Journal of Zoo and Wildlife Medicine 49(1): 143–152, 2018

CLINICAL EFFECT OF HEMOPARASITE INFECTIONS IN SNOWY OWLS (*BUBO SCANDIACUS*)

Kendra C. Baker, D.V.M., Christy L. Rettenmund, D.V.M., Dipl. A.C.Z.M., Samantha J. Sander, D.V.M., Dipl. A.C.Z.M., Anne E. Rivas, D.V.M., Kaitlin C. Green, B.S., R.V.T., Lisa Mangus, D.V.M., Ph.D., Dipl. A.C.V.P., and Ellen Bronson, med. vet., Dipl. A.C.Z.M.

Abstract: Vector-borne hemoparasites are commonly found in avian species. *Plasmodium* spp., the causative agent of avian malaria, are intraerythrocytic parasites that can cause signs ranging from subclinical infection to severe acute disease. In raptor species, most hemoparasites are associated with subclinical infection and are generally not treated when seen on blood evaluation. This case series reviews five cases of hemoparasite infection in snowy owls (*Bubo scandiacus*). These animals were infected with a variety of hemoparasites, including *Plasmodium*, *Haemoproteus*, and *Leukocytozoon* spp. Death of one of these birds due to hemoparasite burden led to a change in the monitoring for and treatment of subclinical hemoparasitic infections in this species. Three subsequently infected snowy owls have been treated with primaquine and chloroquine. The birds that were treated survived infection, and parasite burdens in peripheral blood diminished. Postulated reasons for increased morbidity and mortality associated with hemoparasitic infections in captive snowy owls, as opposed to other raptor species, include stress, concurrent disease, novel pathogen exposure, and elevated environmental temperatures.

Key words: Avian malaria, *Bubo scandiacus*, *Haemoproteus*, hemoparasites, *Plasmodium*, snowy owl.

INTRODUCTION

Plasmodium, *Haemoproteus*, *and Leukocytozoon* spp. are vector-borne hemoparasites frequently observed in avian species. *Plasmodium* organisms are transmitted via mosquitoes (*Culicidae*); *Haemoproteus* via sand flies, also known as biting midges (*Ceratopogonidae*); and *Leukocytozoon* via black flies (*Simulidae*).[11] The life cycle of *Plasmodium* spp. involves replication in host tissues during the exoerythrocytic stage and invasion of circulating blood cells during the erythrocytic phase. In the acute phase of infection, parasite replication in host tissues leads to an inflammatory reaction and subsequent tissue necrosis, while growth of these parasites in erythrocytes can cause hemolysis and anemia.[29] In the host, *Plasmodium* spp. undergo asexual reproduction termed schizogony. The first three stages of schizogony can cause significant damage to parenchymal organs before the parasite can be detected in blood. Complete details of the life cycle are described elsewhere.[3,4,11,29] *Haemoproteus* and *Leukocytozoon* organisms have a similar life cycle to *Plasmodium*; however, in the life cycle of *Haemoproteus*, schizogony is confined to the tissues, and in the life cycle of *Leukocytozoon*, only gametocytes are found in the blood in erythrocytes and leukocytes.[9,29]

In zoological settings, wild passerine birds often serve as reservoirs of hemoparasites.[10,21] Commonly isolated species of *Plasmodium* in North American birds include *P. relictum* and *P. elongatum*.[4,29] Infection typically progresses from a prepatent (exoerythrocytic) stage to an acute (erythrocytic) stage and finally to crisis stage characterized by a peak period of parasitemia. Afterward, a chronic period develops in which parasitemia is adequately controlled by the host immune system.[21] Although frequently detected, these hemoparasites are not generally considered to be pathogenic in most passerine and raptor species. These avian species are more often asymptomatic carriers of *Plasmodium* spp. with low morbidity and mortality despite chronic parasitemia.[17,33] An investigation by Hartup et al found limited correlation between chronic infection of house finches (*Haemorhous mexicanus*) with hematozoans (including *Haemoproteus*, *Plasmodium*, *Leukocytozoon*, and *Trypanosoma*) and host mortality.[30] Conversely, higher morbidity and mortality rates have been shown in naive species,

From the Humane Rescue Alliance, 71 Oglethorpe Street NW, Washington, DC 20011, USA (Baker); the Maryland Zoo in Baltimore, 1876 Mansion House Drive, Baltimore, Maryland 21217, USA (Rettenmund, Sander, Green, Bronson); the Bronx Zoo, 2300 Southern Boulevard, Bronx, New York, NY 10460, USA (Rivas); and Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology, 733 North Broadway, Baltimore, Maryland 21205, USA (Mangus). Correspondence should be directed to Dr. Baker (kendracbaker@gmail.com).

such as Hawaiian honeycreepers (*Drepanididae* spp.), as well as young birds with high parasite loads.[1,3,4,6,10,19] Other studies have indicated that chronic infection can have deleterious long-term effects on life expectancy, body condition, and fitness.[2,9,32]

Snowy owls (*Bubo scandiacus*) have a circumpolar geographic distribution and, in the winter, migrate to the northern parts of North America, Europe, and Asia.[16] During irruption years, snowy owls have been documented in the state of Maryland.[26] Although the vectors known to transmit *Plasmodium*, *Haemoproteus*, and *Leukocytozoon* spp. are found in Arctic conditions, direct transmission of *Plasmodium* in the North American Arctic has yet to be documented.[20,23]

This case series of five snowy owls housed at the Maryland Zoo in Baltimore (MZiB) describes the detection of hemoparasitic infections by blood smear evaluation and polymerase chain reaction (PCR), pathologic findings in two owls that died, and successful management of the three surviving cases.

