogoniodes demersus* were recovered from 93, 63 and 40%, respectively, of the penguins upon admission to the centre. Rescue location and age group were identified as significant determinants of flea abundance, whereas month of admission was a significant determinant of tick abundance. Blood parasites were also common on admission, with *Babesia* being the most frequent (46% prevalence) whereas *Borrelia* was recorded sporadically (1.2%) and *Plasmodium* was recorded once. The prevalence and abundance of ticks on admission was positively associated with *Babesia* infection on admission. Our findings demonstrate the variability and contributing factor of parasite infections in an endangered species of penguin, and highlight the need for additional research on the parasite–host dynamics involving these potential disease vectors.

Introduction

The African penguin (*Spheniscus demersus*) is a seabird native to Southern Africa, and breeds exclusively on the coasts of Namibia and South Africa (Crawford *et al.*, 2013). The species is currently classified as Endangered, having lost nearly 95% of its population since the beginning of the 20th century (Crawford *et al.*, 2011; BirdLife International, 2018). At present, decreased food availability due to competition with fisheries has been identified as the main threat to this species' conservation, along with marine pollution, habitat degradation, climate change and disease (Crawford *et al.*, 2011; Trathan *et al.*, 2015). Roughly one-third of the African penguin population breeds in the Western Cape of South Africa (BirdLife International, 2018), a semi-arid region characterized by relatively warm and dry summers (December to February: average daily maximum temperature 24.9–26.5°C, mean monthly precipitation 15–17 mm) and cold and rainy winters (June to August: 17.5–18.1°C, 77–93 mm) (National Oceanic and Atmospheric Administration, 2019).

Although several studies have investigated the ecology and negative effects that external parasites can have on penguins, most studies focused on hard ticks (Ixodidae) (Gauthier-Clerc *et al.*, 1998, 2003; Frenot *et al.*, 2001; Benoit *et al.*, 2007, 2009; Jansen van Rensburg, 2010; Barbosa *et al.*, 2011), and few have investigated the factors driving the ecology and health effects of soft ticks (Argasidae), fleas (Siphonapthera) and lice (Phthiraptera) on penguins (Duffy and Daturi, 1987; González-Acuña *et al.*, 2013; Espinaze *et al.*, 2019). Three species of external parasites are known to infect wild African penguins: the fleas *Parapsyllus longicornis humboldti* and *Echidnophaga gallinacea*, the soft tick *Ornithodoros capensis* and the chewing louse *Austrogoniodes demersus* (Von Keler, 1952; Zumpt, 1959; Beaucournu and Rodhain, 1990; Espinaze *et al.*, 2019). The tick *O. capensis* is suspected to transmit blood parasites to African penguins, such as the piroplasmid protozoan *Babesia peircei* (Earlé *et al.*, 1993), the spirochete bacterium *Borrelia* sp. (Yabsley *et al.*, 2012) and the rickettsial bacterium '*Candidatus* Anaplasma sphenisci' (Vanstreels *et al.*, 2018*a*). However, there is also speculation that the hard tick *Ixodes uriae*, a frequent parasite of penguins in other regions of the world, might also infect African penguins and transmit these pathogens (Earlé *et al.*, 1993; Peirce, 2000; Yabsley *et al.*, 2012; Vanstreels *et al.*, 2018*a*). Other blood

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parasites known to infect African penguins include the haemosporidian protozoa *Plasmodium* spp. and *Leucocytozoon tawaki* (Fantham and Porter, 1944; Earlé *et al.*, 1992; Parsons and Underhill, 2005), of which mosquitoes and simulid flies are vectors, respectively.

A recent study found that penguin age group (chick *vs* adult), colony location (mainland *vs* island), nest density (total and active nests) and season (spring *vs* autumn/winter) were significant factors determining the abundance of fleas and ticks and the prevalence of blood parasites in wild African penguins at colonies in the Western Cape, South Africa (Espinaze *et al.*, 2019). In this study, we investigate the occurrence of external and blood parasites in African penguins admitted at a rehabilitation centre in the same region, and evaluate whether their prevalence and abundance differ from those reported in wild African penguins and which factors of the individual history of these birds may influence the infection by different external and blood parasites.

