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Molecular Detection of *Haemoproteus* in Two Wild Eurasian Eagle Owls (*Bubo bubo*) in Middle Area of South Korea

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Abstract : This report describes asymptomatic *Haemoproteus* infection in wild Eurasian eagle owls (*Bubo bubo*) diagnosed by blood smear and DNA analysis. This is the first description of natural *Haemoproteus* infection in wild Eurasian eagle owl in South Korea and suggests that the wild resident bird population can be a source for *Haemoproteus* infection of domestic poultry.

Key words **:** *Haemoproteus*, Eurasian eagle owl, resident bird, South Korea.

Introduction

The avian hematozoan parasites *Haemoproteus* spp. have a complex developmental cycle in arthropod vectors and are transmitted when the vector bites a host (6,8). The infected host then facilitates pathogen spread to other susceptible hosts. The prevalence of *Haemoproteus* infection is highly related to host-vector-parasite interactions (11). At a given geographic location, variation in the size of the vector population is positively correlated with variation in the prevalence of the disease in avian communities (3). Altitude, climate, and migratory bird movements also affect the distribution and development of the vectors and parasites (9,13). Therefore, to determine the best strategies for infection control by regional groups, the prevalence of the disease in local bird communities needs to be understood.

In this study, we report the first cases of asymptomatic *Haemoproteus* infection in two wild Eurasian eagle owl (*Bubo bubo*) in South Korea. The infection was diagnosed by blood smear and DNA analysis.

Case

In June 2011, two Eurasian eagle owls captured by a local rescue party on a roadside in a downtown area in Chungbuk province, South Korea, were referred to the Wildlife Center of Chungbuk because of inability to fly. Upon examination, both owls had a drooped wing due to an open fracture of the humerus. Around the fracture, necrotized tissue and myiasis were noted. The owls were emaciated; however, they were sensitive to external stimuli. Peripheral blood was collected from the wing vein and submitted for complete blood count (CBC), serum biochemistry, and blood smear examination. CBC and serum biochemistry showed no significant abnormalities. However, the Wright-Giemsa stained blood smear revealed intraerythrocytic parasites in a cylindrical body (1 to 3 parasites/100 RBCs) (Fig 1), leading to a diagnosis of blood hematozoan parasite infection.

The intraerythrocytic parasites in the blood smears were identified by molecular typing. After the genomic DNA of the parasites was extracted from 10 μL EDTA-treated whole blood using a Dynabeads® DNA DIRECT™ Universal Kit (Invitrogen Life Technologies, Carlsbad, CA, USA), identification of parasites was accomplished by amplification of the mitochondrial large subunit (LSU) ribosomal RNA (rRNA) gene using primers 343F and 496R, as described previously (7). PCR amplification was carried out in a total volume of 50 μL. The final reaction conditions were as follows: 50 mM

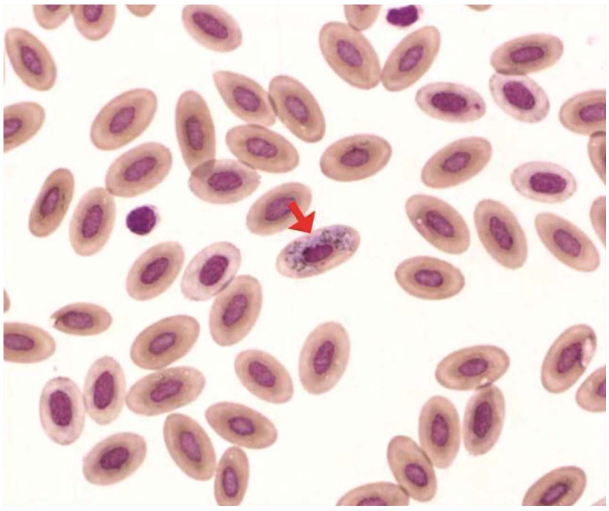

Fig 1. Microscopic finding showing the intracytoplasmic gametocyte of *Haemoproteus* (arrow) that encircle the RBC nucleus, indicating the halter-shaped appearance in an Eurasian eagle owl. Wright-Giemsa stain, × 100 objective.

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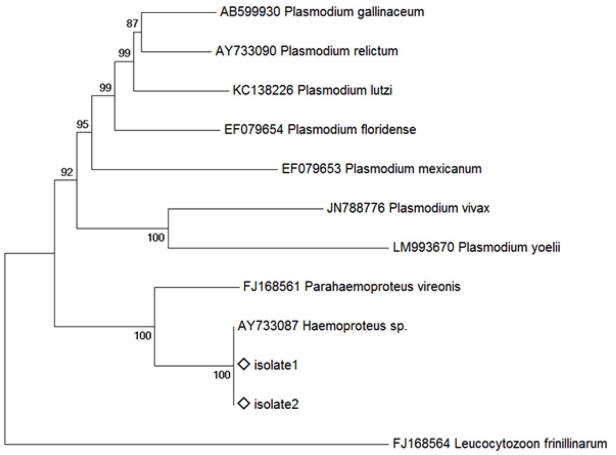

Fig 2. Neighbor-joining phylogenetic analysis based on the mitochondrial large subunit (LSU) ribosomal RNA (rRNA) gene of strains in GenBank databases. Diamonds indicate sequences from the parasite identified in the owls. The first series of letters/numbers represents GenBank database numbers. The LSU rRNA gene sequence of *Leukocytozoon frinillinarum* was used as outgroup. Sequence alignment was performed using CLUSTAL-X v. 1.8 (UCD Conway Institute, Dublin, Ireland), and phylogenetic analysis was performed using MEGA4 v. 4.0.2 (Tempe, AZ, USA).

