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High prevalence of avian hematozoan parasite infection in wild owls in Chungbuk province of Korea (mid-South Korea)

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Abstract

Wild owls are widespread in Korea, even being common in urban areas due to the presence of hills or small mountains in and around the cities. This results in high levels of contact between owls and domestic animals. This study investigated the prevalence of avian hematozoan parasite infection in 2 common owl species in Chungbuk province of Republic of Korea for 3-year period: the Eurasian eagle owl (*Bubo bubo*) and the Brown hawk owl (*Ninox scutulata*). Peripheral blood smears taken from 56 wild owls were examined by microscopic examination and confirmed by molecular technique. Twenty (36%) of these samples tested positive for the *Haemoproteus* infection. The infection rate was higher in Brown hawk owls (50%) than in Eurasian eagle owls (33%). These results indicate that the wild owls may act as a reservoir for *Haemoproteus* infection in susceptible domestic birds.

Key words : Hematozoan parasite, Prevalence, Owl, Republic of Korea

INTRODUCTION

The avian hematozoan parasites *(Plasmodium* spp. and *Haemoproteus* spp.) are transmitted by arthropod vectors and are widespread in domestic and wild bird species across the world (Valkiunas 2005; Ishtiaq et al, 2007). The prevalence of the infection is highly related to host–vector–parasite interactions (Knowles et al, 2010). 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 (Bennett and Cameron, 1974). Altitude, climate, and migratory bird movements also affect the distribution and development of the vectors and parasites (Rogers and Randolph, 2006; Ishtiaq et al, 2007). 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.

Republic of Korea is located in Northeast Asia and has a temperate climate. Each year, numerous species of birds migrate to overwinter or breed in South Korea (Lee et al, 2000). Ishtiaq et al. (2007) reported that 11% of migratory birds in 2 Korean cities tested positive for *Haemoproteus* infection, suggesting that the infection may spread to resident bird communities that come in contact with the migratory birds. However, to date there have been no investigations into the prevalence of the infection in resident bird communities in Republic of Korea.

In this study, we investigated the prevalence of avian hematozoan parasite infection in 2 common wild owl species inhabiting hill or mountain areas in and around urban areas in Korea: the Eurasian eagle owl (*Bubo bubo*) and the Brown hawk owl (*Ninox scutulata*). The study was conducted over a 3-year period to evaluate the prevalence of the infection in resident owl communities.

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MATERIALS AND METHODS

Archived peripheral blood smears of wild Eurasian eagle owls and Brown hawk owls collected at the Wildlife Center of Chungbuk in Chungbuk Province, South Korea were collected by a clinical pathology record search. A clinical record search was performed on archived peripheral blood smears collected from wild Eurasian eagle owls and Brown hawk owls which were collected from 2009 to 2011 by retrieving the information from the database: date of submission, diagnosis, white blood cell (WBC) count, red blood cell (RBC) count, hematocrit, and hemoglobin concentration. The collected smears were originally prepared to obtain differential counts of WBC for complete blood counts (CBCs) using a hemocytometer and Spectronic® genesys™ 5 (Thermo Fisher Scientific, Waltham, MA, USA) at the first presentation.

In brief, the smears were made in the following manner. One drop of collected blood was placed on a clean glass slide and a thin blood smear was prepared. This was stained with Giemsa stain in phosphate buffer (pH 6.8; ratio 1:10) for 40 min. The stained blood smears were then washed with tap water and air dried. The parasitemia was evaluated under a light microscope at magnifications of 40× and 100× (oil immersion). Parasites were identified using morphological characteristics (Garnham, 1966). A minimum of 20,000 RBCs were examined on each slide to provide an average parasite count per 100 RBCs (Atkinson et al, 1995).

Where parasites were detected and fresh peripheral blood was available, the infection was confirmed by molecular analysis. 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, Inc., Carlsbad, CA, USA). The genomic DNA encoding the mitochondrial large subunit (LSU) ribosomal RNA (rRNA) gene was then amplified using primers 343F and 496R, as described previously (Fallon et al, 2003). PCR amplification was carried out in a total volume of 50 μL. The final reaction conditions were as follows: 50 mM KCl; 10 mM Tris-HCl (pH 8.3; 25℃); 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℃ for 2 min was followed by 35 cycles at 94℃ for 1 min, 57℃ for 1 min, and 72℃ for 70 s. A final run at 72℃ 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). The nucleotide sequences were compared with sequences in the GenBank database using the Basic Local Alignment Search Tool (NCBI website, http://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

In total, 56 owl blood films were collected: 40 Eurasian eagle owl films and 16 Brown hawk owl films. All owls had been found wounded or exhausted in or around cities in Chungbuk Province and had been rescued or captured by a local rescue party.

