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Retrospective Study

Empirical Primaquine Treatment of Avian Babesiosis in Seabirds

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Abstract: *Babesia* species are tickborne hemoprotozoans. Although experiments have shown that primaquine is highly effective in the treatment of *Babesia* species infections in mammals, this drug has not been widely used for the treatment of avian babesiosis. Consequently, the treatment of this disease for avian patients has traditionally relied on an empirically established imidocarb treatment. In this study, the authors examined the efficacy of primaquine as a treatment alternative for avian babesiosis (*Babesia peircei* and *Babesia ugwidiensis*) in seabirds. Retrospective analysis was performed on the medical records and blood smears of 446 *B peircei*–positive African penguins (*Spheniscus demersus*) and 41 *B ugwidiensis*–positive Cape cormorants (*Phalacrocorax capensis*) admitted for rehabilitation at the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB, Cape Town, South Africa). Treatment with primaquine (1 mg/kg PO q24h for 10 days) was effective in rapidly and markedly decreasing the proportion of *Babesia*–positive blood smears in African penguins and Cape cormorants. No regurgitation, loss of appetite, or any other signs after administration of primaquine were observed during the study period. The use of primaquine can be a particularly advantageous treatment alternative for avian babesiosis in circumstances in which it is not possible to determine confidently whether the intraerythrocytic inclusions seen in blood smears correspond to *Babesia* or *Plasmodium* or in cases in which there is a coinfection by *Babesia* and *Plasmodium*.

Key words: blood parasite, hemoprotozoans, Piroplasmida, tickborne pathogen, *Babesia*, *Plasmodium*, 8-aminoquinolone, primaquine, aquatic bird, avian, Cape cormorant, *Phalacrocorax capensis*, African penguin, *Spheniscus demersus*

INTRODUCTION

Babesia species are tickborne hemoprotozoans that infect birds and mammals worldwide.[1] Babesiosis is a significant disease in falcons and kestrels (Falconiformes), causing anemia, weakness, and death.[2–5] Several groups of aquatic birds are also susceptible to *Babesia* species, including murres, gulls, and terns (Charadriiformes); herons (Pelecaniformes); darters, gannets, boobies, and cormorants (Suliformes); and penguins (Sphenisciformes).[6,7]

Treatment of avian babesiosis in birds of prey has traditionally relied on the empirically established imidocarb treatment (imidocarb dipropionate, 5–7 mg/kg SC/IM single dose, optionally repeated after 1 week), which was derived from protocols used to treat canine babesiosis.[3,8] This treatment is generally effective in eliminating the disease associated parasitemia, with the only side effect reported being regurgitation after the first injection.[8]

Primaquine has traditionally been used for the treatment of malaria (*Plasmodium* species) in humans[9] and birds.[10–12] Research investigations

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with mammalian species have shown that 8-aminoquinolones, including primaquine, are highly effective treatment options for *Babesia* species infections. However, the 8-aminoquinolones class of drugs also have substantial adverse side effects (eg, emesis, methemoglobinemia, thrombocytopenia, and liver/ kidney degenerative changes).[13–15] As a result, potentially less-toxic drugs are usually preferred for the treatment of babesiosis in mammals,[16,17] even though primaquine is still routinely used to treat feline babesiosis in South Africa.[18] For humans and birds, the use of primaquine has become largely restricted to the treatment of tissue stages of *Plasmodium*, for which there are no other effective parasiticidal drugs.[9,12] Despite the toxic effects reported for some mammalian species, the long-term, daily administration of primaquine in dosages as high as 1.25 mg/kg has been used empirically in captive penguins as a preventative treatment for avian malaria, without significant adverse side effects.[12]

The Southern African Foundation for the Conservation of Coastal Birds (SANCCOB) is a nonprofit organization that regularly receives and rehabilitates oiled, sick, and injured seabirds found along the coast of South Africa. African penguins (*Spheniscus demersus*) and Cape cormorants (*Phalacrocorax capensis*) are 2 of the most frequently admitted species (respectively, approximately 850 and 110 individuals/y) at the facility in Cape Town, South Africa. Babesiosis is a frequent challenge in the rehabilitation of these birds, with 51% of the chicks, 34% of the juveniles, and 15% of the adults of African penguins diagnosed with *Babesia peircei* infections and 59% of the juveniles and 51% of the adults of Cape cormorants diagnosed with *Babesia ugwidiensis* infections while under care at SANCCOB.[19–21]

Avian malaria is also a primary concern for penguins and other seabirds at SANCCOB,[19,21,22] and treatment with chloroquine and primaquine is routinely employed to treat *Plasmodium* infections. During the treatment of individuals coinfected with *Babesia* and *Plasmodium*, it was subjectively noted that primaquine was effective in eliminating the *Babesia* parasitemia. Therefore, an empirical treatment protocol with primaquine to treat babesiosis has routinely been used in penguins and other seabirds since the early 2000s. In this study, the authors retrospectively evaluated the rehabilitation records and blood smears of African penguins and Cape cormorants under rehabilitation at SANCCOB to evaluate the efficacy of primaquine in the treatment of *Babesia*.

