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NOTE

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Haemoproteus columbae infection in a straggler racing pigeon sheltered in Japan

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ABSTRACT. A racing pigeon (*Columba livia* var. *domestica*), a straggler from Taiwan, was sheltered in Nara Prefecture, Japan in 2020. This pigeon showed hemolysis and elevated levels of hepatobiliary and muscle enzymes. Gametocytes of *Haemoproteus columbae* (Apicomplexa: Haemosporida) were observed within the host erythrocytes in thin blood smears. A partial sequence of the mitochondrial cytochrome *b* gene amplified from blood DNA was identical to the lineage HAECOL1 previously reported from pigeons worldwide. This is the first record of *H. columbae* infection in a sheltered bird in Japan.

KEY WORDS: *Columba livia* var. *domestica*, *Haemoproteus columbae*, haemosporidian, pigeon malaria, racing pigeon

Haemoproteus Kruse, 1890 (Haemosporida: Haemoproteidae) is a genus of hematozoan parasites that infect many avian taxa [16]. There are currently seven described species that infect columbiform birds: *Haemoproteus columbae* Kruse, 1890; *Haemoproteus multipigmentatus* Valkiūnas *et al*., 2010; *Haemoproteus multivolutinus* Valkiūnas *et al*., 2013; *Haemoproteus palumbis* Barker, 1966; *Haemoproteus paramultipigmentatus* Valkiūnas *et al*., 2013; *Haemoproteus sacharovi* Novy and MacNeal, 1950; and *Haemoproteus turtur* Ortega and Berenguer, 1950 [16–18]. All these species are considered to be transmitted by hippoboscid flies [1, 16]. In Japan, although *Haemoproteus* spp. have been reported to infect rock doves (*Columba livia*), Japanese wood pigeon (*Columba janthina janthina*), red-headed wood pigeon (*Columba janthina nitens*), Oriental turtle dove (*Streptopelia orientalis*), and white-bellied green pigeon (*Treron sieboldii*) [7, 15], there have been few reports from the main island of Japan, and certainly no clinical cases of the disease. The present study reports a case of *Haemoproteus* infection in a racing pigeon, *C. livia* var. *domestica,* sheltered in Nara Prefecture, Japan, and reveals morphological and molecular features of the *Haemoproteus* parasite.

On July 16, 2020 (day 0), a rock dove was brought to the animal clinic in Osaka Prefecture, Japan. The bird was captured in Nara Prefecture, Japan, earlier in the year, and the leg ring information had revealed that it was a racing bird migrating from Taiwan in 2020. The bird weighed 403 g, had an appetite, and showed no abnormalities on physical examination. Blood films stained with Diff-Quick revealed hemoprotozoan infection in the erythrocytes. Investigation of the complete blood count and serum chemistry revealed elevated levels of liver and biliary duct enzymes (alkaline phosphatase, aspartate aminotransferase, gamma-glutamyl transferase, lactate dehydrogenase, and total bile acid), muscle enzymes (aspartate aminotransferase, creatine phosphokinase, and lactate dehydrogenase), and hemolysis (Table 1) when compared with standard reference values for domestic pigeons [8–10, 14]. Examination of feces showed a roundworm parasite and light-colored feces with yellow-green urates which was attributable to the presence of liver disorder or hemolysis (Fig. 1). Nucleic acid amplification tests conducted at a commercial laboratory were negative for *Chlamydia* and *Mycobacterium*. The bird was brought to the same hospital again (days 24, 53, 81, 112, and 140) and blood tests revealed continuously elevated liver and muscle enzymes, hemolysis, and hemoprotozoan infection (Table 1). Drugs were administered through daily drinking water for liver and bile duct disorders: clarithromycin (days 0–24, 640 mg/l), amoxicillin hydrate (days 25–140, 800 mg/l), glycyrrhizin (days 0–140, 80 mg/l), ursodeoxycholic acid (days 0–24, 160 mg/l), trepibutone (days 0–140, 48 mg/l), pravastatin in sodium (days 24–53, 8 mg/l), and diisopropylamine dichloroacetate (days 0–112, 80 mg/l).