CASE REPORTS

Blood smears were available from four of the five cases discussed and were reviewed by one veterinary technician (KG) to ensure consistency and standardization of the parasitemia level obtained. Slides were reviewed using an Olympus CX-41 microscope using a ×100 objective. An average number of red blood cells (RBC) per high-powered field (HPF) was determined (250 cells/HPF) before reviewing began. Twenty random fields were analyzed for a total of approximately 5,000 RBC. Within each field, the number of each hemoparasite species was recorded. The level of parasitemia for each parasite was then calculated and reported per 1,000 RBC.[8,28]

Samples of frozen whole blood and fixed blood slides were subsequently submitted to the Wildlife Disease Laboratories at the San Diego Zoo Institute for Conservation Research for SYBR® Green real-time PCR evaluation followed by conventional PCR; results were evaluated in conjunction with morphologic identification via microscopy.

The outdoor enclosure for all cases is a single-species wire mesh enclosure with no adjacent animal enclosures and minimal foot traffic. The dimensions for the enclosure are $55 \times 30 \times 16$ feet. Cases 2–5 were housed together in this enclosure prior to the death of case 2 and the release of case 3. Mosquitoes and other insects are not limited by the bars of the enclosure. Mosquitoes are anecdotally most abundant between April and November in Maryland; however, clinical cases of avian malaria do not usually present in collection birds at MZiB until June each year. Case 1 chronologically occurred 2 yr prior to cases 2–5. A time line summary for cases 2–5 can be found in Figure 1 as well as in the descriptions below.

Case 1

A 6-yr-old female captive-bred snowy owl was found dead in the enclosure in December. Pertinent medical history included unilateral ocular trauma leading to severe corneal ulceration, necessitating frequent restraint for medication administration. Treatment for the ocular trauma included topical ophthalmic medications and prophylactic oral itraconazole (Patriot Pharmaceuticals, LLC, Horsham, Pennsylvania 19044, USA; 10 mg/kg po sid) while the bird was housed at the zoo hospital in a quiet stall. Treatments were discontinued 8 days prior to death, and the bird was returned to the primary enclosure 1 day prior to death. Gross necropsy revealed a mild focal corneal opacity of the right eye, moderate transudative pericardial effusion, and multifocal petechiae and fibrin tags on the epicardial surface. Microscopically, 10–15-μm protozoal schizonts containing numerous 1–2-μm merozoites were frequently observed within endothelial cells and other cells in the lungs, liver, spleen, and kidneys, along with moderate subacute inflammation. Subacute epicarditis and myocarditis were also noted, although protozoal organisms were not found in the heart tissue. The cause of death was reported as a disseminated protozoal infection with pericardial effusion and pneumonia. PCR results were inconclusive for this animal.

Case 2

A 1-yr-old free-ranging female snowy owl presented to a wildlife rehabilitation facility with severe left ulnar and radial fractures. After surgical repair, the bird was deemed nonreleasable and entered the zoo collection in April 2014, where it was then housed in an outdoor, wire-sided enclosure. This owl had a history of a mild lingual plaque of unknown etiology in July that resolved within two weeks of presentation, and hemoparasites on regular blood smears morphologically consistent with *Leukocytozoon* and *Plasmodium* spp. Hematologic monitoring 1 mo preceding death showed an elevated hemoparasite burden with 11.4/1,000 RBC *Leukocytozoon* spp. (Fig. 2) and 4/1,000 RBC *Haemoproteus* spp.

Figure 1. Time line of major events occurring in cases 2–5. Markers indicating treatment for hemoparasites and itraconazole include shaded bars to represent treatment length.

Complete blood count (CBC) and biochemistry values were unremarkable per reference interval for this species.[31] No treatment was initiated at that time. A recheck of the hemoparasite burden 2 wk later showed a decline in hemoparasite burden to 6.4/1,000 RBC *Leukocytozoon* spp. and 0.4/1,000 RBC *Haemoproteus* spp.; CBC and biochemistry values remained unremarkable per reference interval for this species.[31] Again, no treatment was initiated, as the bird appeared to be tolerating the infection without evidence of clinical concerns. In August, the bird was transitioned to an adjacent, larger enclosure with cases 3–5. Fifteen days later, case 2 presented moribund. The owl's condition rapidly declined into cardiac and respiratory arrest; resuscitation attempts were unsuccessful. Weight loss was noted at the time of death. An immediate postmortem blood sample was obtained that showed 3.8/1,000 RBC *Leukocytozoon* spp. and 0.4/1,000 RBC *Haemoproteus* spp. Significant findings at necropsy included petechiae and areas of pallor on the epi- and endocardial surfaces. Complete histopathologic evaluation revealed severe lymphohistiocytic inflammation and necrosis affecting the myocardium, moderate reactive gliosis in the brain and brain stem, and varying degrees of lymphoplasmacytic and histocytic inflammation in the kidney, lung, and adrenal tissues. In the liver, there was moderate lymphoplasmacytic periportal hepatitis and widespread coagulative necrosis of hepatocytes. Elongate *Leukocytozoon* gametocytes were frequently observed within blood cells in the vasculature of multiple organs and within the parenchyma of the spleen, which was further characterized by marked lymphoid hyperplasia and large numbers of macrophages containing cellular debris and light brown to black granular pigment. Rare

