The Journal of Veterinary Medical Science

NOTE

Wildlife Science

Detection of avian haemosporidia from captive musophagid birds at a zoological garden in Japan

Masayoshi KAKOGAWA[1,2], Ayana ONO[3], Mizue INUMARU[3], Yukita SATO[3]* and Mitsuhiko ASAKAWA[2]

[1)]Kobe Animal Kingdom, Kobe, Hyogo 650-0047, Japan
[2)]Graduate School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan
[3)]Laboratory of Biomedical Science, Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa 252-0880, Japan

J. Vet. Med. Sci.
81(12): 1892–1895, 2019
doi: 10.1292/jvms.19-0483

Received: 28 August 2019
Accepted: 23 October 2019
Advanced Epub:
4 November 2019

ABSTRACT. One captive musophagid bird at a zoological garden in Japan showed clinical symptoms and was found to be infected with avian haemosporidia. We subsequently collected blood from all musophagid birds kept in the garden and examined for avian haemosporidia using both microscopic and molecular examination. Only *Haemoproteus* gametocytes were observed in the blood of two Guinea turaco (*Tauraco persa*). Three genetic lineages of *Haemoproteus* were identified from three Guinea turacos and one genetic lineage of *Leucocytozoon* was identified from a grey plantain-eater (*Crinifer piscator*). Detected *Haemoproteus* lineages were all identical and completely different from those previously reported in Japan, suggesting that these birds were infected in their original habitat. This is the first record of *Haemoproteus* infection in Guinea turacos.

KEY WORDS: *Haemoproteus*, Japan, *Leucocytozoon*, musophagid bird, zoological garden

Avian haemosporidia, which includes the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*, are common avian blood protozoa that are found in numerous bird species worldwide [14]. Among them, avian *Plasmodium* causes so-called "avian malaria" in host birds; it is a highly pathogenic and lethal infectious disease, especially for several reported naïve bird species [1, 14]. Penguins are typical those naïve species for avian malaria, showing critical symptoms as observed among captive penguins at zoological gardens and aquariums [2, 5, 7, 15]. Moreover, *Haemoproteus* and *Leucocytozoon* infection also seriously affects captive birds [4, 6, 8, 12]. Because many endangered species are kept in zoological gardens and aquariums, it is necessary to monitor the prevalence of those protozoans to promote proper care of these birds and improve their conservation efforts. Some infection cases of those avian haemosporidia were reported among wild and captive birds in Japan [10, 11]. For example, one captive white eared-pheasant (*Crossoptilon crossoptilon*) in a zoological garden of Japan were found to be infected with avian malaria parasite, *P. juxtanucleare* with clinical observation of lethargy and weakness [11]. However, avian haemosporidia prevalence in captive wild birds at zoological gardens and aquariums in Japan has not sufficiently been demonstrated.

At Kobe Animal Kingdom, a zoological garden located in the west part of Japan, *Haemoproteus* infection was found in a captive Guinea turaco (*Tauraco persa*) with clinical symptoms in December 2015. This zoological garden also kept other musophagid birds at that time, but avian haemosporidia prevalence in the zoo was unknown. Therefore, in this study, we examined the blood of the infection-positive bird and other captive musophagid birds to determine their infection status.

First, protozoa were detected in the blood of one individual (No. 1, Table 1) that was kept at Kobe Animal Kingdom in Kobe Prefecture, Japan in December 2015. Then, blood samples of captive musophagid birds were obtained from the wing veins of six individuals, which included one male and three female Guinea turaco (*Tauraco persa*) in May 2016, one female grey plantain-eater (*Crinifer piscator*), and one female violet turaco (*Musophaga violacea*) in June 2016. Those blood collections were implemented as usual health check procedure for kept birds in this zoological garden. Collected blood was used for both microscopic and molecular examination. Blood smears were stained with Diff-Quick solution for morphological observation. DNA was extracted from blood samples and used for nested PCR to amplify haemosporidia mitochondrial DNA, and amplified DNA were sequenced as described previously [9]. Obtained nucleotide sequences were aligned by Clustal W program and compared at 479 bp with sequences in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) and the MalAvi database [3] using the Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov/BLAST/) to construct molecular phylogenetic trees by MEGA6 software.