MATERIALS AND METHODS

While under care at SANCCOB, seabirds were examined by veterinarians upon their admission and weekly thereafter (or more frequently, depending on their clinical condition).[21,22] As part of that examination, blood was collected from the medial metatarsal veins, thin blood smears were prepared, stained with a modified Wright-Giemsa stain (Kyro-Quick, Kyron Laboratories, Benrose, South Africa), and examined for blood parasites under ×500 magnification for about 10 minutes. Once a positive diagnosis was made, a bird would be treated if 1) parasitemia was ≥30 parasites/field (under ×500 magnification), 2) parasitemia was ≥5 parasites/field and packed cell volume was <30% or the bird showed signs of illness (eg, lethargy, decreased appetite, regurgitation, dyspnea), or 3) parasitemia was ≥5 parasites/field and did not diminish in consecutive blood smears obtained 3–7 days apart.

The empirical treatment protocol for babesiosis consisted of primaquine (1 mg/kg PO q24h for 10 days; primaquine phosphate 1.76% m/v in stabilized solution, Primaquin Solution, MedPet Ltd, Benrose, South Africa). This treatment was accompanied by a phospholipid supplement (1 capsule/bird PO q24h for 12 days; deoiled, enriched phospholipids from soybeans, 300 mg/capsule, Essentiale Extreme, Sanofi Aventis Ltd, Midrand, South Africa); as an attempt to mitigate potential hepatotoxic effects of primaquine, however, the efficacy was never thoroughly investigated. Additionally, to prevent transmission of *Babesia* and other tickborne pathogens, all birds with visible ectoparasites are treated with pesticide powder (carbaryl 50 g/kg) upon admission, and the facilities are thoroughly cleaned on a daily basis.

For African penguins, the data were analyzed on 446 *B peircei*–positive individuals, comprising 270 chicks (<90 days old), 138 blues (45–180 days old), 17 juveniles (90 days to 23 months old), and 21 adults (>10 months) (plumage categories established per published criteria)[20,21] received between January 2003 and December 2011. The age ranges above overlap because of the development of the bird's plumage based on individual characteristics regarding growth, nutrition, and environmental conditions. Only *Plasmodium*-negative individuals that received primaquine treatment and for which 3 or more blood smears had been examined within 30 days of the start of the treatment were considered for inclusion in the study. This data set comprised information on the presence or absence of *Babesia* species organisms as recorded

during the routine screening of 1601 blood smears of the selected penguins.

For Cape cormorants, data was analyzed on 41 *B ugwidiensis*–positive individuals received between January 2017 and March 2018 (22 juveniles and 2 adults received primaquine treatment and 16 juveniles and 1 adult did not receive treatment). Only *Plasmodium*-negative individuals that survived to be released and for which 3 or more blood smears had been examined were considered for inclusion in the study. Blood smears of individuals identified as *Babesia* species–positive during routine screening were reexamined to quantify the parasitemia (parasites/100 erythrocytes, as determined by the examination of about 2000 erythrocytes at ×1000 magnification). This data set comprised information on the *Babesia* species parasitemia from 156 blood smears of the selected cormorants.

The date of first day of primaquine treatment was recorded for each penguin and cormorant that received treatment. The *clear* status was assigned to each bird on the day when the first *Babesia*-negative smear was obtained after the start of the treatment; the authors conservatively assumed that the bird had been *Babesia* species–positive until that day, even if no blood smears had been examined. *Relapse/reinfection* was defined as obtaining a *Babesia* species–positive smear after having reached the clear status. For cormorants that did not receive treatment, the date of the first *Babesia* species–positive blood smear was recorded. A lognormal curve was used to represent the changes in the parasitemia of Cape cormorants in relation to the number of days from the start of the primaquine treatment.

To produce photographs that illustrate the morphological changes undergone by *Babesia* species after primaquine treatment, blood smears were obtained from 1 Cape cormorant immediately before and exactly 24 hours after the first dose of primaquine.

