Novel vagrant records and occurrence of vector-borne pathogens in King Penguins (Aptenodytes patagonicus) *in South Africa*

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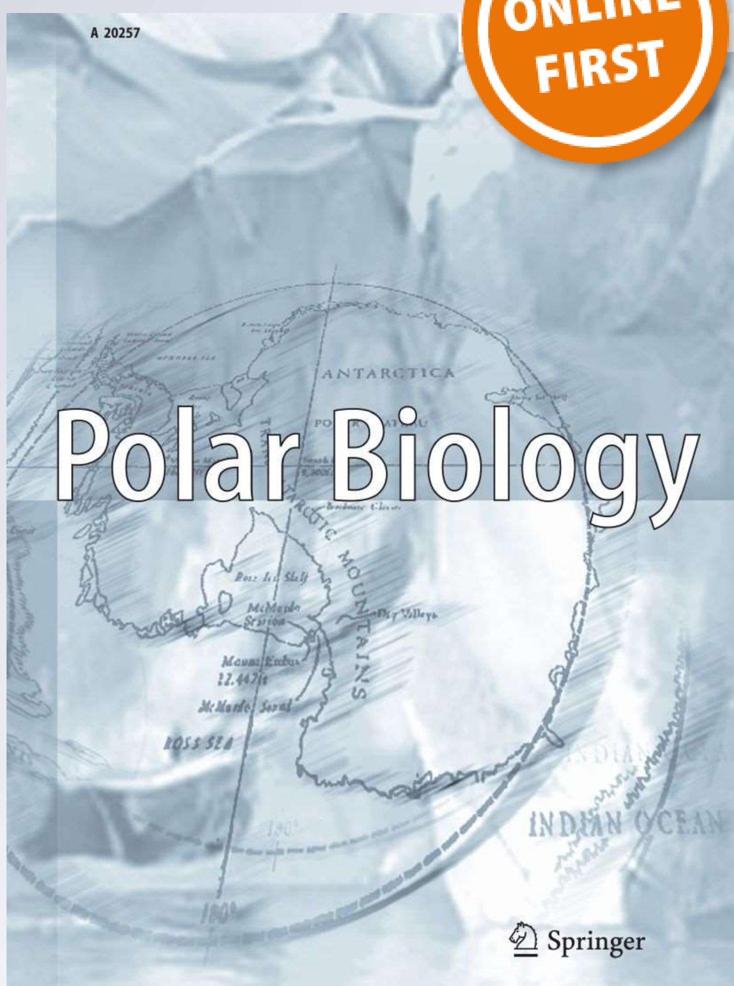

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SHORT NOTE

Novel vagrant records and occurrence of vector-borne pathogens in King Penguins (*Aptenodytes patagonicus*) in South Africa

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Abstract The King Penguin (*Aptenodytes patagonicus*) is a pelagic seabird that breeds on Subantarctic islands and is considered a rare vagrant in South Africa. From 2001 to 2017, six King Penguins were rescued along the South African coast and admitted into rehabilitation centers. These and previous records of King Penguins were obtained near the country's major ports, which suggests that some of these birds may have been ship-assisted. One of the King Penguins evaluated in this study died shortly after being admitted to the rehabilitation center due to extensive hemorrhage caused by a long-line fishing hook, and another had a beak wound consistent with fishing hook injury. Three King Penguins were infected with the tick-borne protozoan *Babesia peircei* and two died as a result of babesiosis. One King Penguin was diagnosed with an infection by *Rickettsia*-like organisms. Pox-like lesions, presumably mosquito-borne, developed on the eyelid skin of one penguin. Additionally, one of two King Penguins permanently captive in Cape Town during the same period also presented a lethal case of spirochetosis, which was possibly tick-borne. These novel records of vector-borne pathogens in King Penguins highlight the risk of seabird rehabilitation centers to serve as potential sources of pathogens to vagrant species, while also illustrating the opportunities that these centers provide for pathogen research and surveillance.

Keywords Blood parasite · Ectoparasite · Fisheries by-catch · Geographic distribution · Southern Ocean · Spheniscidae

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Introduction

King Penguins (*Aptenodytes patagonicus*) are pelagic seabirds that breed on Subantarctic islands (Bost et al. 2014). The species has a global population of approximately 1.6 million breeding pairs, and is not currently considered threatened with extinction (Bost et al. 2014). King Penguins are considered vagrants in South African waters (Hockey et al. 2005); the species' nearest colonies are located at the Prince Edward Islands, more than 1700 km away (Birdlife International 2016).