*Correspondence to: Sato, Y.: sato.yukita@nihon-u.ac.jp

©2019 The Japanese Society of Veterinary Science

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)

Table 1. Detection and identification of avian haemosporidia from captive musophagid birds

Individual birds	DNA detection and identification			Microscopic identification
	Plasmodium	*Haemoproteus*	*Leucocytozoon*	
Guinea turaco (*Tauraco persa*) No. 1	−	+	−	*Haemoproteus*
Guinea turaco (*Tauraco persa*) No. 2	−	+	−	*Haemoproteus*
Guinea turaco (*Tauraco persa*) No. 3	−	+	−	−
Guinea turaco (*Tauraco persa*) No. 4	−	−	−	−
Grey plantain-eater (*Crinifer piscator*)	−	−	+	−
Violet turaco (*Musophaga violacea*)	−	−	−	−

+, positive; −, negative.

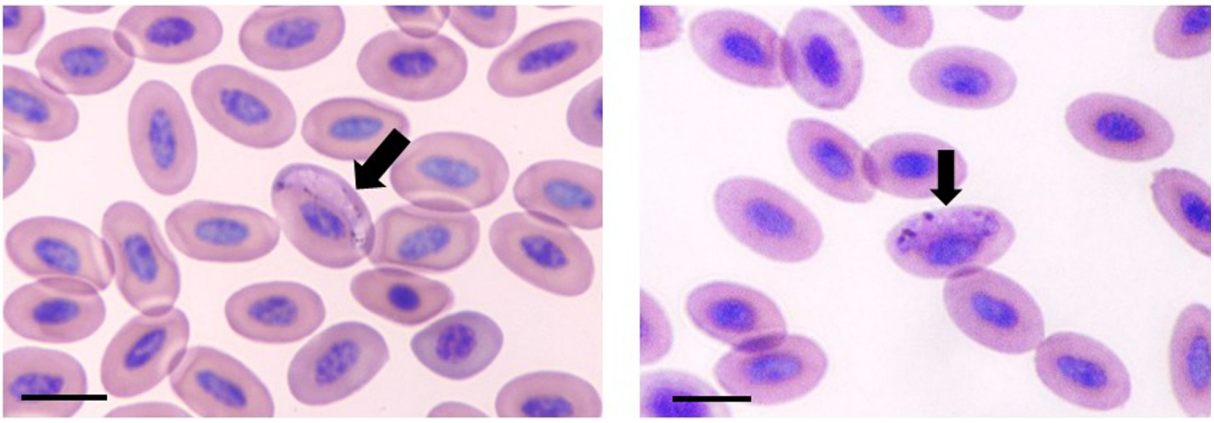

Fig. 1. Gametocytes of *Haemoproteus* sp. found from two captive Guinea turacos (*Tauraco persa*) as indicated by arrows (Left: No.1, Right: No. 2). Bar=10 μm.

Gametocytes that were morphologically similar to *Haemoproteus* were observed from blood smears of two Guinea turacos (Fig. 1) but could not be identified to species (Table 1, Fig. 2). One Guinea turaco that was previously infected with *Haemoproteus* was still positive for *Haemoproteus* (No. 1, Table 1). No *Plasmodium* or *Leucocytozoon* gametocytes were found in any of the blood smears of the studied birds. Four DNA sequences were obtained from four birds, which included three identical *Haemoproteus* lineages from three Guinea turacos and one *Leucocytozoon* lineage from a western plantain-eater (Table 1). Those *Haemoproteus* and *Leucocytozoon* DNA sequences were deposited in GenBank and assigned the accession numbers LC271257 and LC271258, respectively. *Plasmodium* DNA was not amplified from any of the studied samples (Table 1). Two phylogenetic trees were obtained for *Haemoproteus* and *Leucocytozoon* (Figs. 2 and 3, respectively).