RESULTS

The rehabilitators and veterinarians closely observed the treated birds for any signs of toxicity or side effects known to be associated with primaquine treatment. No regurgitation, loss of appetite, or any other adverse physical signs were observed after the administration of primaquine during the study period.

For the *Babesia* species–positive penguins that were treated with primaquine, the proportion of *clear* individuals increased rapidly, as evidenced in blood smears examined during the first 7 days of the treatment (Fig 1A). Fourteen days after starting primaquine treatment, the proportion of individuals that were still identified or presumed to be positive remained consistently low (<1%). Only 2 individuals (0.4%; both of the "blue" plumage category) that received the primaquine treatment did not achieve the *clear* status. Relapse/reinfection was recorded in 12 individuals (2.7%; 10 chicks and 2 blues), occurring as early as 11 days after the start of the treatment (ie, second day after the end of the treatment; Fig 1A).

For the *Babesia* species–positive cormorants that were treated with primaquine, pretreatment parasitemia was, on average, 3.7 ± 1.8 (SD) parasites per 100 erythrocytes (range, 0.1–6.8 parasites). Parasitemia decreased rapidly during the first 3 days of primaquine treatment in all individuals (Fig 1B), and no blood smears were positive after the sixth day of treatment. No cases of relapse/reinfection were observed after that and before cormorants being released back into the wild 12 ± 22 days (range, 1–111 days) following cession of treatment.

Only 1 of the *Babesia* species–positive cormorants that did not receive primaquine treatment had a spontaneous cure (ie, consistently negative blood smears); it is worth noting that this individual was the only adult in the untreated group. The remaining 16 untreated cormorants (all juveniles) continued presenting *Babesia* species–positive blood smears for the remainder of their stay (35 ± 43 days; range, 11–190), and the highest parasitemia diagnosed in these birds was, on average, 1.9 ± 1.2 parasites/100 erythrocytes (range, 0.4–4.0 parasites).

After primaquine treatment, it was generally noted that *Babesia* species parasites in blood smears showed morphological changes suggestive of degeneration: the central cytoplasmic vacuole became enlarged and more clearly outlined, enlarging the parasite, and forcing its cytoplasm and nucleus to the periphery (Fig 2).

DISCUSSION

The results of this study demonstrate that primaquine can be effective in the treatment of *Babesia* species infections in penguins and cormorants. Until detailed pharmacokinetic studies determine the optimum dosage for these 2 avian species, the results of this investigation suggest that the treatment protocol employed in this study (primaquine 1 mg/kg PO q24h for 10 days) produces satisfactory results. The treatment elim-