Known external parasites of wild King Penguins comprise ticks of the *Ixodes auritulus* group and *Ixodes uriae* as well as the lice *Austrogoniodes brevipes* and *Nesiotinus demersus* (Brandão et al. 2014). Information on vector-borne pathogens of King Penguins is scarce, however there is evidence that the species is naturally exposed to tick-borne arboviruses (Major et al. 2009) and tick-borne *Borrelia burgdorferi* sensu lato (the spirochete bacterium that causes Lyme disease) (Olsén et al. 1995; Schramm et al. 2014), and that captive King Penguins are susceptible to mosquito-borne *Plasmodium* spp. (the protozoan parasites that cause avian malaria) (Fantham and Porter 1944; Penrith et al. 1994). Pox-like lesions (skin lesions presumably caused by *Avipoxvirus*) have also been seen on King Penguins at South Georgia (K. Pütz, pers. comm.) and Marion Island (L. Pretorius, pers. comm.), and the anatomical distribution of these lesions on the neck suggest they may have been tick-borne.

In this study, we provide novel records of vagrant King Penguins found along the coast of South Africa and discuss the occurrence of vector-borne pathogens in these birds.

Materials and methods

The Southern African Foundation for the Conservation of Coastal Birds (SANCCOB) is an organization that receives sick and injured marine and coastal birds found along the coast of South Africa, and rehabilitates them at facilities in Cape Town (33°50′02″S 18°29′29″E) and Cape St. Francis (formerly known as Penguins Eastern Cape, 34°12′33″S 24°49′47″E). The uShaka Sea World Aquarium is located in Durban (29°52′02.4″S 31°02′45.2″E) and occasionally receives sick and injured marine and coastal birds. The Two Oceans Aquarium (TOA) is located in Cape Town (33°54′29″S 18°25′03″E). While at these facilities, birds are under continuous veterinary care which includes routine physical examination and hematological exams (hematocrit, plasma protein concentration, blood smear examination).

Penguins were bled (<2 mL) from either the dorsal metatarsal vein or the radial vein. In general, birds were sampled twice monthly during their stay at SANCCOB, or whenever a change in normal behavior was noticed. At TOA, birds were sampled monthly. Blood was collected directly from the hub of the needle into a heparinized capillary tube and a thin blood smear was immediately prepared and air-dried. Blood smears were fixed in methanol and stained with a modified Wright-Giemsa stain (Kyro-Quick stain set, Kyron Laboratories Pty Ltd, Benrose, South Africa). All smears were initially examined for 10–20 min at ×500 magnification (oil immersion), and smears found to contain blood parasites were subsequently re-examined for parasite identification.

Whenever King Penguins died, post-mortem examinations were conducted and tissue samples were collected in 10% buffered formalin for histopathological examination, including trachea, lung, heart, liver, spleen, kidney, proventriculus, small intestine, pancreas, cloaca and brain. The formalin-fixed tissues were embedded in paraffin wax and sections were cut at 5 µm and stained with hematoxylin and eosin and Warthin-Starry, then examined under light microscopy.

Results and discussion

From 1 January 2001 to 01 June 2017, six King Penguins were submitted to rehabilitation centers after having been found on the coast of South Africa (Table 1; Fig. 1); data from another two King Penguins captive at TOA were also considered. Individual history and clinical details of all birds are given in Online Resource 1. No external parasites were observed on any of the King Penguins examined. Some of the individuals presented in this study have been briefly mentioned in Parsons et al. (2017).

Vagrant records of King Penguins in South Africa

To date there are 11 records of King Penguins on mainland South Africa (Table 1). All records were obtained near three of the country's major ports (Cape Town, Port Elizabeth or Richard's Bay) and two of these birds had limitations to their ability to swim (traumatic lesions or having clinical signs of illness); this is consistent with the interpretation that some of these birds may have been ship-assisted (Enticott 1986; Ryan 2015). A possible explanation is that vessels operating in the Southern Ocean might catch these penguins unintentionally while fishing, and keep them on the ship out of curiosity or in an attempt to provide them with some kind of care (e.g. if birds are injured or swallowed a fishing hook), releasing them