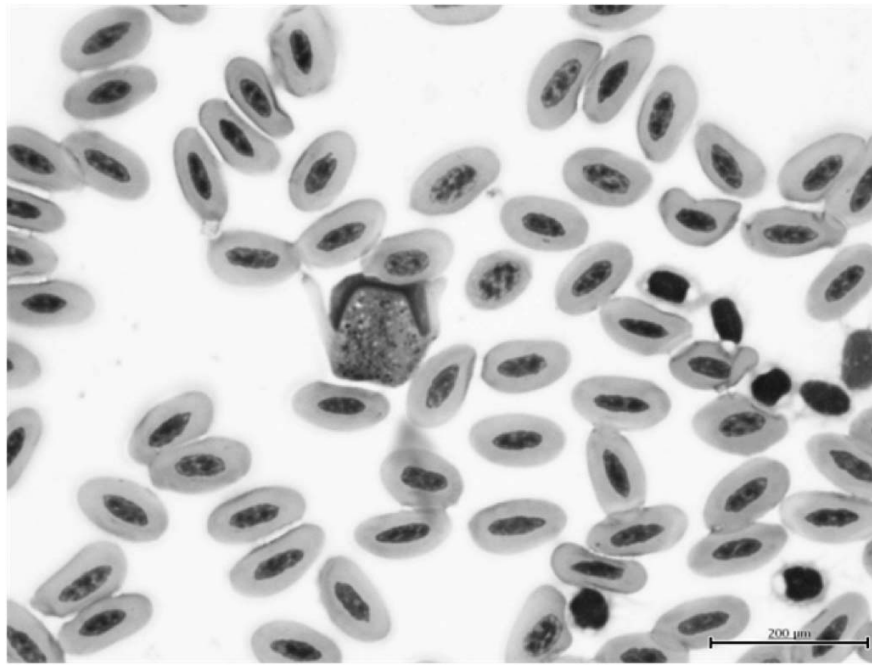

Figure 2. Photomicrograph of a snowy owl (*Bubo scandiacus*) blood smear stained with Wright-Giemsa stain. Intracellular Leukocytozoon (arrow) observed at ×100 (oil immersion).

protozoal cysts without associated inflammation were observed in the myocardium but were not further characterized. West Nile Virus (WNV) antigen was detected in sections of heart and liver tissue by immunohistochemistry. Although WNV was considered to be the primary cause of death in this owl, changes in the spleen suggested that protozoal parasitemia and ongoing erythrocyte destruction were contributory. PCR results from a whole blood sample 1 mo prior to death revealed a positive result with 99% identity with *P. relictum*.

Case 3

A 1-yr-old female free-ranging snowy owl was captured at a local airport with an open left metacarpal-phalangeal I luxation in March. After surgical repair and rehabilitation, the owl shared an enclosure with the birds discussed in cases 2, 4, and 5 from July to December of the same year. Following the death of case 2 in August, this animal was examined closely for hemoparasites. On initial evaluation in August, hemoparasite levels were noted to be 0.6/1,000 RBC *P. relictum* and 1.2/1,000 RBC *Leukocytozoon* spp. The CBC and biochemistry values were unremarkable per reference interval for this species.[31] Considering the potential contribution of these parasites to the death of case 2, treatment with chloroquine phosphate (Rising Pharmaceuticals, Inc., Allendale, New Jersey 07401, USA; 10 mg/kg loading dose, then 5 mg/kg po sid for three doses, then 5 mg/kg po q7 days for four doses) and primaquine phosphate (Taylors Pharmacy, Winter Park, Florida 32789, USA; 1 mg/kg po q7d for five doses) was implemented in this bird. Blood smear evaluation was performed after 10 days of treatment, and parasitemia was reduced to 0.2/1,000 RBC *P. relictum* and 0.6/1,000 RBC *Leukocytozoon* spp. The CBC and biochemistry values remained unremarkable per reference interval for this species.[31] Three and 4 wk after initiation of treatment, blood smear evaluation showed an absence of all hemoparasites. This animal was treated with an additional round of chloroquine phosphate (10 mg/kg po sid for 10 doses) and primaquine phosphate (1 mg/kg po sid for 10 doses) to ensure that the parasitemia had been eliminated. Evaluation of a blood smear 2.5 mo after initial evaluation demonstrated a resurgence of parasitemia (3.5 wk off treatment), with 0.6/1,000 RBC *P. relictum*, 0.4/1,000 RBC *Leukocytozoon* spp., 0.4/1,000 RBC *Haemoproteus* spp. (Fig. 3), and 0.2/1,000 RBC *Babesia* spp. A relative lymphocytosis of 72% was observed. Actual lymphocyte count ($5.46 \times 10^3/\mu l$; reference interval 0.57–$12.17 \times 10^3/\mu l$) and overall white blood cell (WBC) count were within reference interval for this species ($7.59 \times 10^3/\mu l$; reference interval 2.85–$24.74 \times 10^3/\mu l$).[31] Biochemistry values were within reference interval for this species.[31] Blood smear evaluation 1 mo later (3.5 mo after initial evaluation, 7.5 wk off treatment) noted unchanged levels of hemoparasites, and PCR results obtained from fresh frozen heparinized whole blood indicated 97% identity with *Plasmodium* spp. A mild absolute lymphocytosis of was observed ($12.38 \times 10^3/\mu l$), although the WBC count was still within reference interval ($13.75 \times 10^3/\mu l$).[31] The clinical significance of the lymphocytosis in this case is unclear; however, it could be attributed to antigenic stimulation or inflammation secondary to the documented hemoparasitic infection. A blood smear was reevaluated 10 days later (9 wk off treatment) and showed a hemoparasite burden of 0.8/1,000 RBC *P. elongatum*, 0.2/1,000 RBC *Leukocytozoon* spp., 2.4/1,000 RBC *Haemoproteus* spp., and 0.2/1,000 RBC *Babesia* spp. At that time, the lymphocytosis had resolved ($4.41 \times 10^3/\mu l$), and WBC count was still within reference interval ($7.48 \times 10^3/\mu l$).[31] Despite having mild to moderate hemoparasite levels, this bird was determined to be fit for release. Two months following release, in January 2015, this bird was recaptured to check the backpack transmitter placed prior to initial release. Evaluation of a blood smear was performed and showed no hemoparasites. CBC and biochemistry values were unremarkable per reference interval for this species at that time.[31]