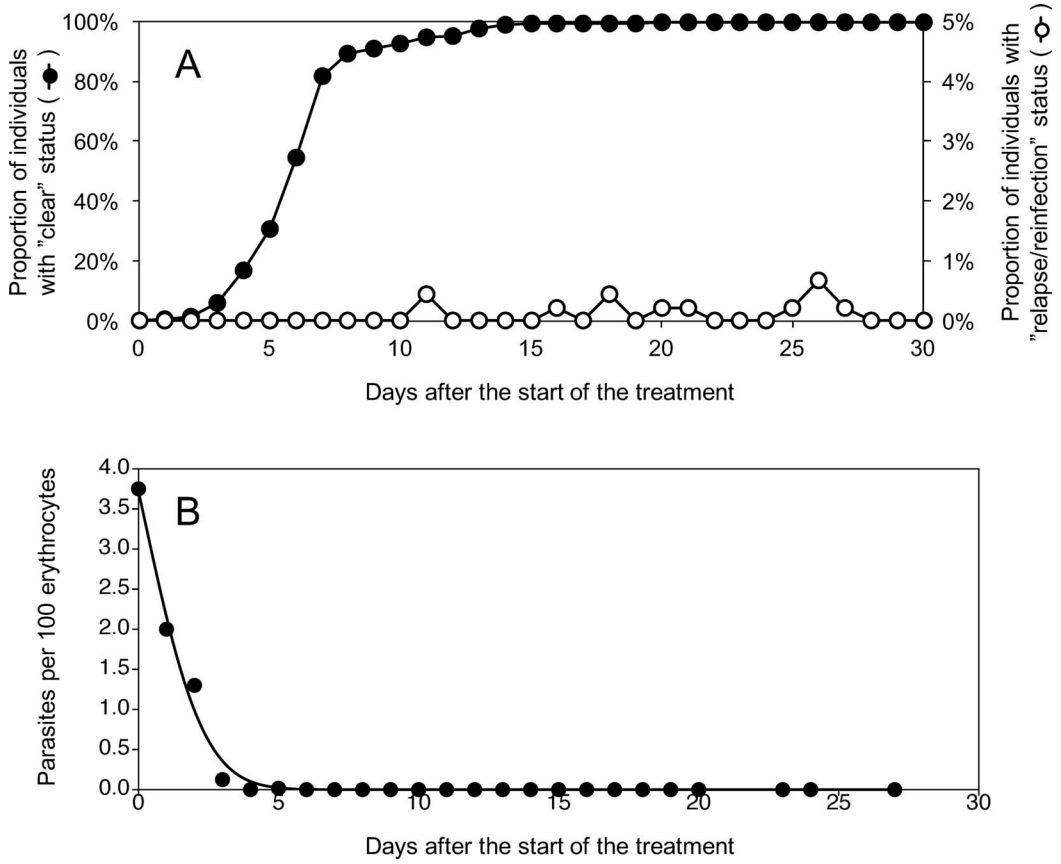

Figure 1. Response of *Babesia*-infected seabirds to treatment with primaquine. (A) Changes in the proportion of African penguins classified as clear for *Babesia peircei* and incidence of relapse/reinfection. (B) Changes in the average parasitemia of *Babesia ugwidiensis* in blood smears of Cape cormorants.

inated *B ugwidiensis* parasitemia in all Cape cormorants and was effective in eliminating parasitemia in 99.6% of African penguins; however, a small proportion (2.7%) of the African penguins showed detectable *B peircei* parasitemia after initially being considered clear of infection. This difference might be related to 1) a relapse, if the parasite was not fully eliminated but eluded detection in blood smears; or 2) reinfection, whereby the infection was eliminated, but the penguins were again exposed to the *Babesia* species organism and infected in captivity. The authors favor the second hypothesis because controlling the soft tick *Ornithodoros capensis*, one of the suspected vectors of *B peircei*,[23] has been a recurrent problem at the facility in which the African penguins were maintained, and there have been instances in which penguins were found to be infected by these ticks during rehabilitation.

Primaquine is readily available in most countries, and it is inexpensive (at present, the 10-day treatment for a 3-kg penguin costs approximately US$ 0.15). The use of primaquine can be particularly advantageous in circumstances in which it is not possible to determine, with confidence, whether the intraerythrocytic inclusions observed in blood smears correspond to *Babesia* species or *Plasmodium* species, or in cases in which there is coinfection by *Babesia* species and *Plasmodium* species. Conversely, a treatment that involves the oral administration of drugs on a daily basis can be problematic in some circumstances. At SANCCOB, administering primaquine orally to penguins and cormorants on a daily basis is usually not a problem because these birds are frequently handled on a daily basis for the administration of oral fluids/formula. In other circumstances (eg, critically ill individuals, stress-sensitive species), the stress associated with the daily handling might be detrimental to the birds' welfare and recovery. In these cases, the treatment traditionally employed for birds of prey with a single, injectable dose of imidocarb may be preferable.

The authors did not observe any adverse physical side effects from the administration of primaquine to the birds in this study. Therefore,

