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# Avian Malaria in African Black-Footed Penguins

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## SUMMARY

Ten captive-reared African black-footed penguins *(Spheniscus demersus)* from a large outdoor colony were monitored for avian malaria, using several diagnostic tests. One treatment regimen was evaluated.

Thin smear blood evaluation enabled detection of seven parasitemias involving *Plasmodium relictum* and *Plasmodium elongatum* in the penguins. Leukocytosis (relative lymphocytosis) was characteristic of infected birds.

Parasitemia was detected as early as 21 days prior to onset of clinical signs (depression, anorexia, regurgitation, pale mucous membranes, and respiratory distress). The single bird that died had clinical signs only a few hours prior to its death. Treatment consisted of 0.03 mg of primaquine phosphate base/kg body weight, administered orally once daily for 3 days. Oral chloroquine phosphate therapy, given simultaneously, was administered in an initial loading dose of 10 mg of chloroquine phosphate base/kg body weight, followed by doses of 5 mg/kg at 6, 18 and 24 hours after the initial chloroquine dose. This treatment regimen prevented mortality and cleared parasites from the blood. Recurrences of malaria occurred in two birds that had received this treatment.


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AVIAN MALARIA is the most important cause of death in captive penguins displayed in open air exhibits around the world. In the past 10 years, 75% of all juvenile penguin mortality and 64% of all adult penguin mortality in the Baltimore Zoo outdoor penguin colony has been associated with avian malaria. Two species of Plasmodium are known to infect captive penguins. *Plasmodium relictum* infections have been reported in six species of captive penguins, including the African black-footed penguin *(Sphensicus demersus)*.[1-7] The only report of a natural infection of *P relictum* in a penguin is of an African black-footed penguin from the southern coast of Africa.[1] *Plasmodium elongatum* infections have been demonstrated in captive African black-footed penguins and Humboldt penguins *(Spheniscus humboldti)*,[4,5,8-10] but there are no reports of this organism infecting wild penguins. Both organisms are known to infect wild passerine birds on all continents.[11,12] The problems of antemortem diagnosis and treatment of avian malaria in penguins are the major concerns for zoo veterinarians responsible for the health of penguin colonies. Either organism is capable of causing rapidly fatal disease in penguins. Premonitory signs are often subtle, and frequently lacking altogether. Depression, anorexia, regurgitation, pale mucous membranes, and respiratory distress rarely appear before the bird is near death. Antemortem diagnosis is further complicated by the prevalence of exoerythrocytic forms and low-grade parasitemias in penguins infected with *P elongatum*.[2,13] Pathologic descriptions of fatal infections have been reported.[2,9] The purpose of this study was to evaluate diagnostic methods available to zoo clinicians, to determine which is the most reliable for diagnosis of avian malaria in penguins, and to evaluate a treatment regimen for the disease.

## Materials and Methods

Seven juvenile and three adult African black-footed penguins were selected from the zoo's open air colony. All study birds were marked and observed daily at two hand feedings.

The study group was evaluated for avian malaria during the season of peak incidence, late August through October. Blood samples were taken from the brachial vein for the evaluation of several blood tests for their relative ability to enable detection of malaria. Isodiagnosis, using young Pekin or Muscovy ducks (5 to 20 days old), was used in an attempt to magnify infections in a more easily evaluated host. This technique was also used as a reference since there is little chance of false-positive results. Susceptible ducks were inoculated with 0.5 ml of heparinized penguin blood, either intravenously or intramuscularly.[14] After inoculation, blood from the ducks was examined three times a week for parasites in thin blood smears. A penguin was considered negative for malaria after nine negative samplings from its isodiagnostic counterpart.

Thin smear diagnosis consisted of making six standard air dried blood smears and fixing them in absolute methanol. These were then stained for 10 minutes with a 10% solution of Giemsa stain in pH 7.2 phosphate buffer. Approximately $4 \times 10^4$ erythrocytes were examined in a minimum of 400 oil emersion fields. Parasitized cells were identified and reported as a percentage of the total number of erythrocytes observed.

Leukocyte and erythrocyte counts were performed with a 1 to 100 dilution technique, utilizing Nat-Herrick avian diluent[15] on a standard hemacytometer. The packed cell volume was determined by microcapillary tube technique. Total protein was evaluated by refractive index, using an

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optical total solids meter. Differential counts were made on heparinized blood smears stained with Wright's stain. The results of these tests were then compared with baseline values for the African black-footed penguin,[16] then correlated with the results of the isodiagnostic technique to establish which tests might be useful in the antemortem diagnosis of malaria in penguins.