Materials and methods

Oiled, sick and injured African penguins found ashore or at colonies in the Western Cape are frequently recovered and sent to the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB), where they are rehabilitated following standard protocols and then released back into the wild (Parsons and Underhill, 2005; Klusener *et al.*, 2018). A total of 171 African Penguins admitted to the SANCCOB facility at Cape Town (33°50′02″S, 18°29′29″E) in the Western Cape, South Africa, from 1 May to 31 December 2017 were evaluated in this study. Sampling took place in every month except for September due to logistical problems. Due to animal welfare concerns, penguins that were severely debilitated (unable to lift themselves), with open wounds, or with body mass lower than 500 g were not screened for parasites and thus not included in the study.

The following individual variables were recorded for each penguin upon admission to the facility: month of admission, rescue location, age group, age subgroup and reason for admission. Rescue location was categorized as: Simon's Town (34°11′50″S, 18°27′04″E; comprising at Seaforth, Windmill, Boulders and Burgher's Walk), Stony Point (34°22′26″S, 18°53′41″E) and other locations (within the Western Cape Province). Age group was categorized into: chick (nestlings, with downy plumage present), blue (young birds about to fledge or recently fledged, having completely lost their downy plumage and with a shiny grey-blueish plumage), juvenile (young birds whose plumage has a dull brown tone) or adult (characteristic plumage with black and white bands) (Whittington *et al.*, 1996). For chicks, the age subgroup was categorized into: P2 (medium chick, secondary down plumage fully developed), P3 (large chick, having lost less than 50% of down plumage) and P4 (large chick, having lost more than 50% of down plumage) (Klusener *et al.*, 2018). Reason for admission was categorized into five previously established categories: pre-emptive removal (chicks removed because their nests were at high-risk areas), abandonment, debilitation, injury and molt (Parsons *et al.*, 2018*a*).

Parasite collection and identification

The dust-ruffling method (Walther and Clayton, 1997) was employed to collect external parasites upon admission to the rehabilitation facility. The entire body (except the mucosae) of each penguin was dusted with pesticide powder (carbaryl 50 g/kg) and placed in a non-transparent 150 L plastic container laid with a sheet, to avoid unnecessary stress on the bird. After 10–20 min, the penguin's feathers were ruffled, to remove both the pesticide powder and external parasites from the penguin while still in the container. The sheet and the container were carefully examined for parasites, as well as the transport box in which the penguins were brought in to the facility (only one penguin was transported per box), and the parasites were carefully collected in 70% ethanol with the assistance of forceps. Additionally, each penguin was physically examined by veterinarians, including full-body palpation (for lesions, deformities, pain response, etc.) that would be expected to identify any attached hard ticks if they had been present. Based on their general morphological characteristics upon examination under a stereomicroscope, parasites were counted and classified as flea, tick or louse. All dust-ruffling procedures and parasite counts were performed by the same person (A. Snyman) to ensure consistency.

A randomly-selected subset of approximately 50 parasites of each category were further examined for species identification based on published morphological keys and descriptions (Jordan, 1942; de Meillon, 1952; Von Keler, 1952; Clay, 1967; Clay and Moreby, 1967; Hoogstraal *et al.*, 1976, 1985; Beaucournu and Rodhain, 1990; Muñoz-Leal *et al.*, 2017). To illustrate the morphological characteristics evaluated for species identification of fleas and ticks, scanning electron microscope photographs were prepared from specimens collected at SANCCOB in previous years; for this purpose, parasites were critical-point dried (CPD 030, Balzers AG, Balzers, Liechtenstein) and sputter-coated (SCD 050, Balzers AG). Lice were slide-mounted using the Canada Balsam technique (Palma, 1978) and photographed under light microscopy.