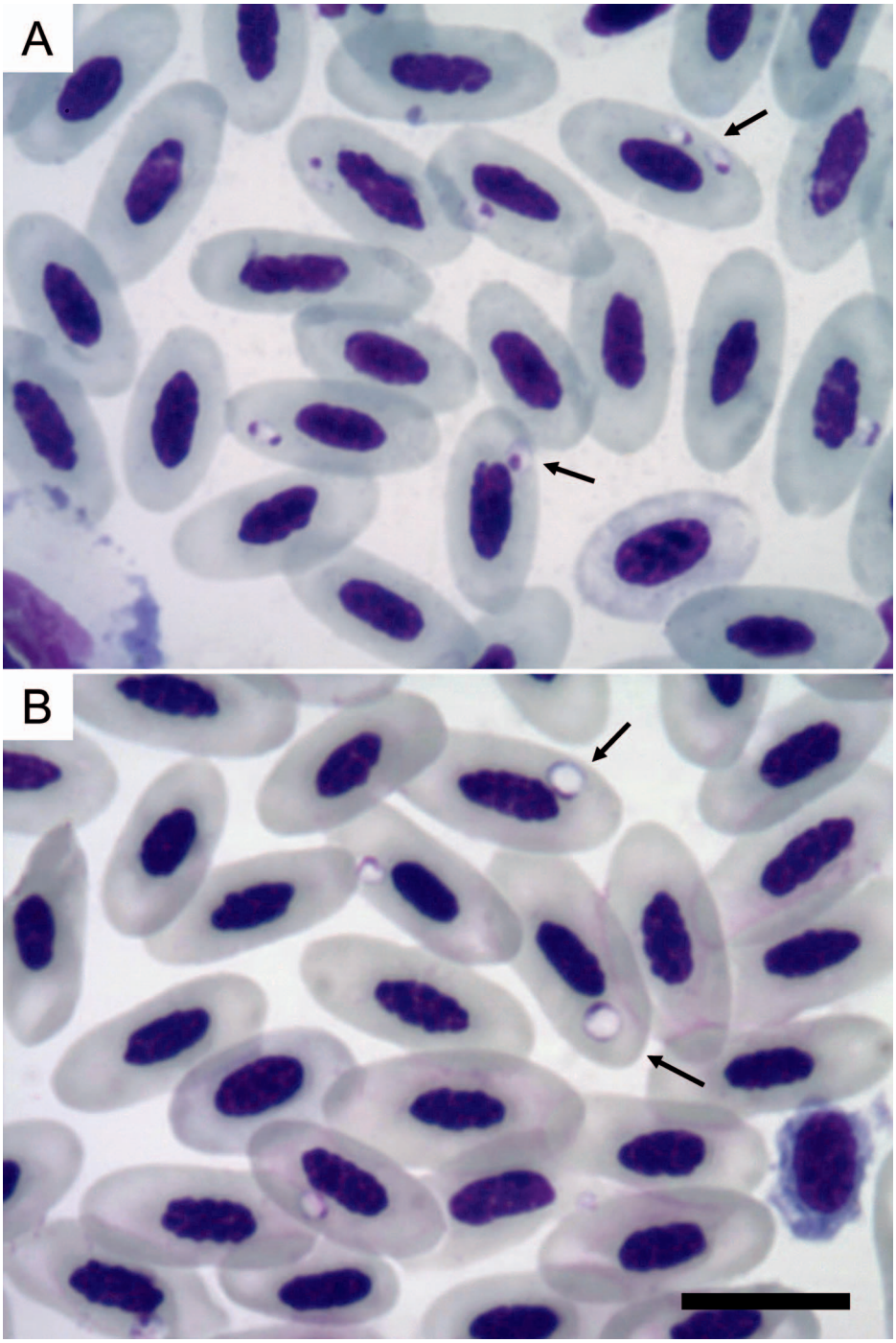

Figure 2. Morphological changes of *Babesia ugwidiensis* (arrows) in the blood smear of a Cape cormorant in response to primaquine treatment. (A) Blood smear obtained immediately before administering the first dose of primaquine to the bird. (B) Blood smear obtained from the same bird 24 hours later. Note that the central cytoplasmic vacuole is enlarged and more clearly outlined, enlarging the parasite and forcing its cytoplasm and nucleus to the periphery (Wright-Giemsa stain; bar = 10 μm).

primaquine appears to be a safe treatment alternative for these 2 avian species. However, it should be noted, that the authors did not monitor plasma biochemistry and enzymes in the treated birds. Consequently, it would be valuable to have future studies investigate the pharmacokinetics of primaquine, its potential adverse physical effects in seabirds, and if present, what measures can be

taken to mitigate those adverse effects. Because the juvenile cormorants admitted for rehabilitation at SANCCOB are often severely anemic (occasionally with hematocrits as low as 8%–15%), an additional factor to be considered is whether the primaquine treatment could lead to destruction of erythrocytes, which would be detrimental to the birds' initial recovery.

Lastly, it is worth noting that although this study does not address the extent to which *Babesia* species infections are pathogenic, there is evidence that these parasites can be pathogenic to the 2 avian species included in this study. *Babesia peircei* infections can cause mild anemia, leukocytosis, and impairment of hepatic function in African penguins,[24] and the survival rate of juvenile Cape cormorants during rehabilitation is lower for *B ugwidiensis*–positive individuals than it is for negative individuals.[19] Furthermore, there are cases of acute *Babesia* species infections leading to the death of common murres (*Uria aalge*)[25] and in a king penguin (*Aptenodytes patagonicus*) during rehabilitation.[26] Thus, it is clear that having therapeutic alternatives to treat these *Babesia* species infections is beneficial to the rehabilitation and medical care of these birds.

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