Parasitological examinations of fecal and blood specimens collected on day 0 were performed at the Nippon Veterinary and Life Science University. The parasite found on the fecal specimen was identified morphologically as pigeon roundworm, *Ascaridia columbae* (Nematoda: Ascaridida), commonly found in pigeons and doves. Examination of the peripheral blood film by light

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Fig. 1. Gross findings of fecal specimens showing a roundworm and yellow-green urates.

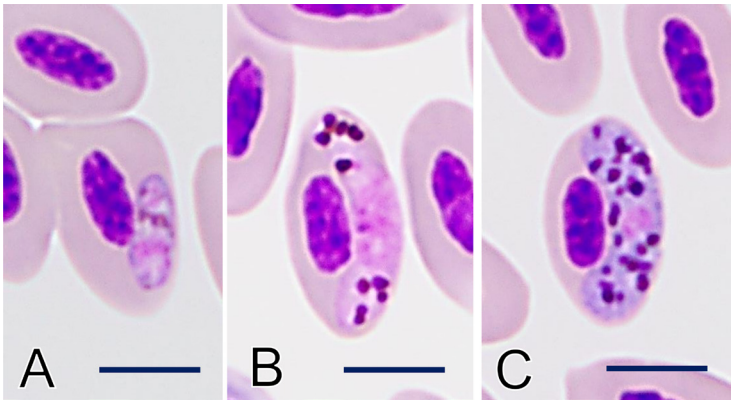

Fig. 2. Intraerythrocyte gametocyte of *Haemoproteus columbae* in the thin blood film. **(A)** Young gametocyte. **(B)** Microgametocyte. **(C)** Macrogametocyte. Giemsa stain. Bar=5 µm.

microscopy revealed the presence of *Haemoproteus* gametocytes in 3.3% of the erythrocytes, including young (0.2%; 1/415) and mature gametocytes (2.9%; 12/415) (Fig. 2A–C). Mature gametocytes were sausage-shaped, halteridial in position, and touched the nuclei and envelope of erythrocytes (Fig. 2B and 2C). Pigmented granules in the macrogametocyte cytoplasm were round, unequal in size, and sometimes aggregated into compressed masses, with approximately 30 randomly dispersed per parasite. The parasite nucleus was small and was located at the median center. The granules in the microgametocytes were round and frequently exceeded 1 µm in diameter, and aggregated in the peripheral cytoplasm. Based on the morphological keys by Valkiūnas *et al*. [16–18], the present parasite was identified as the gametocyte of *H. columbae.* To examine the genetic characteristics of *H. columbae,* a partial fragment of the mitochondrial cytochrome *b* gene (*cytb*) was amplified and sequenced. Genomic DNA was extracted from the blood using the QIAmp DNA Blood Mini Kit (Qiagen, Venlo, Netherlands). The *cytb* was amplified by PCR using the primer set HAEMF (5′- ATGGTGCTTTCGATATATGCATG-3′) and HAEMR2 (5′- GCATTATCTGGATGTGATAATGGT-3′) [2]. Reactions were performed using TaKaRa Ex Taq polymerase (TaKaRa Bio, Kusatsu, Japan). The amplification program consisted of initial denaturation at 95°C for 5 min, followed by 40 cycles at 95°C for 30 sec, 50°C for 30 sec, and 72°C for 1 min, with a final extension step at 72°C for 10 min. The products were electrophoresed on 1.5% agarose gel, purified with ExoSaP-IT (Applied Biosystems, Waltham, MA, USA), and sequenced on a 3730x DNA analyzer (Applied Biosystems), after labeling with BigDye terminator (Applied Biosystems), using PCR primers. The 478 bp-long sequence was deposited in the DNA Data Bank of Japan under Accession no. LC647343. Comparison with the International Nucleotide Sequence Databases using the BLAST program showed 100% identity with the haplotype HAECOL1 (Accession no. KU131583), which is reported in *C. livia* from a wide range

Table 1. Hematologic parameters from a racing pigeon infected with *Haemoproteus columbae*