Blood samples were collected upon admission and weekly during rehabilitation for each individual. Thin blood smears were freshly prepared and stained with a modified Wright–Giemsa stain (Kyro-Quick, Kyron Laboratories, Benrose, South Africa). Blood smears were examined under a light microscope with 500× magnification for approximately 10 min, and blood parasites were identified based on their morphological characteristics (Earlé *et al.*, 1993; Peirce, 2000; Valkiūnas, 2005; Valkiūnas and Iezhova, 2018). The parasitaemia was roughly estimated by counting the number of parasites in 10 adjacent microscope fields on the tail of the blood smear (500× magnification, each field covering an area of 0.565 mm^2 or *c.* 900 erythrocytes).

Statistical analyses

For the purpose of the analysis, 'prevalence' was expressed as the number of infected penguins divided by the number of penguins examined. For each external parasite, 'intensity' was calculated as the total number of individual parasites divided by the number of penguins infected by that parasite, and 'abundance' was defined as the total number of individual parasites divided by the number of penguins examined. 'Interval to first diagnosis' was used to refer to the interval (in days) from admission to the first time when a blood smear from a penguin was identified as positive. 'Infection ratio' was calculated by dividing the number of penguins positive for a blood parasite at any moment throughout their stay at the rehabilitation centre (including on admission) by the number of penguins examined. For blood parasites, 'prevalence on admission' and 'highest parasitaemia on admission' refer to blood smears obtained on the day of admission to the rehabilitation centre, and 'overall highest parasitaemia' refer to all blood smears obtained for each penguin throughout its stay at the rehabilitation centre (including on admission).

The χ^2 or Fisher exact tests were used to evaluate whether there were relationships between the presence of different parasite types in relation to one another. Anderson–Darling tests were used to determine whether external parasite intensity or abundance was normally distributed. The following transformations were attempted: natural logarithm, three-parameter lognormal,

exponential, two-parameter exponential, Box–Cox and Johnson. Data on abundance of fleas was normalized following Johnson transformation, whereas the abundance of ticks and lice could not be normalized.

Forward selection General Linear Models were used to evaluate which individual variables (month of admission, rescue location, age group, reason for admission) could be used to predict the abundance (or transformed abundance, for fleas) of each external parasite type, followed by χ^2 or Fisher post-hoc tests. P value threshold for variable inclusion in the model was 0.2. Binary logistic regression was used to evaluate whether the individual variables and the abundance of external parasites (transformed flea abundance, tick abundance, louse abundance) could predict *Babesia* prevalence on admission or its infection ratio throughout rehabilitation. *Borrelia* and *Plasmodium* could not be included in this analysis due to an insufficient number of infected penguins. Significance level was 0.05 for all tests.

Results

Three species of external parasites were identified, one flea species, *P. longicornis humboldti*, one tick species, *O. capensis* and one louse species, *A. demersus*. Supplementary File S1 provides detailed photographs illustrating the diagnostic morphological features of these species.

The raw data evaluated in this study are provided in Supplementary File S2. Table 1 summarizes the prevalence, intensity and abundance of external parasites. Maximum recorded intensity was 311 fleas, 154 ticks and 142 lice (different penguins). Only one penguin (0.6%) did not have external parasites, and the following parasite combinations were recorded: only fleas (25.7%), only ticks (4.7%), only lice (1.2%), fleas and ticks (28.7%), fleas and lice (9.4%), ticks and lice (0.6%), fleas, ticks and lice (29.2%). There was no significant relationship between the presence of fleas and ticks ($P = 0.539$) or of fleas and lice ($P = 0.365$). In contrast, there was a significant association between the presence of ticks and lice ($\chi^2 = 5.001$, $P = 0.025$): louse-infected penguins had a higher prevalence of ticks (74%) than did louse-free penguins (56%), and tick-infected penguins had a higher prevalence of lice (47%) than did tick-free penguins (29%).