Table 1 Summary of records of King Penguins along the coast of South Africa, 1982–2017

Record	Date	Location	Remarks	Reference
A	12 Jan 1977	Bloubergstrand, Western Cape (33°47′S, 18°27′E)	Adult. Body mass 12.4 kg. Started molting 16 days later. Transferred to permanent captivity in Denmark in Jan 1977	Cooper (1978)
B	28 Jan 1982	Sundays River mouth, Eastern Cape (33°42′S, 26°02′E)	Adult. Body mass 14.5 kg. Started molting eight days later. Transferred to permanent captivity in the UK in Apr 1982	Ross and Cockcroft (1985)
C	Jul 1985	Cape Town harbor, Western Cape (33°51′S 18°26′E)	Sighted at sea. Presumed to have been ship-assisted	Enticott (1986)
D	Aug 1985	Cape Town harbor, Western Cape (33°51′S 18°26′E)	Sighted at sea. Presumed to have been ship-assisted	Enticott (1986)
E	10 Aug 2005	Camps Bay beach, Western Cape (33°57′S, 18°22′E)	Adult female. Body mass 9.7 kg. Deceased after one day due to extensive hemorrhage caused by a long-line fishing hook perforating the esophagus and liver. No blood smears were examined	This study
F	19 Jan 2007	Thyspunt, Eastern Cape (34°12′S, 24°43′E)	Adult, unknown sex. Body mass 11.4 kg. Upon admission presented an abscess on the right shoulder. Started molting three days after admission. *Babesia* sp. infection was detected 63 days after admission. Transferred to permanent captivity in the USA in Jan 2008.	This study
G	20 Mar 2007	Cape Point, Western Cape (34°20′S 18°28′E)	Adult, unknown sex. Pre-molt	Ryan (2015)
H	04 Oct 2007	Milnerton beach, Western Cape (33°52′S, 18°29′E)	Adult male. Body mass 15.1 kg. Upon admission presented coughing and ocular discharge. Pox-like lesions developed on the eyelids of both eyes seven days after admission, and healed completely after one month. Death occurred 67 days after admission and was attributed to systemic inflammation, probably caused by severe *Babesia peircei* infection	This study
I	12 Feb 2009	Eerste River mouth, Eastern Cape (34°04′S, 24°13′E)	Adult male. Body mass 11.6 kg. Upon admission presented cut wounds on the right foot. Started molting two days after admission. Blood smear collected five days after admission revealed infection by *Babesia* sp. Death occurred 99 days after admission and was attributed to babesiosis	This study
J	22 Feb 2016	St Lucia beach, KwaZulu-Natal (28°23′S, 32°25′E)	Adult male. Body mass 12.45 kg. Found during a very hot day, survived for a few hours after being captured and placed in a facility with air conditioning, but died during transport to a rehabilitation facility. Cause of death could not be established but heat stress was suspected	This study
K	27 Apr 2017	Hout Bay beach, Western Cape (34°03′S, 18°21′E)	Adult male. Body mass 9.88 kg. Had arrested molt and a small wound to the side of the mouth that was consistent with a fishing hook injury. Started molting 12 days after admission. Death occurred 19 days after admission and was attributed to bacterial septicemia and bacterial enteritis. Post-mortem blood smears revealed a severe infection by *Rickettsia*-like organisms and a concurrent mild infection by *Babesia* sp	This study