In this study, we found that *Haemoproteus* gametocytes from two captive Guinea turacos were identical to the *Haemoproteus* genetic lineages amplified from three Guinea turacos. One *Haemoproteus*-positive Guinea turaco showed clinical symptoms including anorexia or diarrhea, which indicated possible pathogenicity in this bird species caused by infection. These captive musophagid birds in this zoological garden were kept with other bird species such as Ramphastidae in the semi free-ranging area and visitors were allowed to feed to these captive birds directly. These rearing environment might be possible stress factors to those captive birds occasionally, inducing some symptoms for infected individuals. The detected *Haemoproteus* lineages from Guinea turacos were not found in other birds in Japan, and these captive Guinea turacos were introduced to the studied zoological garden in 2006; therefore, we suggest that these protozoa-positive birds were infected in their region of origin. However, some lethal cases were reported when non-adapted bird species were infected with *Haemoproteus* spp. [4, 8]. Consequently, it is also necessary to identify and estimate the prevalence of avian *Haemoproteus* in the native habitats of Guinea turacos.

Haemoproteus gametocyte detection indicated that the protozoans multiplied in at least two Guinea turacos (Nos. 1 and 2), and those birds may be reservoirs of *Haemoproteus* for other captive individuals during the blood-sucking season of vectors, such as biting midges or louse flies [14]. Individual No. 1 was likely infected with *Haemoproteus* from at least December 2015 to May 2016, because it first showed clinical symptoms of *Haemoproteus* infection in December 2015 with observation of gametocytes of *Haemoproteus* in the blood film; therefore, continuous examination of blood with special attention to body condition is needed for this and other individuals to improve treatment strategies for infected individuals and conservation of affected species. Some medical treatments can be applied to those infected birds, i.e., symptomatic treatments at first, and then usage of anti-protozoal drugs such as chloroquine and primaquine after detection of haemosporidia.

Contrary to the observed *Haemoproteus* infection prevalence, we only detected *Leucocytozoon* DNA from the western plantain-eater, but we did not detect any gametocytes. The detected lineage was genetically similar to those from free-ranging raptors