General Linear Models identified rescue location and age group as significant predictors of flea abundance, whereas month of admission and reason for admission were included in the model but were not statistically significant (Fig. 1A). Month of admission was a significant predictor of tick abundance, and age group was included in the model but was not statistically significant (Fig. 1B). Finally, for louse abundance, the month of admission was the only variable included in the model, even though it was not significant (Fig. 1C).

Blood parasites were not thoroughly characterized in each case, but were considered morphologically consistent with *B. peircei* and Relapsing Fever *Borrelia* as previously recorded in African penguins at the same facility (Earlé *et al.*, 1993; Yabsley *et al.*, 2012). Only one case of *Plasmodium* sp. infection was recorded, but the species involved could not be identified due to the low parasitaemia. No other blood parasites were detected.

Table 2 summarizes the prevalence of blood parasites upon admission, the infection ratio, the interval to first diagnosis and the highest parasitaemia. Maximum interval to the first diagnosis was 45 days for *Babesia* and 13 days for *Borrelia*. Maximum parasitaemia recorded in this study was 40 parasites per field for *Babesia*, six parasites per field for *Borrelia*, and one parasite per field for *Plasmodium*.

Eighty-nine penguins (52.0%) did not have blood parasites on admission, and for those that were infected the following parasite combinations were recorded on admission: only *Babesia* (44.4%), only *Borrelia* (1.2%), only *Plasmodium* (0.6%), *Babesia* and *Borrelia* (1.8%). There was no significant relationship between the presence of different blood parasites on admission (all $P > 0.9$). Forty-seven penguins (27.5%) did not have blood parasites detected during rehabilitation. The following parasite combinations were recorded with regards to their infection ratio: only *Babesia* (69.0%), only *Plasmodium* (0.6%), *Babesia* and *Borrelia* (2.9%). There were no significant relationships in the infection ratio of these blood parasites to one another (all $P > 0.2$).

The presence of ticks on admission was not associated with the prevalence of *Babesia* on admission ($\chi^2 = 3.177$, $P = 0.075$), but it was associated with the infection ratio of *Babesia* ($\chi^2 = 4.210$, $P = 0.040$): tick-infected penguins had a higher infection ratio of *Babesia* (78%) than did tick-free penguins (62%), and penguins recorded as *Babesia*-positive during rehabilitation had a higher prevalence of ticks on admission (68%) than did penguins that remained *Babesia*-negative throughout rehabilitation (50%). Although all penguins that were *Borrelia*-positive on admission or became *Borrelia*-positive during rehabilitation had ticks on admission, the presence of ticks of admission was not significantly associated with this parasite's prevalence on admission ($P = 0.532$) nor infection ratio ($P = 0.159$). Binary logistic regression analysis identified month of admission as the only significant predictor of *Babesia* on admission, whereas flea and tick abundance were included in the model but were not significant (Fig. 2).

Table 1. Epidemiological parameters on the occurrence of ectoparasites recovered from African penguins (*Spheniscus demersus*) admitted for rehabilitation

Parasite	Prevalence on admission (%)	Intensity on admission	Abundance on admission
Fleas	93.0	49.0 ± 58.1 (8, 27, 72)	45.5 ± 57.4 (7, 22, 61)
Ticks	63.2	10.4 ± 18.7 (2, 5, 12)	6.6 ± 15.7 (0, 1, 7)
Lice	40.4	5.8 ± 17.1 (1, 2, 5)	2.4 ± 11.2 (0, 0, 2)

Intensity and abundance are represented as: mean ± S.D. (first quartile, median, third quartile).

Discussion

The vast majority (99%) of the African penguins examined in this study had external parasites, with fleas being most frequently recorded (93%) followed by ticks (63%) and lice (40%). The three taxa of external parasites identified in this study (*P. longicornis humboldti*, *O. capensis* and *A. demersus*) are in agreement with previous studies on the external parasites of the African penguin (Von Keler, 1952; Zumpt, 1959; Beaucournu and Rodhain, 1990).