Case 4

A 4-yr-old male snowy owl, captive bred in northern Germany prior to transfer to Maryland at the age of 1 yr, was screened for hemoparasites following the death of case 2, as these birds were housed together. On initial evaluation in August, case 4 had a hemoparasite burden of 1.8/1,000 RBC *Plasmodium* spp. trophozoites and 2.4/1,000 RBC *Leukocytozoon* spp. A mild leukocytosis ($26.6 \times 10^3/\mu l$) with a relative and absolute lymphocytosis (72%, $19.15 \times 10^3/\mu l$) was observed.[31] As in case 3, the significance of the lymphocytosis is unclear but suspected to be related to the concurrent hemoparasite infection. Biochemistry values were unremarkable per reference interval for this species.[31] PCR results from fresh frozen heparinized whole blood indicated 99% identity with *Haemoproteus syrnii* cytochrome b gene. Treatment was initiated in the same manner as described in case 3. Blood smear evaluation 3 wk

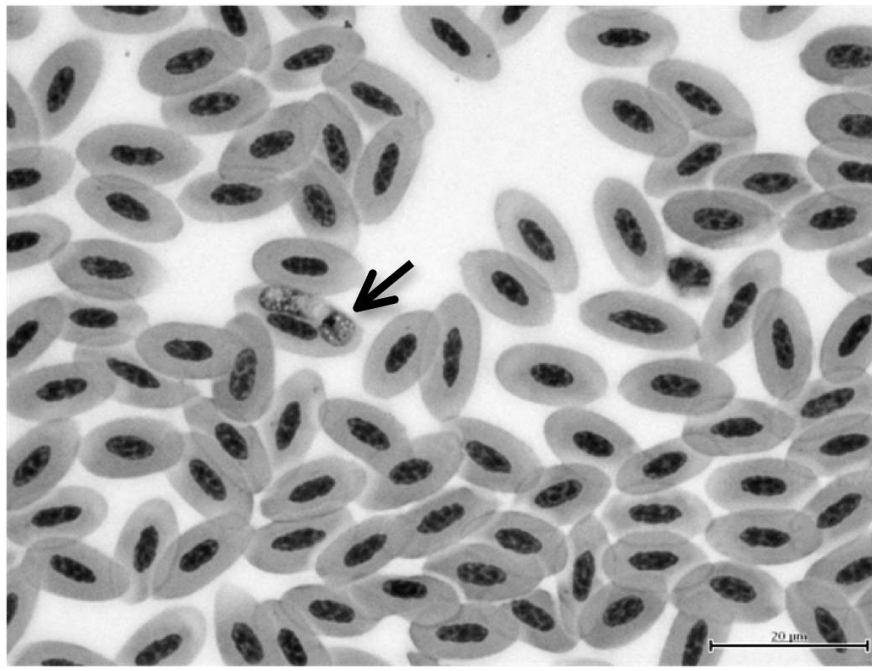

Figure 3. Photomicrograph of a snowy owl (*Bubo scandiacus*) blood smear stained with Wright-Giemsa stain. Intraerythrocytic *Haemoproteus* spp. (arrow) observed at ×100 (oil immersion).

after initial evaluation showed a reduction in hemoparasite burden, 0.2/1,000 RBC *P. elongatum* and 0.2/1,000 RBC *Plasmodium* spp. trophozoites. However, the previously noted leukocytosis and lymphocytosis had increased ($45.54 \times 10^3/\mu l$ and $30.05 \times 10^3/\mu l$, respectively).[31] One week later, a blood smear showed 0.2/1,000 RBC *P. relictum* with improvement but persistence of the previously noted leukocytosis ($27.7 \times 10^3/\mu l$) and lymphocytosis ($18.29 \times 10^3/\mu l$).[31] Empiric treatment with itraconazole (Patriot Pharmaceuticals, LLC, Horsham, Pennsylvania 19044, USA; (10 mg/kg sid 14d, per os) was implemented for possible *Aspergillus* spp. infection. An additional 10-day sid course of chloroquine phosphate and primaquine phosphate as described above was implemented 1 wk later. No hemoparasites were observed on repeated blood smear evaluations repeated three times from October to the following April despite cessation of prophylactic antimalarial medication. This bird is currently in good health, and blood smear evaluations are performed annually.