Microscopic examination revealed that 20 (36%) of the birds tested positive for avian hematozoan parasite

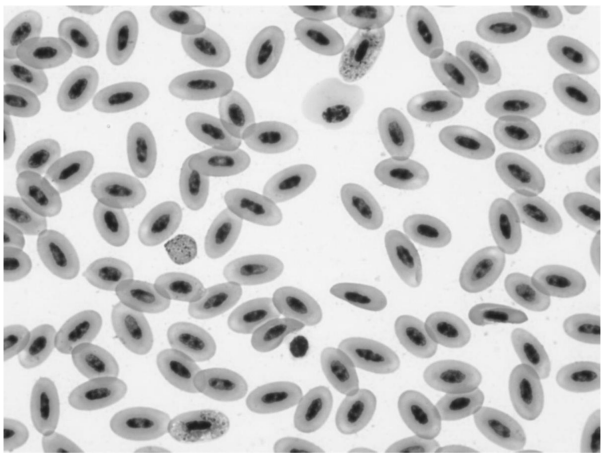

Fig. 1. Microscopic finding showing the intracytoplasmic gametocyte of *Haemoproteus* (arrow) that encircle the RB nucleus, indicating the halter-shaped appearance in an Eurasian eagle owl.

infection (Fig. 1). The infection rate of Brown hawk owls (8 individuals; 50%) was higher than that in Eurasian eagle owls (12 individuals; 30%). Notably, all birds that tested positive were collected between May and September (i.e., late spring to summer season) in both bird species, with the exception of 2 Eurasian eagle owls. Table 1 indicates the information of the birds positive to avian hematozoan parasite infection.

Molecular analysis using fresh blood samples was performed in randomly selected two blood samples of Eurasian eagle owls. Both analyses showed that the identified organisms are *Haemoproteus* spp. Both of the LSU rRNA sequences from the parasites were 99% similar to that of *Haemoproteus* sp. examined in Bridled honeyeaters (*Lichenostomus frenatus*) in Australia (GenBank accession number AY733087; Beadell and Fleisher, 2005).

DISCUSSION

Haemoproteus spp. have a complex developmental cycle in arthropod vectors and are transmitted when the vector bites a host (Friend and Franson, 1999; Eldridge and Edman, 2000). The infected host then facilitates pathogen spread to other susceptible hosts. Republic of Korea has a large population of resident wild 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 fowl on farms, but they also contact other domestic animals and humans. In our study, 38% of the owls examined, all of which had been captured or rescued in or around cities, tested positive for intracytoplasmic gametocytes of avian hematozoan parasites. This suggests that the parasites may be widespread in the wild owl population, and that regardless of their pathogenicity in these birds, owls may act as reservoirs for the infection in susceptible domestic birds. It is supported by the high similarity between the LSU rRNA sequences of the parasites we examined and those of *Haemoproteus* parasites identified in Bridled honeyeaters (Beadell and Fleisher, 2005).

It was found that the prevalence of parasites was higher during the late spring to summer season (90%).

Table 1. Information of *Haemoproteus*-positive owls examined in this study

Sample information		Test results				
Species	No.	No. of parasite (/100 RBCs)	WBC ($\times10^3/\mu L$)	RBC ($\times10^6/\mu L$)	Hct (%)	Hgb (g/dL)
Eurasian eagle owl	6893_23	3-4	3.50	0.38	15	1.4
	7797_01	0-1	1.64	1.61	35	7.1
	8767_04	1-2	1.42	0.85	22	5.2
	8455_71	1-2	1.00	1.40	29	4.8
	8509_07	Not counted	7.78	0.36	10	2.3
	4981_02	Not counted	9.11	1.80	40	9.9
	1779_25	0-1	6.67	1.38	40	9.1
	4981_03	6-7	11.78	1.50	37	7.7
	3765_181	6-7	24.20	2.49	35	7.8
	10484_01	5-6	7.11	1.48	36	9.0
	9809_24	1-2	6.89	1.54	35	6.5
	4981_34	7-8	7.78	1.60	34	13.1
Brown-hawk owl	6849_51	0-1	3.78	2.06	46	10.7
	8455_54	3-4	3.78	2.81	58	13.3
	8074_10	3-4	11.33	1.94	45	9.7
	9820_20	3-4	2.44	2.01	46	10.5
	3765_170	Not counted	6.67	1.87	35	8.0
	9867_07	Not counted	3.11	1.71	34	7.7
	3765_232	6-7	13.56	2.13	39	8.8
	9867_11	1-2	NT	NT	NT	NT

NT, no test because of insufficient sample.

This matches previous findings that the prevalence of avian hematozoan parasites (*Plasmodium* and *Haemoproteus*) increases in spring–summer due to increased numbers of arthropod vectors and suppression of birds' immune systems as a result of high levels of sex hormones in their blood (Bennett and Cameron, 1974; Saino et al, 1995; Wedekind and Folstad, 1994). In addition, 85% of the owls that tested positive for the infection were collected in the last 2 years of the study. Usually, avian hematozoan parasites are more prevalent in the tropics than in temperate regions and at lower altitudes (i.e., at comparatively higher temperatures) within a region (Durrant et al, 2006; Ishtiaq et al, 2007). Therefore, this finding raises the possibility that the prevalence of the parasite may be increasing as a result of an increase in average temperature in South Korea.

Avian hematozoan parasite infection can be diagnosed by microscopic examination of a peripheral blood smear (Friend and Franson, 1999; Bensch et al, 2000; Eldridge and Edman, 2000). Often, *Haemoproteus* spp. and *Plasmodium* spp. are very similar, 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, which suggests that the prevalence of infection found in this study may be an underestimate. Therefore, further studies will be carried out in the future to examine the prevalence of avian hematozoan parasite infection in wild resident and migratory birds, and to examine the genetic relationship between isolated parasites.

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