The prevalence of external parasites in this study was remarkably higher than that reported for wild African penguins at the Western Cape (Table 3). In part, this may be related to the fact that our sample largely corresponded to penguins from mainland colonies, which are known to have a higher prevalence of fleas, ticks and *Babesia*/*Plasmodium* than island colonies (Espinaze *et al.*, 2019). Perhaps more significantly, however, the generally higher prevalence of parasites seen in this study might be related to the fact that our sample consisted of individuals that were brought for rehabilitation. As such, our sample is bound to overrepresent the sicker and weaker individuals of the population, and the epidemiological parameters estimated in this study (e.g. prevalence, intensity, abundance) are thus likely to be higher than those of the overall population. It is also worth noting that even though the dust-ruffling method is generally considered a reliable method to estimate the external parasite load of birds, it is known to slightly underestimate the parasite load when compared to other methods such as body washing of carcasses (Clayton and

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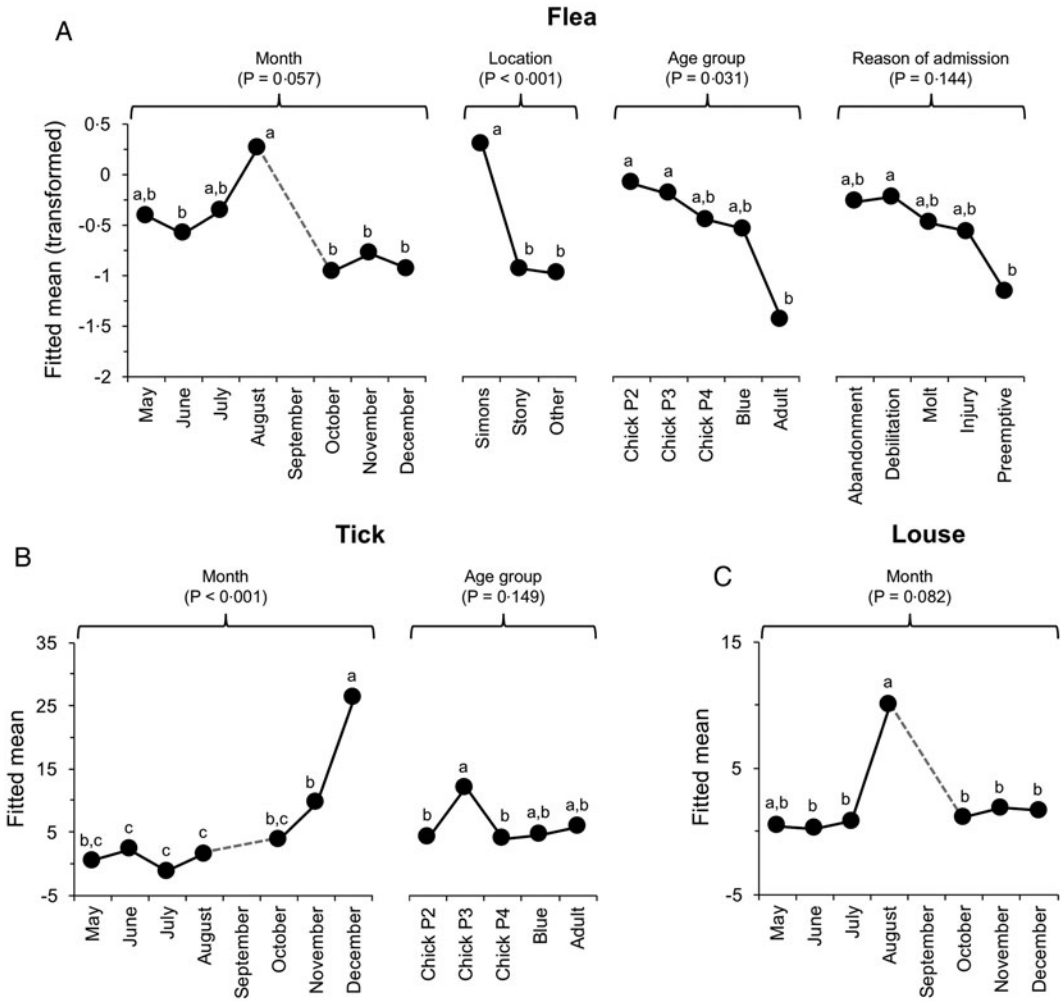

Fig. 1. Main effects plots of the individual variables on the abundance of external parasites recovered from African penguins (*Spheniscus demersus*) admitted for rehabilitation. *P* values represent the significance of each variable in the General Linear Model and different letters adjacent to a node represent a significant difference in χ^2 or Fisher post-hoc tests.