Case 5

A 4-yr-old female snowy owl, also captive bred in northern Germany with the bird described in case 4 prior to transfer to Maryland, was examined and treated for hemoparasites following the death of case 2. Case 5 had the lowest burden of hemoparasites with 0.2/1,000 RBC *Plasmodium* spp. trophozoites (Fig. 4) and 1.2/1,000 RBC *Haemoproteus* spp. observed. CBC and biochemistry values were unremarkable per reference interval for this species.[31] PCR results were later obtained from fresh frozen heparinized whole blood 1 mo prior to this date that showed a 99% identity with *P. relictum*. Treatment was initiated in the same manner as described in case 3 with chloroquine and primaquine for 4 wk. Two weeks into the treatment course, this bird was started on itraconazole (Patriot Pharmaceuticals; 10 mg/kg po sid for 28 doses). Blood smear evaluation at 3 and 4 wk after initial evaluation showed no hemoparasites and an unremarkable CBC and biochemistry panel.[31] This animal was treated with an additional round of chloroquine phosphate and primaquine phosphate as described in case 3 to ensure that the parasitemia had been eliminated. Six weeks after initial evaluation, 58/1,000 RBC *Babesia* spp. were observed on blood smear evaluation (Fig. 5). WBC count was increased ($21.6 \times 10^3/\mu l$) as compared to the previous sample ($18.9 \times 10^3/\mu l$) but still within normal limits. Due to this, the chloroquine phosphate and primaquine phosphate treatments

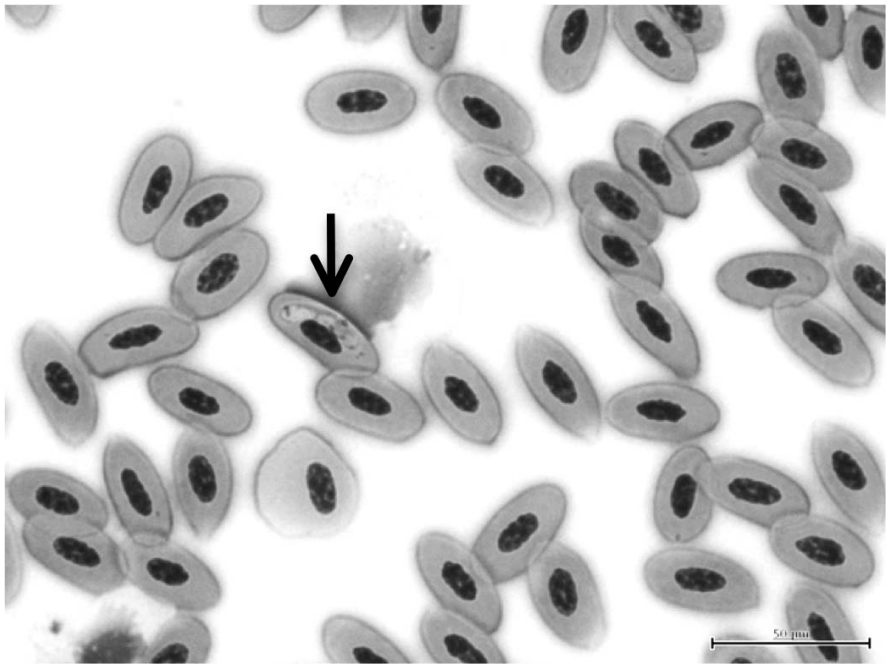

Figure 4. Photomicrograph of a snowy owl (*Bubo scandiacus*) blood smear stained with Wright-Giemsa stain. Intraerythrocytic *Plasmodium elongatum* (arrow) observed at ×100 (oil immersion).

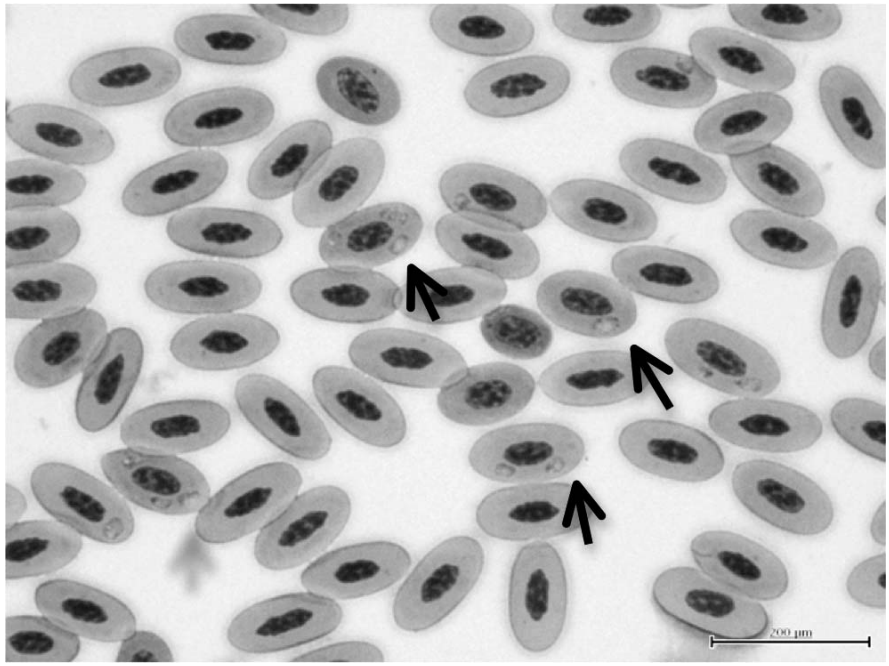

Figure 5. Photomicrograph of a snowy owl (*Bubo scandiacus*) blood smear stained with Wright-Giemsa stain. Intraerythrocytic *Babesia* spp. (arrows) observed at ×100 (oil immersion).

were extended for an additional 5 days. A blood smear was evaluated 10 days later, and the *Babesia* spp. load had decreased to 1.2/1,000 RBC, with a concurrent decrease in the WBC count (15×10^3/μl).[31] Throughout the following fall and winter, three blood smear evaluations revealed low levels of hemoparasites (0.4/1,000 RBC *Plasmodium* spp. trophozoites and 0.2/1,000 *Haemoproteus* spp.) and a WBC count within reference intervals.[31] This bird is currently in good health, and blood smear evaluations are performed annually.