Blood samples were taken from the medial tarsal vein of the subjects every 14 days. All blood sampling sessions were scheduled for midmorning, the projected time of maximal peripheral parasitemia.[4,11] Each bird's behavior was observed closely for clinical signs. In the event of detection of parasitemia, a second blood sample was obtained from the affected bird to allow duplicate tests to be run.

Originally, initiation of treatment was to be delayed until the onset of clinical signs, but the death of an infected penguin only a few hours after the onset of clinical signs prompted a change in protocol. Subsequently, treatment was begun as soon as parasitemia was detected. Treatment consisted of oral intubation with physiologic saline suspensions of chloroquine phosphate and primaquine phosphate. Primaquine phosphate was administered at a dosage of 0.3 mg/kg, once daily for 3 days. The first dose of chloroquine phosphate administered concurrently with the primaquine therapy was given at a dosage of 10 mg/kg. This was followed by dosages of 5 mg/kg at 6, 18, and 24 hours after the initial dose.

After completion of treatment, blood samples were obtained from affected birds twice, 7 days apart. If either sampling revealed parasitemia, the treatment regimen was repeated. Otherwise, the bird was returned to the study group.

## Results

Five of the ten penguins developed parasitemias detectable by thin smear analysis. In two birds, the parasitemia was separated by a month or more of negative results on thin smears. The parasitemias ranged from 0.001% to 1.88% of infected erythrocytes, with parasitemias of less than 0.01% being most frequently observed.

Six of seven parasitemias involved *Plasmodium elongatum*. One parasitemia was too low for accurate speciation of the parasite. *Plasmodium relictum* was identified in only one bird, as a mixed infection. This bird had the highest percentage of infected erythrocytes detected, and was the only study bird to die.

Isodiagnosis agreed with thin smear diagnosis in 10 of 13 trials; three parasitemias were detected by thin smear, but not detected by Pekin duck isodiagnosis. Muscovy ducks proved refractile to infection for isodiagnosis, failing to develop parasitemias when inoculated with a relatively large number of parasites, when compared to that for Pekin duck inoculations. Positive results from isodiagnosis were not obtained sooner than 6 days after inoculation. Nine or 10 days were often required before diagnosis was possible by this method.

Appreciable changes were not seen in the total erythrocyte count, packed cell volume, or total serum protein content of infected birds. Birds with parasitemias had a marked relatively lymphocytosis when compared with baseline values. Birds in the study also had varying degrees of relative lymphocytosis, as compared with baseline values before parasitemia was detected. Thus, it might be that infections exist even though they are not detectable by isodiagnosis or by direct thin smear analysis. Three arbitrary levels of relative lymphocytosis were correlated with the eventual detection of parasitemia in a bird (Table 1).

TABLE 1—Degree of Relative Lymphocytosis Related to Confirmed Parasitemia

| Degree of relative lymphocytosis | No. of trials over threshold/ No. of trials | Parasitemia without relative lymphocytosis above threshold (% of total trials) | Relative lymphocytosis above threshold but parasitemia never demonstrated (% of total trials) | Relative lymphocytosis above threshold and parasitism eventually demonstrated (% of total trials) |
|---|---|---|---|---|
| > 50% | 47/65 | 4.6 | 20 | 47.7 |
| > 60% | 40/65 | 9.2 | 17 | 35.3 |
| > 70% | 16/65 | 9.2 | 4.6 | 10.7 |

The total leukocyte count in infected birds was elevated above the baseline mean in 5 of the 7 trials wherein parasitemia was confirmed. In the two trials without the expected leukocytosis, the same bird was involved. This bird had a relative lymphocytosis in only one of these two trials. Total leukocyte counts as high as 98,000/mm$^3$ were obtained from infected birds, but counts in the range of 20,000 to 30,000/mm$^3$ were more common.

Four birds were given the experimental treatment regimen after detection of parasitemia. Two birds were given two rounds of treatment, as they each had parasitemias a second time. One bird with a parasitemia was not treated and died 21 days after detection of the infection. This bird became anorectic and depressed a few hours before death. None of the treated birds died. Anorexia, depression, pale mucous membranes, regurgitation, and respiratory distress were evident in one bird in which parasitemia developed twice. The other bird in which parasitemia developed twice was anorectic and had convulsions prior to the detection of the first infection. A third bird was anorectic and depressed prior to treatment. Twice, penguins had no clinical signs at the time of detection of parasitemias. Affected birds became clinically normal within 4 days of beginning treatment.