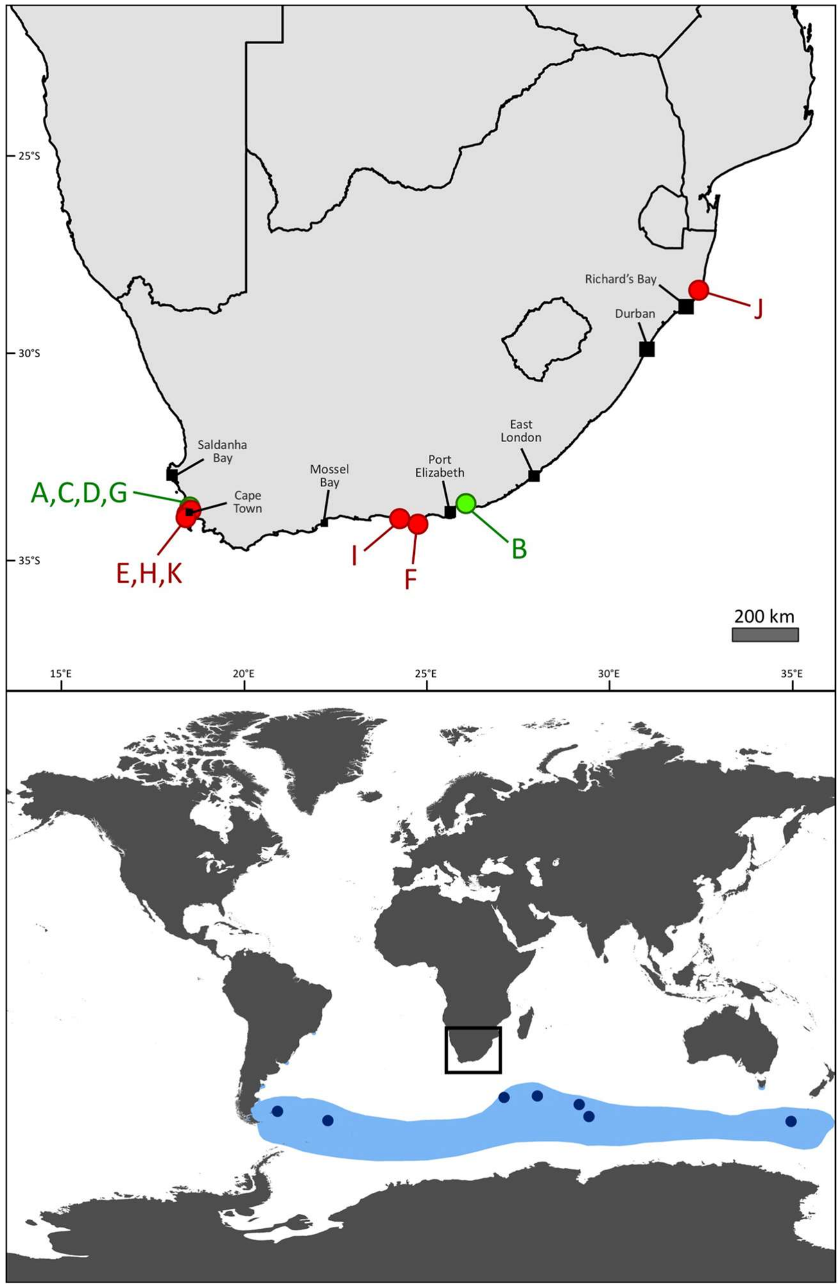

Fig. 1 Records of King Penguins along the coast of South Africa, 1982–2017. *Top* past (*light gray/green circles*) and novel records (*dark gray/red circles*) of King Penguins; ports (*squares*) are also shown, with the square size being proportional to the total cargo volume handled per year (adapted from Africa Ports 2017). *Bottom* natural distribution of King Penguins (*gray/blue area*) and their breeding colonies (*black dots*) (adapted from Birdlife International 2016). (Color figure online)

shortly before docking to avoid problems with port authorities.

One of the King Penguins in this study (record E) died shortly after being admitted to the rehabilitation center due to extensive hemorrhage caused by a long-line fishing hook perforating the esophagus and liver (Fig. 2). Another King Penguin (record J) presented an oral wound under the left side of the beak (through-and-through 2 × 1 cm cut to the oral mucosa and skin, with re-epithelized margins) that was consistent with a fishing hook injury. To our knowledge, these are the first cases reported of King Penguins negatively affected by long-line fisheries.

Babesia infections

Blood smears were examined for four of the six King Penguins admitted for rehabilitation, and all four were *Babesia*-positive at some point during rehabilitation. The interval between admission and the first Babesia-positive blood smear was: 5 days (record I), 19 days (record J), 63 days (record F) and 67 days (record H). The prepatent period of *Babesia* in penguins is not known; in domestic mammals, it is generally between 6 and 21 days (Griffiths 1978). It therefore seems likely that, for record I, the infection occurred prior to admission to the rehabilitation center.