DISCUSSION

Birds of prey are not typically considered susceptible to clinical infection with hemoparasites. In healthy birds, parasite burden is typically maintained at low levels by innate and adaptive immune responses.[21,29] However, Arctic, Antarctic and island species are believed to be more susceptible to infection by hemoparasites, particularly *Plasmodium* spp.[21,23,24] While an obvious stressor is not always evident in conjunction with increased parasitemia, increases in parasite burden can be a valuable indicator of underlying disease or disease severity.[5]

Based on the cases presented here and by Evans et al, hemoparasitic disease is not benign in snowy owls as it is believed to be in other raptor species. Stressors such as high environmental temperatures likely contributed to several of the described cases. Arctic temperatures during the summer months (June–September) typically vary from 0°C to 17°C.[7,15] Heat stress may play a role in reactivation of chronic disease into acute infection, especially in bird species that are housed outside of their preferred thermoneutral zone.[12,13] The mortality of case 2 occurred during the very warm and humid summer months in Maryland, as did the increased parasitemia observed in cases 3–5. Maryland temperatures during summer months (June–September) range from 27°C to 38°C.[35] Compounding this, restraint and administration of oral medication may be stressful in some birds, such as snowy owls, and the cost–benefit ratio should be considered to balance the need to medicate with keeping stress to a minimum. The necessary handling in order to treat the ocular trauma in case 1 likely contributed to increased stress and may have led to the overwhelming parasitemia. Emphasizing this point, case 1 was the only case discussed where parasitemia was the primary cause of death. In case 2, WNV was determined to be the primary cause of death, although comorbid hemoparasitic infection and resulting erythrocyte destruction were considered contributory. In addition, 15 days prior to death, this bird was moved into the same enclosure as cases 3–5, and this could have contributed to increased stress due to the novel housing situation. Cases 3–5 were initially evaluated and treated following the mortality of case 2. Parasitemia was noted, and treatments were initiated during the summer months, when environmental temperatures and higher vector densities may have been a factor in the fluctuating parasitemia. The introduction of the birds in cases 2 and 3 to the birds in cases 4 and 5 may have created additional stress, as these birds had not been historically housed together. The increase in hemoparasite burden and addition of new hemoparasites despite treatment in cases 3–5 can be attributed to continued exposure to vectors as well as identification of hemoparasites based on the stage of infection (tissue vs blood stage).

Vector control, cooling, and reducing handling are further measures that can be implemented to decrease hemoparasitic disease. Environmental management, including the addition of fans, removal of standing water, and the use of insect growth inhibitors and pyrethrin insect spray around the perimeter of the enclosure, is now utilized at MZiB to minimize the risk of vector exposure to cases 4 and 5. Lower parasite burdens have been noted on blood smears during subsequent summers, and the owls have remained asymptomatic. This decrease in parasite burden is likely attributed to the additional environmental vector control. Vector control should be implemented in susceptible climates, as prevention is far more effective than treatment.

Effects of hemoparasite infection can be subtle, especially in subclinical cases. Asghar et al investigated short- and long-term fitness effects of chronic infection with *Plasmodium* and *Haemoproteus* spp. in great reed warblers (*Acrocephalus arundinaceus*). While this study found no obvious short-term effects of chronic infection, long-term effects included telomere shortening, which has been correlated with organ dysfunction and accelerated aging.[2] Another study evaluated the effects of infection with one versus two different species of hemoparasites (*Haemoproteus* and *Plasmodium* spp.) in house martins (*Delichon urbicum*). This study found that birds with two species of hemoparasites had lower body condition and decreased survival times compared to those with a single-species infection; however, birds with double infection showed higher reproductive success.[22] In contrast to these two studies, there are studies indicating that low-level chronic

hemoparasitemia has no effect on survival of multiple bird species.[14]

There are few documented reports of acute hemoparasite-associated disease in raptors. Evans et al reported mortality of two juvenile snowy owls following a combined infection with *Haemoproteus* and *Leukocytozoon* spp.[9] In another case, heat stress is mentioned as a factor contributing to the mortality of a snowy owl concurrently infected with WNV and *Haemoproteus* spp.[13] Tavernier et al report a case of an overwhelming *P. supraecox* infection in addition to traumatic injuries in an Eastern screech owl (*Megascops asio*). After implementing treatment with mefloquine, the owl showed rapid clinical improvement and decreased parasitemia.[32]

Gyrfalcons (*Falco rusticolus*) are suspected to be more susceptible to *Plasmodium* spp. infection than other raptor species and show clinical disease as well as mortality with lower parasite loads, presumably due to their stressed temperament in captivity.[27,29] The Raptor Center in St. Paul, Minnesota, distinguishes snowy owls as a species that is also more susceptible than other raptors to clinical disease from hemoparasite infection and recommends routine antimalarial prophylaxis during insect season in both gyrfalcon and snowy owls in captivity.[27]

Diagnosis of hemoparasites can be achieved via microscopy based on morphology as well as molecular diagnostics. Morphologic diagnosis in conjunction with PCR identification of hemoparasites increases confidence in diagnosis, as studies have shown that using PCR alone can underestimate the occurrence of mixed infections.[24,33,34] The samples submitted for PCR identification in the preceding cases had been collected 2 yr prior to laboratory submission. As such, there is a potential for DNA degradation. For these reasons, inclusion of both morphologic and PCR identification was utilized in this case series.

