Plasmodium (*Bennettinia*) *juxtanucleare* INFECTION IN A CAPTIVE WHITE EARED-PHEASANT (*Crossoptilon crossoptilon*) AT A JAPANESE ZOO

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Introduction

The white eared-pheasant is a Galliformes bird native to the Qinghai, Sichuan, Yunnan and Tibet regions of mainland China, and it is listed as a Near Threatened (NT) species in the 2006 IUCN Red List Category. A captive propagation plan has been designed for this species at zoos worldwide, and captive breeding has been succsssful in some zoos. Currently, there are 47 captive birds worldwide, and 18 birds are present in 3 Japanese zoos. *Plasmodium* (*Bennettinia*) *juxtanucleare* is mainly found in Phasianidae bird species, including domestic fowl, in the widespread zoogeographical regions of the neotropics, Ethiopia and the Orient. This parasite was also found to infect a variety of wild and captive birds mainly belonging to the Phasianidae. A case of infection in a captive black-footed penguin (*Spheniscus demersus*) was also reported. The effect of the parasitic infection on captive endangered species poses not only individual health risks but also propagation problems. Here, we present a case of *P.* juxtanucleare infection in a captive white eared-pheasant in order to contribute to the associated veterinary management and the ex *situ* conservation of this rare species.

Materials and methods

The affected bird was an 11-year-old male white eared-pheasant at Yokohama Zoological Garden, The bird was hatched at Yokohama Municipal Nogeyama Zoo in June 1995 and was transported to the present facility for a captive propagation program in March 1999. In March 2006, the bird exhibited lethargy and weakness. Since a haemosporidian parasite was detected by blood examination, medical treatment with an anti-malarial drug was started. During the duration of therapy, blood samples were collected from the brachial wing vein. Stained blood films were scanned for the presence of the blood parasite with an optical microscope. The WinROOF™ was used to take measurements from the digital photo micrographs of the parasite. The ratio of infected blood cells was calculated after observing 5,000 blood cells. DNA was extracted from whole blood samples at diagnosis and from a frozen blood sample collected at arrival by PCI method. The nested PCR reaction targeted a partial region of the cytochrome *b* genes of *Plasmodium* was performed using primer sets designed by Hellgren et *al.* (2004). The nested PCR products were directly sequenced (Murata et *al.* 2008).

Results

Examination of the stained blood films revealed that the avian malarial parasite of the genus Plasmodium was present in the cytoplasm of the red blood cells. During the clinical period, the extent of parasitemia was 4.4 ± 4.51% (range: 0.41–14.7%, *n*=14). No other haernosporidian parasites was detected. Small trophozoites of the parasite were observed adjacent to the erythrocyte nuclei. The gametocytes were round or oval and small. Most of their size did not exceed the host cell nucleus, but some gametocytes were larger than the nucleus of its host erythrocyte in size and slightly displaced it to the periphery. From these morphological characteristics and morphometrics of the parasite, it was identified as P. *juxtanucleare*(Fig. 1). Plasmodium genes were detected in both the samples collected at the time of diagnosis and arrival. Alignment of *the* partial cyt b gene of Plasmodium from the white eared-pheasant resulted in 478 bp in length. The NJ analysis placed the parasite from this individual in the same cluster as *P. juxtanucleare* isolated from fowls in Japan and Brazil, and the percent identity of the parasite

from this study with the 478bp Japanese strain and the 474bp Brazilian strain was 100% and 99%, respectively (Fig. 2).

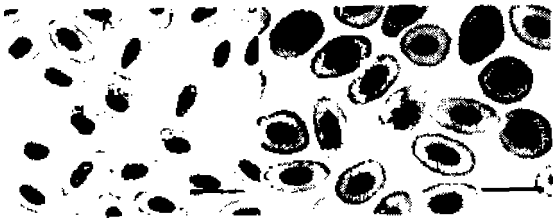
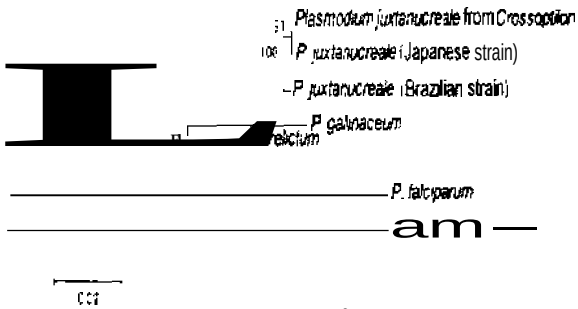


Fig. 1. Several stages, trophozoite and gametocytes, *of Plasmodium juxtanucleare* in the blood of *Crossoptilon crossoptilon*. Bar = 10µm



Frg. 2. The **sequences obtained** from this study **and** those of other *Plasmodium* species in GenBank and DDBJ (Accession nos. DQ017964, AB250415, AY099029, AY733090, AJ276844) were aligned using the ClustalW, and phylogenetic analysis was performed using MEGA version 3.1 Distance analyses were performed using the Kimura 2-parameter model with the neighbor-joining (NJ) method. *Leucocytozoon dubreuli* sequence (GenBank Accession no. AY099063) was **used as** an outgroup to root the tree Bootstrap analyses used 10,000 replicates. The DDBJ accession number for the partial region of cytochrome *b* is AB302893.

Discussion

P. juxtanucleare was first described in fowls (*Gallus gallus* var. *domesticus*) in Brazil. Although the parasite was found in 6 species from 5 avian orders of Phasianidae after the first report, this is the first documentation of *P. juxtanucleare* infection found in a bird of the genus *Crossoptilon*. In the present study, molecular analysis revealed that the partial mitochondria1 cyt b gene sequences of the parasite detected in the white eared-pheasant were identical to those of *P. juxtanucleare* isolated from fowls in Japan. Although few informative data are available from the DNA database in order to compare among strains of the parasite, our molecular analysis data demonstrated that the parasite belongs not to the Brazilian strain but to the Asian strain. More molecular data is required on the haernosporidian parasite and its natural reservoir since it would provide useful information on the evolution, distribution and origin of *P. juxtanucleare*. The clinical symptoms associated with *P. juxtanucleare* infection were not frequently observed in cases where in the infection occurred naturally. However, severe cases were reported in immunecompromised birds and/or birds under stress. Pathogenicity varies among the *P. juxtanucleare* strains, and high mortality was reported in young experimentally infected chicks. In our case, the diseased bird showed intermediate grade parasitemia (4.4 ± 4.51 %) and clinical symptoms, including lethargy. Although the virulence of *P.* juxtanucleare in the white eared-pheasant has not been cleared yet, the attention is needed in zoos where keep the endangered Phasianidae and non-Phasianidae birds susceptible to avian malaria from the standpoints of conservation and veterinary medicine. Surveillance for *P. juxtanucleare* infection at zoos together with vector research is required in order to understand the infection route of the parasite and to take preventive measures.

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