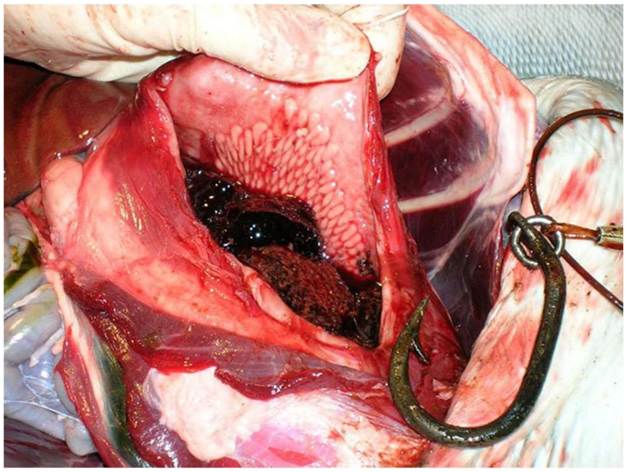

Fig. 2 Extensive bleeding into the esophagus and stomach of a King Penguin caused by a long-line fishing hook (record E)

Previous studies have failed to detect blood parasites in the blood smears of wild King Penguins at Macquarie (Laird 1952; Jones 1988), South Georgia (Peirce and Prince 1980), and Heard islands (Jones 1988), with a combined sample size of 103 individuals. In contrast, *Babesia* spp. are relatively common parasites of African penguins (*Spheniscus demersus*) and other species of seabirds routinely admitted at SANCCOB (Parsons et al. 2016, 2017). To our knowledge, these are the first recorded cases of *Babesia* in King Penguins. The parasites were generally consistent with *Babesia peircei* based on the presence of the characteristic amoeboid tetrads (Fig. 3a, b) and chromatin-free cytoplasm remains at the base of the group of elongated merozoites (Earlé et al. 1993; Peirce 2000). However, in contrast to the original description of *B. peircei* in African Penguins (Earlé et al. 1993), we observed a large proportion of elongated merozoites where the chromatin was concentrated near the proximal end (Fig. 3c, d). This difference could reflect earlier stages of merozoite development or could be related to autolysis since we examined post-mortem smears. Detailed photographs of the parasites are provided in Online Resource 2.

There is no evidence that *Babesia* infections are lethal to other species of penguins (Montero et al. 2016; Parsons et al. 2016, 2017; Vanstreels et al. 2016). In this study, however, babesiosis was considered to have caused (record I) or significantly contributed (record H) to the death of King Penguins. In the first of these cases (record I), no *Babesia* was detected in the blood smears routinely obtained in the 67 days during which the bird was maintained in captivity, and *Babesia* infection was detected only upon death. In the other case (record H), the interval between the first *Babesia*-positive blood smear and mortality was 95 days. Ante-mortem clinical signs of these two individuals were lethargy, hyporexia and regurgitation. Post-mortem findings included generalized congestion of the carcass, enlarged and pale liver, enlarged spleen, and enlarged and pale kidneys. Histopathology revealed large numbers of *Babesia* sp. visible within the cytoplasm of erythrocytes, and vasculitis and/or fibrin thrombosis in various tissues.

Rickettsia-like infection

Post-mortem blood smears of one King Penguin (record K) revealed a large number of *Rickettsia*-like cytoplasmic inclusions within the cytoplasm of erythrocytes (>60% infected erythrocytes) (Fig. 3e–h); a concurrent mild *Babesia* infection was also noted (<1% infected erythrocytes). The *Rickettsia*-like inclusions were pleomorphic, comprising round shapes (ranging from 0.9 to 2.0 μm in diameter) with a central vacuole (Fig. 3e, f) to heterogenous masses containing two to six denser particles (Fig. 3g, h). Additional photographs are provided in Online Resource 2. The affected penguin was concluded to have died as a result of bacterial enteritis with complicating septicemia, to which the *Rickettsia*-like infection may have been related.

To our knowledge there are no previous records of *Rickettsia*-like infection in penguins, however such organisms have been reported in hard ticks *Ixodes uriae* at the Kerguelen Archipelago (Chastel et al. 1993), where King Penguins breed (Birdlife International 2016). Furthermore, *Rickettsia* spp. has been widely documented in soft ticks *Ornithodoros capensis* on oceanic islands at the Southwest Indian Ocean (Dietrich et al. 2014) and in hard ticks *Amblyomma* spp. throughout the African continent (Mediannikov et al. 2010). Fleas can also vector some avian *Rickettsia* (Sekeyová et al. 2012).