When determining whether treatment for *Plasmodium* spp. or other hemoparasites is indicated, considerations must include the impact of treatment on the well-being of the animal and the low likelihood of clearing the infection. The individual animal should be taken into account, as not all infections will require pharmacological treatment. Treatment is typically well tolerated in avian species, although primaquine phosphate can cause gastrointestinal upset, myelosuppression, methemoglobinemia, and hemolysis, whereas chloroquine can cause retinopathy, neuromyopathy, and hepatotoxicity in humans.[18,25] Due to the dual stages of *Plasmodium* infection, medications that target each stage are recommended in acute infections, namely, chloroquine phosphate for blood stages and primaquine phosphate for tissue stages of the parasite.[4,21,29,30] Other drugs, such as mefloquine and doxycycline, have been used anecdotally with success in select avian species for prophylaxis and treatment of *Plasmodium* spp. infections; however, pharmacologic data are lacking for antimalarial drugs in birds. It is interesting to postulate whether the birds described in cases 4 and 5 would have tolerated their hemoparasite burdens without pharmacologic assistance, as they showed no clinical signs of concurrent disease and were captive bred.

CONCLUSIONS

The potential for clinical disease with hemoparasite infection in snowy owls is supported by one case of mortality in a snowy owl and a second case in which hemoparasitic infection contributed to the death of a snowy owl. Three further cases demonstrated high parasite burdens of five hemoparasite species during a period of stress related to high seasonal temperatures, close proximity to conspecifics, and stress related to medical management. After mitigation of most of these factors, monitoring in subsequent years revealed lower levels of hemoparasitemia. This case series highlights the potential sensitivity of this species to hemoparasites and the effective management in clinically asymptomatic individuals to reduce the parasite burden. Because of their natural history, snowy owls may be affected clinically during times of stress or concurrent disease more so than other raptor species.

Acknowledgments: The authors would like to acknowledge the animal care staff, including keepers, animal managers, veterinarians, and veterinary technicians at the Maryland Zoo in Baltimore, for the diligent care they continue to provide for these birds. The authors also acknowledge the valuable collaboration of Project SNOWstorm, particularly with Dr. Cindy Driscoll, David Brinker, and Dr. Erica Miller.

LITERATURE CITED

1. Alley MR, Fairley RA, Martin DG, Howe L, Atkinson T. An outbreak of avian malaria in captive yellowheads/mohua (*Mohoua ochrocephala*). N Z Vet J. 2008;56:247–251.

2. Asghar M, Hasselquist D, Hansson B, Zehtindjiev P, Westerdah H, Bensch S. Hidden costs of infection:

chronic malaria accelerates telomere degradation and senescence in wild birds. Science. 2015;347:436–438.

3. Atkinson C. Avian malaria. In: Atkinson C, Thomas N, Hunter D (eds.). Parasitic diseases of wild birds. Ames (IA): Wiley-Blackwell; 2008. p. 35–53.

4. Atkinson CT, van Riper C. Pathogenicity and epizootiology of avian haematozoa: *Plasmodium, Haemoproteus*, and *Leucocytozoon*. In: Loye JE, Zuk M (eds.). Bird-parasite interactions: ecology, evolution and behavior. London (United Kingdom): Oxford University Press; 1991. p. 19–48.

5. Bowman DD (ed.). Protozoans. In: Georgis' parasitology for veterinarians, 6th ed. Philadelphia (PA): W. B. Saunders; 1995 p. 83–111.

6. Cranfield MR, Graczyk TK, Beall FB, Ialeggio DM, Shaw ML, Skjoldager ML. Subclinical avian malaria infections in African black-footed penguins (*Spheniscus demersus*) and induction of parasite recrudescence. J Wildl Dis. 1994;30:372–376.

7. Climate Kids [Internet]. Earth Science Communications Team at NASA's Jet Propulsion Laboratory/California Institute of Technology [cited 2017 June 6]. Available from http://climatekids.nasa.gov/polar-temperatures

8. DPDx Laboratory Identification of Parasites of Public Health Concern. *Plasmodium* spp. Determination of Parasitemia [Internet]. Centers for Disease and Control Prevention [cited 2016 October 1]. Available from https://www.cdc.gov/dpdx/resources/pdf/benchAids/malaria/Parasitemia_and_LifeCycle.pdf

9. Evans M, Otter A. Fatal combined infection with *Haemoproteus noctuae* and *Leukocytozoon ziemanni* in juvenile snowy owls (*Nyctea scandiaca*). Vet Rec. 1998; 143:72–76.

10. Ferrell ST, Snowden K, Marlar AB, Garner M, Lung NP. Fatal hemoprotozoal infections in multiple avian species in a zoological park. J Zoo Wildl Med. 2007;38:309–316.

11. Friend M, Franson JC (eds.). Hemosporidiosis. In: Field manual of wildlife diseases—general field procedures and diseases of birds. Madison (WI): U.S. Geological Survey; 1999. p. 193–199.

12. Gonzalez-Quevedo C, Davies R, Richardson D. Predictors of malaria infection in a wild bird population: landscape-level analyses reveal climatic and anthropogenic factors. J Anim Ecol. 2014;83:1091–1102.