KCl; 10 mM Tris-HCl (pH 8.3; 25°C); 1.5 mM $MgCl_2$; 200 μM of each dNTP; 100 ng of each primer; and 2.5 units Taq polymerase (iNtRON Biotechnology, Seongnam, South Korea). The PCR was performed in a TaKaRa Thermal Cycler Dice (TaKaRa Bio Inc., Otsu, Shiga, Japan). An initial denaturation at 94°C for 2 min was followed by 35 cycles at 94°C for 1 min, 57°C for 1 min, and 72°C for 70 s. A final run at 72°C for 3 min completed the program. The PCR product was separated by electrophoresis for 40 min at 100 V in a 2% agarose gel and stained with ethidium bromide for visualization under UV light. Following electrophoresis, all positive products were bi-directionally sequenced using an ABI PRISM™ Bigdye™ Terminator Cycle Sequencing Ready Reaction Kit V.3.1 (PE Applied Biosystems, Foster City, CA, USA). When compared to the the GenBank database, the deduced sequences were 99% similar to that of *Haemoproteus* sp. examined in Bridled honeyeaters (*Lichenostomus frenatus*) in Australia (GenBank accession number AY733087) (2). Phylogenetic analysis of the identified LSU rRNA gene sequence also showed that the intraerythrocytic parasites were *Haemoproteus* sp. (Fig 2).

Discussion

The avian hematozoan parasites *Haemoproteus* spp. are transmitted by arthropod vectors and are widespread in domestic and wild bird species across the world (9,12). At a given geographic location, variation in the size of the vector population is positively correlated with variation in the prevalence of the disease in avian communities (3). Altitude, climate, and migratory bird movements also affect the distribution and development of the vectors and parasites (9,13).

South Korea has a large population of resident wild Eurasian eagle owls, which have been sighted even in urban areas due to the presence of numerous hills or small mountains in and around the cities. Usually, the owls invade or attack domestic poultry on farms, but they also contact other domestic animals and even humans frequently. Although the *Haemoproteus* species have been considered as host-specific parasites (1), several studies have also indicated that the host shifts of the hematozoan including *Haemoproteus* and *Plasmodium* species seem to have occurred repeatedly in the parasite-host system (4,5,10). When considering the fact that our cases had no parasite-specific clinical or hematological abnormalities, the host shifts suggest that the asymptomatic owls may act as a reservoir for *Haemoproteus* infection in domestic poultry in South Korea.

Haemoproteus infection can be diagnosed by microscopic examination of a peripheral blood smear (4,6,8). Often, *Haemoproteus* spp. appear similar to *Plasmodium* spp., but can be differentiated by the following features: (i) the gametocytes of *Haemoproteus* are larger than those of *Plasmodium* and encircle the RBC nucleus, giving a "halter-shaped" appearance that occupies over half of the RBC cytoplasm with little displacement of the nucleus; (ii) after invading the RBC, the *Plasmodium* merozoites form trophozoites, schizonts, or gametocytes (infrequently observed), whereas *Haemoproteus* only forms gametocytes; and (iii) the insoluble refractile, yellow to brown pigments (hemozoin) that are derived from the digestion of hemoglobin are more dispersed in *Haemoproteus*. However, it should be noted that microscopic examination is less sensitive than molecular analysis, as it depends on the peripheral parasitemia. In this cases, we also suspected hematozoan parasites in the owls because of the peripheral parasitemia. This finding suggests that many of asymptomatic wild owls may be misdiagnosed by blood smear examination, resulting in the underestimated prevalence of the parasite in the population of wild owls in South Korea. Therefore, a further study will be carried out in the future to examine the prevalence of *Haemoproteus* infection in wild resident and migratory birds by molecular methods with high sensitivity, and to examine the genetic relationship between isolated parasites.

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대한민국 중부지역의 2 마리 야생 수리부엉이에서 헤모프로테우스의 분자검출

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요 약 : 본 증례는 야생 수리부엉이에서 혈액도말과 유전자 분석으로 무증상의 헤모프로테우스 감염을 확인한 것이다. 이것은 대한민국의 야생 수리부엉이에서의 헤모프로테우스 감염을 처음 보고한 것으로 야생 텃새들이 가금의 헤모프로테우스 감염원이 될 수 있음을 제시한다.

주요어 : 헤모프로테우스, 수리부엉이, 텃새, 대한민국