Spirochaetales infection

One of the two King Penguins at TOA was diagnosed with an infection by Spirochaetales after having been captive at that facility for two years. On histopathology, these spirochetes were thicker (approx. 0.26 μm), longer (up to 18 μm), and more consistently undulated (wave length approx. 1.65 μm) than the Relapsing Fever *Borrelia* previously reported in blood smears of African Penguins at SANCCOB (see Yabsley et al. 2012). However, the measurements were within the range of Lyme Disease *Borrelia* (*Borrelia burgdorferi* sensu lato) and Animal Spirochetosis *Borrelia* (*Borrelia anserina*) (Olsén 2007; Meriläinen et al. 2015); of these, only Lyme Disease *Borrelia* has previously been recorded in King Penguins (Schramm et al. 2014).

The spirochetal infection was considered to have played a primary role in the death of the studied penguin. Post-mortem findings were generalized congestion and

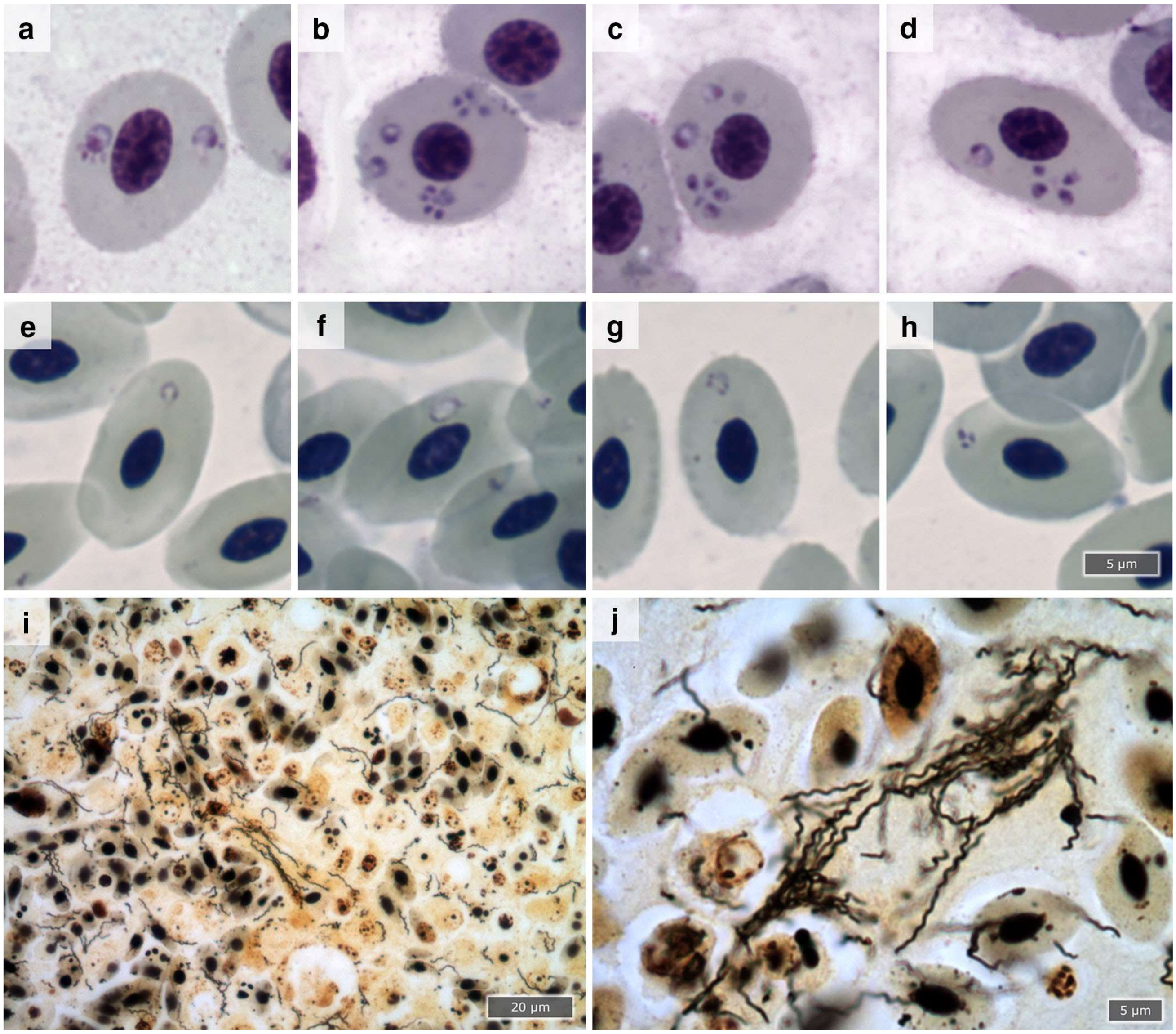

Fig. 3 Evidence of vector-borne pathogens in King Penguins in South Africa: **a–d** *Babesia peircei* in the liver impression smear of a King Penguin deceased while undergoing rehabilitation (record H, modified Wright-Giemsa stain); **e–h** *Rickettsia*-like inclusions in the post-mortem blood smear of a King Penguin deceased while undergoing rehabilitation (record K, modified Wright-Giemsa stain); **i, j** Large quantities of spirochetes in the liver of a permanently captive King Penguin (case 2, Warthin-Starry stain)

extensive petechiation over the visceral peritoneum, and enlargement of the liver and spleen. Histopathology revealed diffuse moderate congestion of organs, hepatocyte necrosis and apoptosis, mild leucostasis and fibrin thrombosis in the lungs, mild lymphoplasmacytic interstitial nephritis and multifocal villi necrosis in the small intestine. Silver histochemistry (Warthin-Starry stain) revealed numerous spirochetes in the spleen, liver, lungs and kidneys (Fig. 3i, j). These histopathological findings are distinct to those previously reported in an African Penguin deceased during rehabilitation due to relapsing fever, which presented with splenic reticuloendothelial hyperplasia with hemosiderosis, lung edema and moderate subacute lymphocytic meningoencephalitis (Yabsley et al. 2012).

Pox-like lesions

While undergoing rehabilitation at SANCCOB, one King Penguin (record H) developed small pale reddish papules to nodules on both eyelids, ranging from 5 to 10 mm in diameter. These growths had a friable consistency, and appeared diffusely hemorrhagic. These lesions were similar to avian pox lesions that are occasionally seen in African Penguin chicks undergoing rehabilitation at SANCCOB (Kane et al. 2012). In contrast, they were distinct from the pox-like lesions seen in King Penguins at South Georgia and Marion Island, which tend to be larger (20–50 mm) and occur on the neck (K. Pütz and L. Pretorius, pers. comm.).