13. Harasym C. West Nile Virus and hemoparasites in captive snowy owls (*Bubo scandiacus*)—management strategies to optimize survival. Can Vet J. 2008;49: 1136–1138.

14. Hartup BK, Oberc A, Stott-Messick B, Davis AK, Swarthout EC. Blood parasites of house finches (*Carpodacus mexicanus*) from Georgia and New York. J Wild Dis. 2008;44:469–474.

15. Hebert PDN (ed.). Canada's Polar Environments [Internet]. CyberNatural Software, University of Guelph; c2002 [cited 2017 June 6]. Available from http://www.arctic.uoguelph.ca/cpe/environments/climate/climte_present/temp/arc_summ.htm

16. Holt DW, Larson MD, Smith N, Evans DL, Parmalee DF. Snowy owl (*Bubo scandiacus*). In: Rodewald PG (ed.). The birds of North America. Ithaca (NY): Cornell Lab of Ornithology; 2015 [cited 2016 October 30]. Available from https://birdsna.org/Species-Account/bna/species/snoowl1

17. Howe L, Castro IC, Shoener ER, Hunter S, Barraclough RK, Alley MR. Malaria parasites (*Plasmodium* spp.) infecting introduced, native and endemic New Zealand birds. Parasitol Res. 2012;110:913–923.

18. Invanz. Chloroquine phosphate. In: Physicians' desk reference, open access website version; 2012. Available from http://www.pdr.net

19. Krams IA, Suraka V, Rantala MJ, Sepp T, Mierauskas P, Vrublevska J, Krama T. Acute infection of avian malaria impairs concentration of haemoglobin and survival in juvenile altricial birds. J Zool. 2013;291: 34–41.

20. Laird M. A lack of avian and mammalian haematozoa in the Antarctic and Canadian arctic. Can J Zool. 1961;39:209–213.

21. Levin I, Parker G. Haemosporidian parasites: impacts on avian hosts. In: Miller RE, Fowler ME (eds.). Zoo and wild animal medicine, Volume 7, Current therapy. St. Louis (MO): Elsevier; 2012. p. 356–362.

22. Marzal A, Bensch S, Reviriego M, Balbontin J, De Lope F. Effects of malaria double infection in birds: one plus one is not two. J Evol Biol. 2008;21:979–987.

23. Meixell BW, Arnold TW, Lindberg MS, Smith MM, Runstadler JA, Ramey AM. Detection, prevalence and transmission of avian hematozoa in waterfowl at the Arctic/sub-Arctic interface: co-infections, viral interactions and sources of variation. Parasit Vectors. 2016;9:390.

24. Parker D; updated by Valkiûnas G. Plasmodium. In: American Association of Zoo Veterinarians Infectious Disease Committee Manual [Internet]. c2013 [cited 2016 October 1]. Available from http://c.ymcdn.com/sites/www.aazv.org/resource/resmgr/IDM/IDM_Plasmodium_2013.pdf

25. Plumb DC. Primaquine phosphate. In: Plumb's veterinary drug handbook, 7th ed. Ames (IA): Wiley; 2011. p. 854–855.

26. Project SNOWstorm [Internet]. Project Owlnet; c2017 [cited 2017 June 6]. Available from http://www.projectsnowstorm.org

27. Redig P, Ponder J. Raptors: practical information every avian practitioner can use. Proc Assoc Avian Vet. 2010:176.

28. Reinoso-Pérez MT, Canales-Delgadillo JC, Chapa-Vargas L, Riego-Ruiz L. Haemosporidian parasite prevalence, parasitemia, and diversity in three resident bird species at a shrubland dominated landscape of the Mexican highland plateau. Parasit Vectors. 2016;9:307.

29. Remple J. Intracellular hematozoa of raptors: a review and update. J Avian Med Surg. 2004;18:75–88.

30. Sohsuebngarm D, Sasipreeyajan J, Nithiuthai S, Chansiripornchai N. The efficacy of artesunate, chloroquine, doxycycline, primaquine and a combination of artesunate and primaquine against avian malaria in broilers. J Vet Med Sci. 2014;76:813–817.

31. Sponsoring Institution of data access. [Internet]. (Version 1.7, updated 2014 January 27). Zoological Information Management System [cited 2016 December 14] Available from http://zims.Species360.org

32. Tavernier P, Sagesse M, Van Wettere A, Redig P. Malaria in an Eastern screech owl (*Otus asio*). Avian Dis. 2005;49:433–435.

33. Valkiunas G, Bensch S, Iezhova TA, Krizanauskiene A, Hellgren O, Bolshakov CV. Nested cytochrome B polymerase chain reaction diagnostics underestimate mixed infections of avian blood haemosporidian parasites: microscopy is still essential. J Parisitol. 2006;92:418–422.

34. Valkiunas G, Iezhova TA, Krizanauskiene A, Palinauskas V, Sehgal RN, Bensch S. A comparative analysis of microscopy and PCR-based detection methods for blood parasites. J Parisitol. 2008;94: 1395–1401.

35. Weather Underground [Internet]. The Weather Company, LLC [cited 2017 June 6]. Available from https://www.wunderground.com/history/airport/KDMH/2016/6/1/CustomHistory.html?dayend=30&monthend=9&yearend=2016&req_city=&req_state=&req_statename=&reqdb.zip=&reqdb.magic=&reqdb.wmo=&MR=1

Accepted for publication 09 October 2017