NOTE

Wildlife Science

The first clinical cases of *Haemoproteus* infection in a snowy owl (*Bubo scandiacus*) and a goshawk (*Accipiter gentilis*) at a zoo in the Republic of Korea

Seung-Hun LEE[1,2], Dongmi KWAK[1] and Kyoo-Tae KIM[3]*

[1]College of Veterinary Medicine, Kyungpook National University, Daegu 41566, Republic of Korea
[2]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan
[3]Animal Health Center of Zoo Land, Daejeon O-World Theme Park, Daejeon 35073, Republic of Korea

ABSTRACT. This study reports two clinical cases of avian haemosporidian infection caused by a *Haemoproteus* sp., involving a snowy owl (*Bubo scandiacus*) and a goshawk (*Accipiter gentilis*), at a zoo. The snowy owl died after presenting with anorexia, depression and lethargy. A blood smear with Wright's staining confirmed *Haemoproteus* infection. Necropsy of the snowy owl revealed hypertrophy of the internal organs, including the liver, gallbladder, kidney and adrenal glands. The goshawk showed anorexia, depression and a lowered head position, and was diagnosed with a *Haemoproteus* infection based on a blood smear. The goshawk was completely cured by treatment with a combination of atovaquone and proguanil hydrochloride. Both cases showed decreased erythrocytes, hemoglobin and hematocrit values on complete blood count.

KEY WORDS: *Accipiter gentilis,* avian malaria, *Bubo scandiacus,* captive bird, *Haemoproteus* infection

Avian haemosporidian parasites (*Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp.) are vector-borne pathogens belonging to the families Plasmodiidae, Haemoproteidae and Leucocytozoidae, and are also known as avian malaria parasites [4, 25]. Parasites are transmitted through exclusive vectors, such as mosquitoes (Culicidae) for *Plasmodium*, blood-sucking simuliid flies (Simuliidae) for *Leucocytozoon* and biting midges (Ceratopogonidae) and hippoboscid flies (Hippoboscidae) for *Haemoproteus* [7, 24].

Avian malaria is a cosmopolitan avian disease and more than 250 species of avian haemosporidian parasites have been reported in 40 countries [4, 25]. The distribution of haemosporidian parasites can be affected by various factors, including climate, vector distribution, genetics and host factors, such as age or immunity [11]. Recent studies have shown that migratory birds can act as important carriers of the parasites, transporting the parasites from one geographical region to another. Moreover, migratory birds can cause cross-species transmission to resident birds [7, 8].

Diagnosis of haemosporidian infection is mainly based on the identification of parasites in blood samples. Because the recent development of molecular techniques has allowed veterinarians and researchers to diagnose these parasites, their species and lineages have been identified [25].

In the Republic of Korea (ROK), some previous studies have identified avian haemosporidian parasites at morphological and molecular levels in wild birds; however, those studies focused on the detection of the pathogens and the hosts were asymptomatic [5, 8, 9]. A sole case of *Plasmodium* infection in a captive penguin has been reported [12]. To the best of our knowledge, no clinical cases of *Haemoproteus* infection in birds have been reported in the ROK. In the present study, we report two clinical cases of haemosporidian infection caused by a *Haemoproteus* sp. in captive birds in the ROK.

The first case, a 10-year-old male snowy owl (Strigiformes; *Bubo scandiacus*), housed at a zoo in Daejeon O-World, Daejeon, the ROK, presented with anorexia, depression and lethargy for two days before dying (Fig. 1A). To identify the cause of death, a necropsy, complete blood count (CBC), blood chemistry examination and blood smear were performed. Blood was collected from the cutaneous ulnar vein as soon as possible after the bird's death.