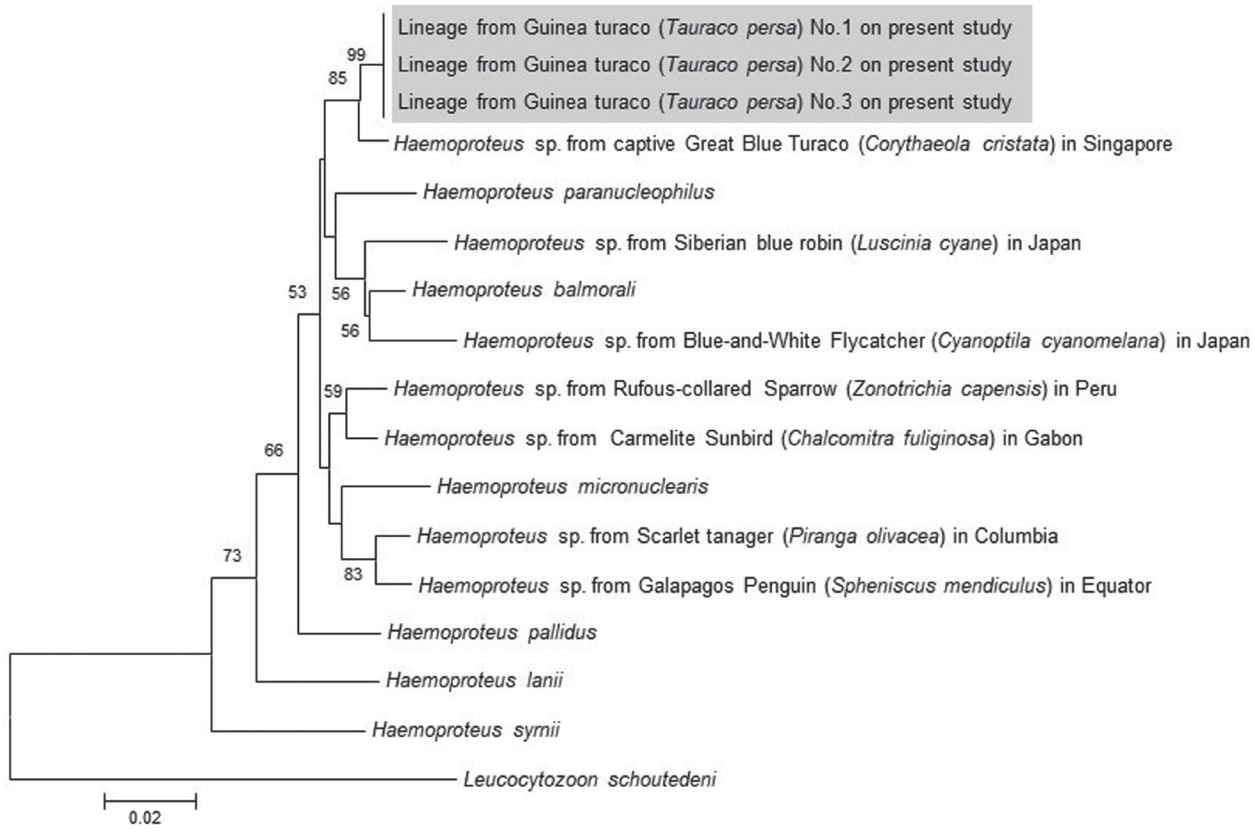

Fig. 2. Phylogenetic status of detected lineages from 3 captive Guinea turacos (*Tauraco persa*). All DNA sequences of detected lineages were identical each other and classified as genus *Haemoproteus*.

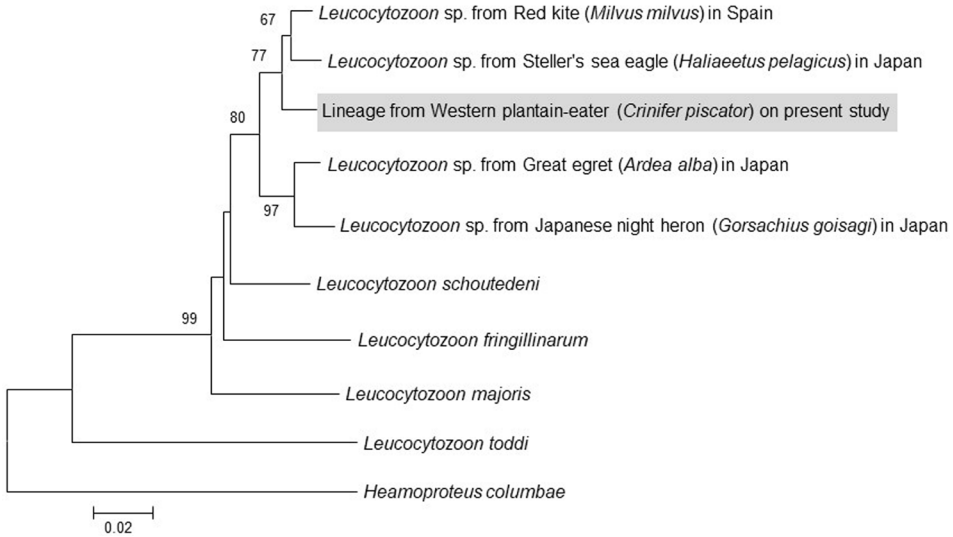

Fig. 3. Phylogenetic status of a detected lineage from captive Western plantain-eater (*Crinifer piscator*). Detected lineage was classified as genus *Leucocytozoon* but no parasites were found in the blood film.

worldwide, including in Japan (Fig. 2). However, the reservoir birds, areas of infection origin, and pathogenicity to host birds are unknown. Because raptors are also kept at Kobe Animal Kingdom and black flies (Simuliidae), which are vectors of *Leucocytozoon*, are distributed in Japan [13], it is necessary to determine if those captive raptors can be reservoirs. Because the prevalence of haemosporidia in arthropod vectors and wild bird hosts both inside and outside of the studied zoological garden have not been examined, future research should demonstrate if there is transmission and consider adequate strategies to prevent protozoan infection.

ACKNOWLEDGMENTS. This study was partially supported by the Japan Society for the Promotion of Science, KAKENHI (26450484), the Strategic Research Base Development Program "International Research on the Management of Zoonosis in Globalization and Training for Young Researchers" from the MEXT of Japan and Nihon University Research Grants.