Table 2. Epidemiological parameters on the occurrence of blood parasites detected in blood smears obtained from African penguins (*Spheniscus demersus*) during rehabilitation

Parasite	Prevalence on admission (%)	Parasitaemia on admission	Infection ratio (%)	Overall highest parasitaemia	Interval to first diagnosis
Babesia	46.2	9 (5–13)	71.9	8 (5–15)	0 (0–8)
Borrelia	1.2	1.5	2.9	2 (1–4)	7 (0–12.5)
Plasmodium	0.6	1	0.6	1	0

Infection ratio, parasitaemia and interval to first diagnosis are represented as: median (first quartile–third quartile)

Drown, 2001). However, we examined live specimens of an endangered species, most of which were in a debilitated health condition, and dust-ruffling was determined as the best feasible method in consideration to animal welfare and ethical concerns.

While lice remain on the penguins throughout their life cycle, fleas and soft ticks will alternate between feeding on the penguins (soft ticks usually feed at night) and hiding in the nest materials (Duffy and Daturi, 1987; Whitehead *et al.*, 1991; Walther and Clayton, 1997). As a result, the proportion of the population of fleas and ticks that will be found on a penguin at any given time relative to the population at the nest is likely to vary substantially. In this study, flea abundance was greater in penguins admitted from Simon's Town than in those from Stony Point or other locations, but a similar effect was not detected for ticks. The reasons for this are unclear, but could be related to differences in the humidity and coarseness of the nesting substrate (Whitehead *et al.*, 1991; Kemper *et al.*, 2007), vegetation (Dawson, 2004) or nest cover (Murray and Vestjens, 1967). Some researchers have also suggested that artificial nests used for African penguins might favour the build-up of external parasite loads (Kemper *et al.*, 2007; Sherley *et al.*, 2012); however, this remains to be adequately evaluated.

Age group was an important determinant of the abundance of fleas but not of ticks and lice. Fleas do not need to attach to their hosts for extended periods of time; however, the fact that they are found most frequently on chicks might be related to the fact that the downy plumage of penguin chicks might be easier to penetrate than the dense waterproof plumage of juvenile and adult penguins.

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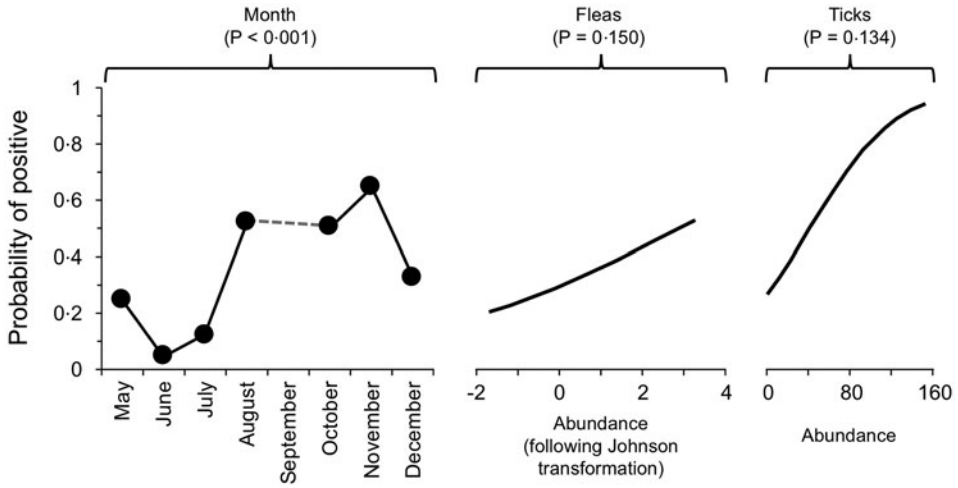

Fig. 2. Main effects plot of the individual variables on the positivity to *Babesia* sp. in African penguins (*Spheniscus demersus*) admitted for rehabilitation. *P* values represent the significance of each variable in the binary logistic regression analysis.