The appearance of the body was normal upon visual inspection; the bird weighed 1.3 kg and no specific gross lesions were observed. Necropsy revealed enlargements of the liver, gallbladder (Fig. 1B), kidney and adrenal glands. Necrosis was observed in the pancreas and the proventriculus was found to be congested (Fig. 1C). The large and small intestines were almost empty and the
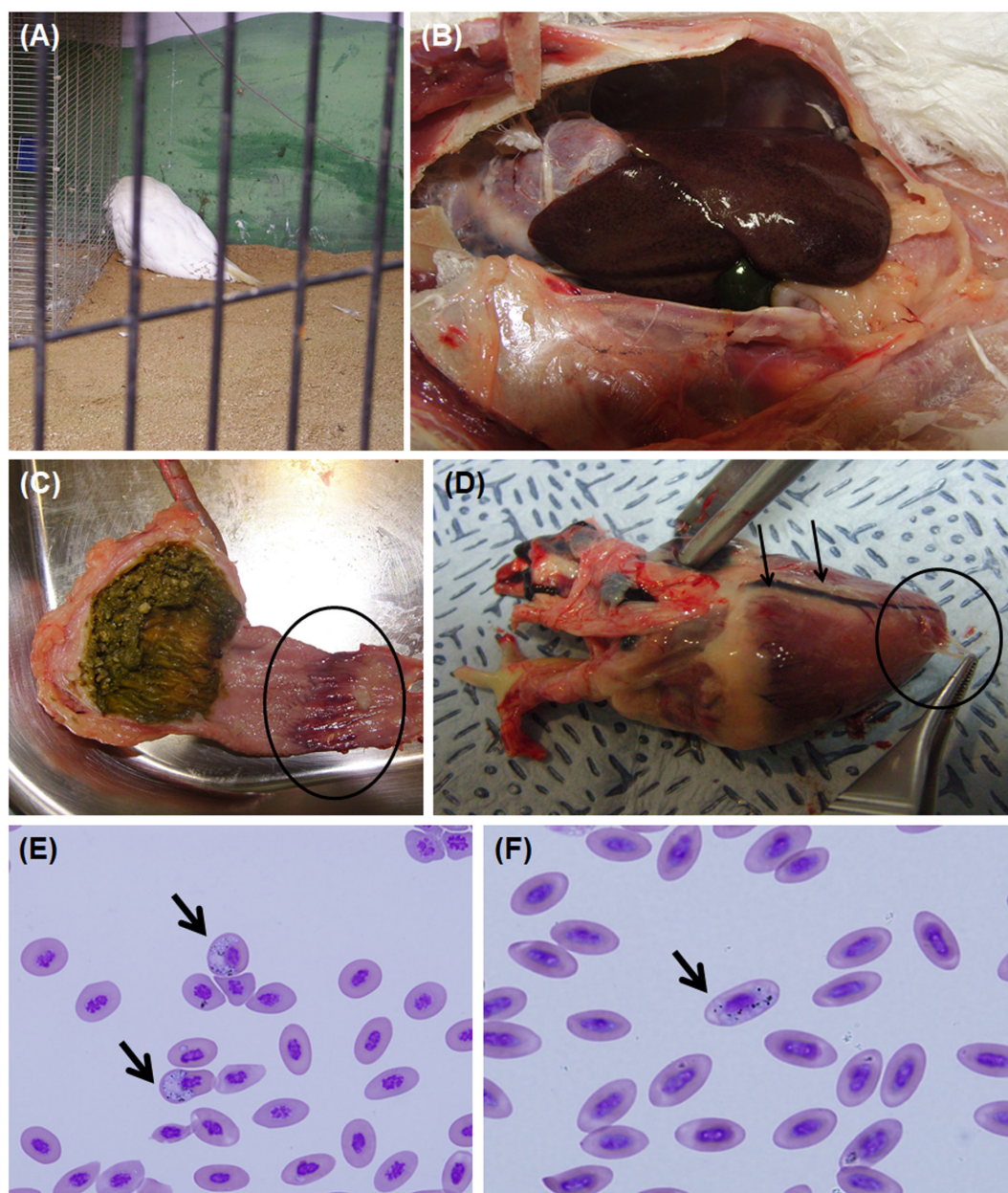
Fig. 1. *Haemoproteus* infection in a snowy owl (*Bubo scandiacus*) and a goshawk (*Accipiter gentilis*). (A) A snowy owl presenting with lethargy, dullness and anorexia before death. Necropsy of the snowy owl showed (B) discoloration of the liver and enlargement of the gall bladder, (C) congestion and hemorrhage of the proventriculus, (D) fat infiltration in the epicardium (dark arrows) and fibrous inflammation at the apex of the heart (dark circle). A blood smear, stained with Wright's solution, of the (E) snowy owl and (F) goshawk. Arrows (E, F) indicate *Haemoproteus* infection in erythrocytes.

duodenalis was slightly enlarged. In the epicardium, fibrous materials at the apex of the heart and fat infiltration were observed and the epicardium itself was superficially uneven (Fig. 1D). When the heart was dissected, the atrium and ventricles were found to be enlarged.