REFERENCES

1. Atkinson, C. T., Thomas, N. J. and Hunter, B. D. 2009. Parasitic Diseases of Wild Birds, Wiley-Blackwell, Ames.
2. Bak, U. B., Park, J. C. and Lim, Y. J. 1984. An outbreak of malaria in penguins at the Farm-land Zoo. *Korean J. Parasitol.* **22**: 267–272. [Medline] [CrossRef]
3. Bensch, S., Hellgren, O. and Pérez-Tris, J. 2009. MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. *Mol. Ecol. Resour.* **9**: 1353–1358. [Medline] [CrossRef]
4. Cannell, B. L., Krasnec, K. V., Campbell, K., Jones, H. I., Miller, R. D. and Stephens, N. 2013. The pathology and pathogenicity of a novel *Haemoproteus* spp. infection in wild little Penguins (*Eudyptula minor*). *Vet. Parasitol.* **197**: 74–84. [Medline] [CrossRef]
5. Cranfield, M. R., Graczyk, T. K., Beall, F. B., Ialeggio, D. M., Shaw, M. L. and Skjoldager, M. L. 1994. Subclinical avian malaria infections in African black-footed penguins (*Spheniscus demersus*) and induction of parasite recrudescence. *J. Wildl. Dis.* **30**: 372–376. [Medline] [CrossRef]
6. Ferrell, S. T., Snowden, K., Marlar, A. B., Garner, M. and Lung, N. P. 2007. Fatal hemoprotozoal infections in multiple avian species in a zoological park. *J. Zoo Wildl. Med.* **38**: 309–316. [Medline] [CrossRef]
7. Grilo, M. L., Vanstreels, R. E. T., Wallace, R., García-Párraga, D., Braga, É. M., Chitty, J., Catão-Dias, J. L. and Madeira de Carvalho, L. M. 2016. Malaria in penguins - current perceptions. *Avian Pathol.* **45**: 393–407. [Medline] [CrossRef]
8. Halpern, N. and Bennett, G. F. 1983. *Haemoproteus* and *Leucocytozoon* infections in birds of the Oklahoma City Zoo. *J. Wildl. Dis.* **19**: 330–332. [Medline] [CrossRef]
9. Hellgren, O., Waldenström, J. and Bensch, S. 2004. A new PCR assay for simultaneous studies of *Leucocytozoon, Plasmodium*, and *Haemoproteus* from avian blood. *J. Parasitol.* **90**: 797–802. [Medline] [CrossRef]
10. Murata, K. 2002. Prevalence of blood parasites in Japanese wild birds. *J. Vet. Med. Sci.* **64**: 785–790. [Medline] [CrossRef]
11. Murata, K., Nii, R., Sasaki, E., Ishikawa, S., Sato, Y., Sawabe, K., Tsuda, Y., Matsumoto, R., Suda, A. and Ueda, M. 2008. *Plasmodium* (*Bennettinia*) *juxtanucleare* infection in a captive white eared-pheasant (*Crossoptilon crossoptilon*) at a Japanese zoo. *J. Vet. Med. Sci.* **70**: 203–205. [Medline] [CrossRef]
12. Ortiz-Catedral, L., Brunton, D., Stidworthy, M. F., Elsheikha, H. M., Pennycott, T., Schulze, C., Braun, M., Wink, M., Gerlach, H., Pendl, H., Gruber, A. D., Ewen, J., Pérez-Tris, J., Valkiūnas, G. and Olias, P. 2019. *Haemoproteus minutus* is highly virulent for Australasian and South American parrots. *Parasit. Vectors* **12**: 40. [Medline] [CrossRef]
13. Sato, Y., Tamada, A., Mochizuki, Y., Nakamura, S., Okano, E., Yoshida, C., Ejiri, H., Omori, S., Yukawa, M. and Murata, K. 2009. Molecular detection of *Leucocytozoon lovati* from probable vectors, black flies (Simuliudae) collected in the alpine regions of Japan. *Parasitol. Res.* **104**: 251–255. [Medline] [CrossRef]
14. Valkiūnas, G. 2005. Avian Malaria Parasites and Other Haemosporidia, CRC Press, Boca Raton.
15. Vanstreels, R. E. T., Braga, É. M. and Catão-Dias, J. L. 2016. Blood parasites of penguins: a critical review. *Parasitol.* **143**: 931–956. [Medline] [CrossRef]