## Discussion

Five of 10 birds developed naturally occurring malarial parasitemias during the course of this study. *Plasmodium elongatum* was responsible for most of the parasitemias detected in this study, with *Plasmodium relictum* being observed only once. The mixed infection of *P elongatum* and *P relictum* was noteworthy because it confirmed a low degree of cross immunity conferred by *P elongatum* infections against other avian malarias.[17] *Plasmodium elongatum* is rapidly fatal in Pekin ducks[13] but causes only low parasitemias.[3] It has been postulated that its pathogenicity is due to the predominant exoerythrocytic forms.[13] Exoerythrocytic forms of *P elongatum* cause extensive pathologic changes in black-footed penguins,[9] and this study confirms that peripheral parasitemias tend to be low in these birds,

usually less than 0.01%. Observations on the single bird that died showed that parasitemia can be demonstrated up to 21 days before the onset of clinical signs. They also demonstrated that the interval between the onset of clinical signs and death can be as short as a few hours.

Diagnosis from clinical signs alone is not accurate. Penguins have been known to die from avian malaria without any premonitory signs.[2] When signs do develop, they are nonspecific and can be confused with other penguin diseases such as aspergillosis and bacterial gastroenteritis. Twice in this study, birds had a parasitemia without any clinical signs.

Observing a parasite in a thin smear establishes malarial infection with certainty, but a negative result does not rule out the possibility of an exoerythrocytic form of the disease. Thin smear technique is capable of detecting parasitemias as low as 0.001% in penguins if smears are of good quality and well-stained. A minimum of 40,000 erythrocytes should be examined to be able to detect parasitemias this low. Properly trained technicians can use this technique to identify infected birds weeks before signs appear. Isodiagnosis by inoculation of infected blood into susceptible hosts such as Pekin and Muscovy ducks has been used in avian malaria research to increase the sensitivity of thin smear diagnosis.[18] The false-negative results with this technique and the prohibitively long time required to read the tests make it undesirable as a clinical tool. The technique can be of value when speciation of the malarial parasite is important, but the results may not always be available before the affected bird dies. This is certainly true if the penguin has signs of illness when the test is initiated.

Studies on baseline hematologic values have shown an appreciable rise in total leukocyte count and differential lymphocyte count in the fall of the year. It has been postulated that these increases may be related to avian malaria, since most cases of fatal penguin malaria are also seen in the fall.[14] This study lends support to that hypothesis. Leukocyte counts of 19,000/mm$^3$ or more were indicative of malarial infections. All known infected penguins, except one, demonstrated total leukocyte counts above the baseline range of 11,566 ± 6,427/mm$^3$.[14] The one exception may have been depleted of leukocytic precursors by the time of sampling. It did not have a leukocytosis but did have a relative lymphocytosis.

Relative lymphocytosis was promising as a diagnostic criterion for penguin malaria. A healthy penguin has a relative lymphocyte count of 33% to 37%.[14] A relative lymphocytosis of 50% or more in this study gave few false-negative results when compared to eventual confirmation of parasitemia. Many birds with 50% or more lymphocytes failed to develop a parasitemia initially, but most of these were eventually confirmed to have infections, indicating that relative lymphocytosis might be a more sensitive indicator of infection than thin smear diagnosis or isodiagnosis. An increase in circulating lymphocytes may be a response to exoerythrocytic forms of the disease occurring before parasitemias are demonstrable. If this is the case, false-positive results or birds with increased lymphocyte counts, but never confirmed to have parasitemia, probably indicated subclinical exoerythrocytic infections. Studies in other birds indicate that chronic low-level exoerythrocytic infections are responsible for premunition against further infection.[18] A similar situation may exist in the penguin. A relative lymphocytosis of 70% or more was seen in most smears associated with parasitemias, suggesting that the degree of lymphocytosis is an indicator of the severity of infection.

Severe anemia is a sign of malaria in ducks.[13] In this study, packed cell volumes, total erythrocyte counts, and total serum protein determinations failed to confirm anemia as a component of malaria in penguins. This study indicates that the most useful profile for diagnosing avian malaria in penguins would include thin smear diagnosis and differential and total leukocyte counts. A leukocytosis correlated with a lymphocytosis should be considered indicative of avian malaria, even in the absence of documentable parasitemia.

The therapeutic regimen was based on standard human infant treatment schedules for the drugs used. Although early treatment appeared to decrease expected mortality, it failed to free the host of malarial organisms in two cases. The lymphocyte data from this study clouds the evaluation of the effectiveness of this treatment regimen, since it indicates that penguins may develop nonfatal subclinical infections. Most treated birds had developed clinical signs prior to the beginning of therapy and were considered gravely ill. We can say that the treatment cleared the subjects of any observable parasitemia, and that none of the treated patients died of malaria. The apparent failure to free the penguins of all malarial organisms may be an advantage if immunity is indeed based on premunition from chronic infection.[18,19] The question unanswered is whether the two recurrences of malaria were due to reinfection or recurdescence. No toxic side effects of either of the drugs used were seen in any of the treated birds.

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