	Unit	Day 0	Day 24	Day 53	Day 81	Day 112	Day 140	Reference ranges
ALP	U/l	1,862	1,066	2,099	1,717	1,270	2,506	160–780[a]
AST	U/l	661	263	150	162	119	114	45–123[b]
CK	U/l	693	477	494	>2,000	664	290	110–480[b]
GGT	U/l	529	576	113	16	4	5	0–3[b]
LDH	U/l	568	429	178	406	107	64	30–205[b]
BA	µmol/l	109	62	nt	nt	nt	nt	22–60[b]
TCHO	mg/dl	662	536	352	238	226	317	na
TG	mg/dl	130	676	198	183	165	198	na
TP	g/dl	5.4	6.8	6.2	4.8	4.4	4.4	2.1–3.5[b]
PCV	%	52	52	65	63	61	60	40–57[c]
WBC	10^3/µl	11.0	11.6	nt	nt	12.6	nt	2.6–22.3[c]
Het	%	50.9	44.8	nt	nt	61.5	nt	4.5–43.5[c]
Lym	%	49.1	55.2	nt	nt	38.5	nt	52.5–90.5[c]
Gametocytes		Present	Present	Present	Present	Present	Present	
Parasitemia	%	3.1	3.3	nt	nt	nt	nt	
Color of the plasma		Red	Red	Red	Red	Red	Yellow	

ALP, alkaline phosphatase; AST, aspartate aminotransferase; CK, creatine phosphokinase, GGT, gamma-glutamyl transpeptidase; LDH, lactate dehydrogenase; BA, total bile acid; TCHO, total cholesterol; TG, tryglyceride; TP, total protein; PCV, packed cell volume; WBC, white blood cells; Het, heterophils; Lym, lymphocytes; nt, not tested; na, not available. [a] Lumeiji & Bruijine (1985) [8]; [b] Lumeiji (2008) [10]; [c] Scope *et al*. (2002) [14].

Table 2. Survey and documentation of *Haemoproteus* infection in rock dove, *Columba livia,* in Japan

Localities	Origin	Diagnosic method	Infection status (species)	References
Hyogo	Wild	Microscopy	0/278	[11]
Hokkaido	Wild	PCR	0/27	[20]
Okinawa	na	PCR	1/2 (*H. columbae*)	[15]
Kanto region	na	PCR	0/15	[15]
Kanto region	Wild	PCR	0/34	[7]

na: not available.

of tropical and subtropical areas [3, 6, 12, 13].

Haemoproteus columbae has a complex life cycle involving two hosts: merogony in internal organs and gametogony in red blood cells of pigeons, and sporogony in the midguts of the pigeon fly *Pseudolynchia canariensis* [16]. Cepeda *et al*. (2019) showed the full lifecycle of the lineage *H. columbae* HAECOL1 [4]. Merogony is found mainly in the lungs and liver and causes pneumonia and hepatitis, respectively. Gametocytes appear in the peripheral blood 19 to 20 days post infection (d.p.i.), followed by a rapid increase 22 to 25 d.p.i. and a rapid decrease 29 to 30 d.p.i., and then return to the chronic phase a week later. In the chronic phase, parasitemia is low (<10%) and lasts for over several months. In the present case, the infection phase is considered to be chronic because the parasitemia is maintained at a low level. Histological examination of a biopsy is necessary to determine whether the prolonged elevation of liver and muscle enzymes is due to merogony-induced disorder or some other disease such bacterial and viral infections and poisoning [10]. The treatment of *Haemoproteus* infections has not been sufficiently studied. Treatment with buparvaquone, a second-generation hydroxynaphthoquinone antiprotozoal drug related to atvaquone and parvaquone, reduces the number of gametocytes [5]; however, complete cure is difficult and is not approved for use in animals in Japan.

Infection with *H. columbae* is widely reported in tropical and subtropical areas, which coincides with the distribution of pigeon flies [16]. On the other hand, because pigeon flies are not seen in main island of Japan [19], there are no reports on the detection of *H. columbae* there (Table 2) [7, 11, 15, 20]. Matsumura louse fly, *Ornithomya avicularia aobatonis* (Diptera: Hippoboscidae), often parasitizes pigeons in main island of Japan, but the species is considered an unsuitable host for *H. columbae* [1]. Therefore, it is presumed that the present case was sucked by pigeon flies carrying *H. columbae* in Taiwan and then migrate to main island of Japan.

In summary, this is the first molecularly confirmed case of *H. columbae* in the main island of Japan. The present case demonstrated the potential risk of transboundary introduction of *H. columbae* by racing pigeons.

CONFLICT OF INTEREST. The authors have nothing to disclose.

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