Table 3. Comparison of the prevalence of external and blood parasites in African penguins (*Spheniscus demersus*) in this and previous studies

	Parsons *et al.* (2016) (wild birds)	Espinaze *et al.* (2019) (wild birds)		This study (birds admitted for rehabilitation)		
	Adults (*N* = 100) (%)	Chicks (*N* = 583) (%)	Adults (*N* = 210) (%)	Chicks (*N* = 141) (%)	Juveniles (*N* = 25) (%)	Adults (*N* = 5) (%)
P. longicornis	Not evaluated	81.9[a]	45.2[a]	93.6	96.0	60.0
O. capensis	Not evaluated	13.0	1.0	63.8	68.0	20.0
A. demersus	Not evaluated	0	0	39.7	44.0	40.0
Plasmodium sp.	0	43.0[b]	8.1[b]	0	0	20.0
Babesia sp.	3.0			48.9	40.0	0
Borrelia sp.	0	2.1	0	1.4	0	0

[a]Data from Dassen island was not included because flea larvae were not counted.
[b]*Babesia* and *Plasmodium* were not differentiated.

This is corroborated by previous observations that in adult penguins the fleas are usually restricted to the brood patch (Murray, 1967). Furthermore, penguin chicks are nest-bound and would thus be exposed to fleas for a greater proportion of the time than adults, which periodically leave the nest to forage at sea.

In the Western Cape, egg laying of African penguins usually peaks in March and April followed by a secondary peak in October to December (Crawford *et al.*, 1999; Wolfaardt and Nel, 2003). Incubation lasts 37–38 days and is followed by an average 74–90 days of chick rearing (Williams, 1995), and it may therefore be predicted that most chicks from the March–April egg laying peak will fledge in July and August. The peak of the abundance of fleas and lice in penguins admitted for rehabilitation in August can therefore be interpreted as the result of a gradual build-up in the population of these parasites in response to the breeding seasonality of their host, possibly also with a contribution from the favourable humidity conditions from the rainy winter. On the other hand, tick abundance remained low during most of the breeding season and then increased at the end of the year and peaked in December. Considering that soft ticks tend to be more abundant in nests with dry substrate (Daturi, 1986; Kemper *et al.*, 2007), it is possible that the increase in tick abundance from October onwards reflects the progression towards the drier summer weather.

Previous studies have shown that the reason for admission is a strong predictor of haematological parameters and rehabilitation outcomes for African penguins admitted for rehabilitation at SANCCOB, with abandoned and debilitated penguins consistently in poorer health status than those in the other categories (Parsons *et al.*, 2018*b*; Vanstreels *et al.*, 2018*b*). The greater abundance of fleas, ticks and lice in these categories is therefore not surprising considering the opportunistic nature of these parasites. However, because fleas and ticks can also have a substantial impact on the health of their hosts and potentially even contribute to nest desertion (Duffy, 1983; Oppliger *et al.*, 1994), it is difficult to ascertain the direction of this causal relationship.

Blood parasites were remarkably common in African penguins examined in this study, with *Babesia* being the most frequent (46% prevalence on admission, 72% infection ratio), whereas *Borrelia* was recorded only sporadically (1% prevalence on admission, 3% infection ratio) and *Plasmodium* was only recorded once. Although these parasites have long been known to infect wild African penguins (Coles, 1941; Fantham and Porter, 1944), their prevalence in this study was markedly higher than in apparently wild healthy adult African penguins sampled in previous studies (Parsons *et al.*, 2016; Espinaze *et al.*, 2019).