The CBC showed increased mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, and reduced erythrocyte, hemoglobin and hematocrit levels (Table 1). In addition, the blood chemistry examination revealed elevated levels of gamma glutamyl transferase (9 U/*l*; reference values: 7−7 U/*l*) and alkaline phosphatase (129 U/*l*; reference values: 21−51 U/*l*) [6]. Wright's staining of a blood smear revealed inclusion bodies in the erythrocytes (Fig. 1E). Based on the CBC results and the blood smear, *Haemoproteus* sp. infection was confirmed [24]. After confirmation of *Haemoproteus* infection in the snowy owl, other birds in the same aviary were tested for *Haemoproteus* infection using the same method; however, no other cases were identified.

To rule out the possibility of bacterial infection, tissue samples collected from the lung, liver, kidney, proventriculus and small

Table 1. Complete blood count values from the snowy owl (*Bubo scandiacus*) and goshawk (*Accipiter gentilis*) infected with a *Haemoproteus* sp.

Variables	Snowy owl		Goshawk	
	Values	Reference range [6]	Values	Reference range [6]
WBC ($\times 10^3$ cells/μl)	15.2	4.0–37.6	11.0	4.0–11.0
Neutrophil ($\times 10^3$ cells/μl)[a]	10.0	-	8.0	-
Lymphocyte ($\times 10^3$ cells/μl)[a]	4.6	-	1.5	-
Monocyte ($\times 10^3$ cells/μl)[a]	0.5	-	0.9	-
Eosinophil ($\times 10^3$ cells/μl)[a]	0.2	-	0.6	-
Basophil ($\times 10^3$ cells/μl)[a]	0.0	-	0.0	-
Erythrocytes ($\times 10^6$ cells/μl)	1.35	2.2–3.7	2.04	2.6–3.48
Hemoglobin (g/dl)	10.3	12.2–12.2	12.2	12.1–17.7
Hematocrit (%)	29.0	33.0–51.0	41.5	43.0–53.0
MCV (fl)	214.8	118.9–190.9	203.0	141.0–156.0
MCH (%)	76.3	-	59.8	44.5–51.6
MCHC (g/dl)	35.5	29.0–29.0	29.3	30.5–34.3

a) Reference values are not available. MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

and large intestines were cultured on blood agar and MacConkey agar at 37°C for 12–18 hr. No pathogenic bacteria were identified from any of the cultures.

In addition to the microscopic examination, DNA was extracted from whole blood and liver tissue using a DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) for molecular diagnosis and characterization. Polymerase chain reaction (PCR) was used in order to amplify the mitochondrial cytochrome *b* region specific to haemosporidian parasites, as previously described [25], without a positive control; no PCR products were identified.

The second case was a 3-year-old male goshawk (Falconiformes; *Accipiter gentilis*), housed at the same zoo; the bird showed anorexia, depression and a permanently lowered head position. For diagnosis, blood was collected from the cutaneous ulnar vein, and a CBC and Wright's staining of a blood smear were performed. The CBC revealed an elevated level of eosinophils and lowered levels of erythrocytes, hemoglobin and hematocrit (Table 1). Inclusion bodies were observed in the erythrocytes and a *Haemoproteus* sp. infection was confirmed (Fig. 1F) [24]. As in the first case, DNA was extracted from whole blood and PCR was attempted with the same method as mentioned; however, again, no PCR products were obtained.

To treat the *Haemoproteus* sp. infection in the goshawk, a combination of atovaquone and proguanil hydrochloride (Malarone™, GlaxoSmithKline, Mississauga, ON, Canada) was administered orally. Specifically, half a tablet was given with feeding once a day for three days. After a break of one week, another treatment course was administered in the same way for another three days. After two weeks, the *Haemoproteus* sp. infection was completely cured, and no haemosporidian parasites were observed in a blood smear stained with Wright's solution.