Avian pox viruses can be transmitted through direct contact between infected birds, however the spread of the virus can be greatly enhanced by biting insects (Van Riper III and Forrester 2007). In this study, we consider the fact that the pox-like lesions were on the eyelids is suggestive of mosquito transmission, as has been speculated to occur in Magellanic Penguins (*Spheniscus magellanicus*) (Kane et al. 2012). The lesions started developing approximately 7 days after admission to the rehabilitation center, which suggests the infection occurred at the center. They were treated with topical chloramphenicol, and after peaking in size approximately 12 days after admission, they gradually receded until becoming completely healed one month after admission. This progression is similar to that usually witnessed in African Penguins (Kane et al. 2012; Parsons, unpubl. data), suggesting a similar etiology.

Implications for disease surveillance and rehabilitation efforts

This study found a remarkably high frequency of vector-borne pathogens. It was not possible to conclusively determine whether these infections occurred prior to or following admission to the studied facilities. For *Babesia*, there is reason to believe that at least in one case the infection occurred in the wild. For the *Rickettsia*-like and Spirochaetales infections and the pox-like lesions, however, the long interval between admission to the facility and the first diagnosis suggests that the King Penguins might have been infected while in captivity. This raises concern on the possible role played by these centers as potential sources of pathogens to vagrant species, which could in turn negatively affect their conservation. In the past, this concern led the Scientific Committee of Antarctic Research (SCAR) to issue Recommendation XXIV-3, discouraging attempts to rehabilitate seals and seabirds indigenous to the Antarctic continent and Subantarctic islands that were kept in captivity.

Furthermore, it is worth noting that the number of novel host-pathogen associations recorded in this study, despite the small sample size, illustrates the potential of seabird rehabilitation centers for parasitological and pathological research and surveillance. While the data obtained from rehabilitation centers cannot be considered to faithfully represent the prevalence and epidemiological patterns that occur in natural populations, this form of surveillance allows for an increased probability of detecting rare or emerging pathogens that could otherwise be difficult to detect through studies sampling healthy individuals in their natural environment.

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