The fact that the prevalence and abundance of *O. capensis* ticks on admission were associated with *Babesia* infection on admission and with its infection ratio throughout the rehabilitation supports the hypothesis that this tick species is the main vector of *Babesia* to African penguins (Brossy *et al.*, 1999), rather than *I. uriae* (Earlé *et al.*, 1993). Interestingly, the month of admission was the strongest determinant of *Babesia* infections on admission, being lower in May to June than in August to December. Because tick and flea abundance were also included in the model, this seasonal profile must be related to factors other than the abundance

of these parasites. A possible explanation is that the seasonal emergence of *Babesia* at the breeding colonies relies on the increase in the population of penguin chicks in July–August (related to the March–April egg laying peak, as previously discussed) in order to provide a sufficient density of susceptible hosts to allow for the effective transmission of this parasite.

A similar association could not be demonstrated between *Borrelia* infections and tick prevalence or abundance, even though this bacterial pathogen is also suspected to be transmitted by *O. capensis* (Yabsley *et al.*, 2012). However, the lack of a statistically-significant association could be related to this blood parasite's relatively low prevalence, and it should be noted that all penguins that were *Borrelia*-positive on admission or that became *Borrelia*-positive during rehabilitation were infected with *O. capensis* on admission. It is also worth noting that our failure to find *I. uriae* on the examined penguins suggests that this tick species might be extremely rare in African penguins, if it occurs at all. In fact, we suspect that the records of *I. uriae* in seabirds in 'South Africa' provided by Arthur (1965), which are the only records of this species for the country (Muñoz-Leal and González-Acuña, 2015), actually referred to the Prince Edward Islands, a Subantarctic archipelago that belongs to South Africa. We are therefore sceptical that *I. uriae* is present in mainland South Africa.

While fleas, soft ticks and lice have yet to be demonstrated to be significantly pathogenic to penguins, it seems reasonable to assume that these parasites are accompanied by some level of discomfort, and potentially by health effects such as blood loss and damage to the skin or feathers (Duffy, 1983; Oppliger *et al.*, 1994; Barbosa *et al.*, 2002), especially in some of the higher intensities of infection documented in this study (e.g. >100 parasites/infected host). Further studies would be valuable to determine whether the skin inflammation associated with flea and tick bites could influence the feeding requirements of chicks or contribute to nest desertion, as documented in other birds (Duffy, 1983; Bouslama *et al.*, 2002). On the other hand, although lice are generally considered to have a lesser health impact, they could be a contributing factor to the penguin chick feather-loss disorder, a syndrome for which the aetiology is unknown (Kane *et al.*, 2010), or to other instances of feather damage (Barbosa *et al.*, 2002; Vanstreels *et al.*, 2018*b*).

The greatest impact of these external parasites is probably related to their role as pathogen vectors. *Borrelia* infections are significantly associated with a decreased survival probability in African penguin chicks admitted for rehabilitation at SANCCOB (Vanstreels *et al.*, 2019), and in some cases, *Borrelia* infections are lethal to penguins (Yabsley *et al.*, 2012; Parsons *et al.*, 2018*b*). Although *Babesia* infections have yet to be implicated in the death of African penguins, they have been linked to haematological abnormalities in otherwise apparently healthy African penguins (Parsons *et al.*, 2016) and their potential lethality has been demonstrated in other penguin species (Parsons *et al.*, 2017, 2018*b*). Furthermore, the flea *P. longicornis* is speculated to serve as a mechanical facilitator to the transmission of *Avipoxvirus* to penguins (Kane *et al.*, 2012). It is therefore clear that further studies on the ecological dynamics involving these pathogens and their vectors could be valuable in order to better understand and protect the endangered African penguin.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182020000141.

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Conflict of interest. None.

Ethical standards. This research project was approved by the Animal Research Ethics Committee of the Nelson Mandela University (A17-SCI-ZOO-003) and was conducted under national and provincial permits (TOPS permit 03314, CapeNature permit 0023-AAA004a00121).

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