For differential diagnosis of *Haemoproteus* spp. infection, infection with *Plasmodium* spp. and *Leucocytozoon* spp. should be considered. In this study, the parasites in both cases were identified as belonging to *Haemoproteus*, based on the following points: the presence of an iron pigment, called hemozin, caused a black color in the internal organs (Fig. 1B, 1E and 1F); gametocytes took up a large portion of the erythrocytes, without displacing the nucleus (Fig. 1E and 1F); and asexually reproducing stages (schizonts) were absent [1, 20, 24]. Species identification may be achieved based on microscopic observations; however, it requires live parasites and technical skill, as the species differ only subtly [24].

The pathogenicity of avian haemosporidian infections varies according to the parasite and host species. Generally, *Haemoproteus* infection is considered a benign condition; in contrast, *Plasmodium* infection can have severe outcomes [13]. However, studies have reported the clinical relevance of *Haemoproteus* infection to anemia, reluctance to move, hypertrophy of the internal organs, watery blood, low reproductivity, reduced female body mass and reduced survival [1–3, 14, 18, 19]. Those clinical symptoms are consistent with our findings, such as enlargement of the liver, gallbladder, kidney and adrenal glands, and necrosis of the pancreas.

In this study, the two cases of *Haemoproteus* infection had different outcomes. In the first case, the bird showed only general symptoms (anorexia, depression and lethargy) and suddenly died after two days, while its progress was under observation, and *Haemoproteus* infection was confirmed by necropsy. The second case, which showed similar symptoms to the first case, was diagnosed with *Haemoproteus* infection early on, based on microscopic examination and a CBC, and was treated appropriately. Based on these difference, the authors believe that early diagnosis and treatment are important in cases of *Haemoproteus* infection.

As the main site of infection of *Haemoproteus* is the erythrocytes of avian species, the infection causes hematological abnormalities. Consistent results were observed in both presented cases, based on CBC and hematological examination. Specifically, decreased erythrocytes, hemoglobin and hematocrit values might be attributed to the *Haemoproteus* infection. In addition, elevated gamma glutamyl transferase and alkaline phosphatase levels suggest decreased liver function.

For avian anti-malaria medication, chloroquinine, primaquine, atovaquone and proguanil hydrochloride are recommended [15, 22]. The combination of atovaquone and proguanil hydrochloride (Malarone™, GlaxoSmithKline) used in this study was

originally developed as a human antimalarial medication, but also shows high effectivity against avian malaria [10, 17, 22]. Interestingly, La Puente *et al.* [14] reported that *Haemoproteus* treatment using primaquine showed stronger medication effects in female than male wild blue tits (Passeriformes; *Cyanistes caeruleus*). Because few studies are available on experimental infection with *Haemoproteus*, additional studies are required to assess the effects of host species and sex on the course of the disease.

In the present study, DNA was extracted from whole blood and liver tissue, and PCR was performed for molecular diagnosis and characterization. However, neither of the cases revealed positive PCR products. Consistent results were described in several previous studies, which reported that the PCR assay and microscopic examination had similar sensitivity for diagnosing haemosporidian parasites; however, these studies showed false-negative PCR results [2, 13, 23]. A possible reason for the false-negative results in the PCR assay may be the particularly high concentration of DNA in erythrocyte nuclei in birds, DNA degradation or the genetic diversity of haemosporidian species [13, 16, 21, 23]. Unfortunately, no positive control was available in the present study, and unexpected error in the PCR process cannot be excluded.

In conclusion, we here reported the first clinical cases of haemosporidian infection caused by a *Haemoproteus* sp. in two captive birds in the ROK. The results of this study suggest that early diagnosis and treatment are important for the recovery of birds from haemosporidian infection. Because the ROK is one of the stopover sites for migratory birds and *Haemoproteus* spp. have been identified in wild birds in this country [5, 8, 9], continuous monitoring for haemosporidian parasites in captive and domestic birds is required.

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