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An Outbreak of Malaria in Penguins at the Farm-land Zoo

Ung-Bok Bak, Jae-Chan Park
College of Veterinary Medicine, Seoul National University, Suwon, Korea

and Young-Jae Lim

Farm-land Zoo, Yongin-gun, Geonggi-do, Korea

INTRODUCTION

Since malaria in penguins was first reported in 1926 in a King penguin at the London Zoo, avian malaria had been accepted to be the most important parasitic agent causing severe epizootics at the Zoos in Europe and United States (Griner and Sheridan, 1967; Fowler, 1978). Plasmodium(P.) relictum was identified in an epizootic at the Schoenbrunn Zoo of Viena (Griner and Sheridan, 1967). British and European Zoos have experienced seasonal epizootic of malaria caused by P. relictum (Griner and Sheridan, 1967). Huff and Shiroshi (1962) found that the Humboldt penguins at the National Park Zoo in Washington were infected with P. elongatum and P. relictum. Herman et al. (1968) and Fleischman et al. (1968) reported epizootics of malaria caused by P. elongatum in the African penguins at the Baltimore Zoo. Regarding on avian malaria in Korea, presence of Plasmodium sp. has been described in the blood films of the sparrows presumably as P. cathemerium (Honda, 1939).

Authors investigated an outbreak of highly infective disease in the penguins at the Farmland Zoo in Yongin district of central Korea, confirming involvement of *Plasmodium sp.* with the blood and visceral organs.

MATERIALS AND METHODS

Animals inspected:

Four Humboldt penguins (Spheniscus humboldti)

aged 3 years that had been imported from Japan were inspected.

Pathological examination:

The visceral organs except brain and bone of 3 cases which were necropsied at the Zoo were subjected to histopathological examination at the College of Veterinary Medicine in Suwon. The lesions were fixed with 10% buffered formalin and prepared into paraffin sections and stained with hematoxylin and eosin for microscopic examination. The thin blood smears were made from two cases and treated with Wright stain.

RESULTS

Clinical history:

Four Humboldt penguins were imported from Japan in June 30, 1983 to the Farm-land Zoo in Yongin district. All of them exhibited illness suddenly including anorexia, depression, weakness and lethergy on a week after keeping them in the cage. Three cases of the affected birds died one after one from 4 to 6 days after onset of illness.

Gross findings of the lesions:

Gross lesions observed at necropsy were consistently similar in the 3 cases. The dominant gross changes were noted at the liver, spleen, heart and lung. The liver appeared to be swollen with round edge of the lobes and also slightly lighter in color and friable. The spleen was enlarged with the tense capsule and showed soft and friable consistency. The cut surface was

convex and granular. Increased amount of the pericardial fluid, clear and yellowish in color, were observed in each case. The lung showed swollen, reddened appearance and doughy consistency. The collection of clear yellowish fluid around the lung was noticed. The air sacs were filled with clear fluid.

Microscopic findings:

Peripheral blood picture: The affected erythrocytes showed poikilocytosis and marked polychromatophilia. Numerous erythrocytes appeared to have basophilic cytoplasm (Fig.1). The plasmodial parasites showing different developmental stages were confirmed in the numerous mature erythrocytes (Fig. 1, 2, 3). The parasitic bodies were measured from 1.5 µm up to 7.8 µm in diameter and bluish in Wright stain. An occasional erythrocyte was found to contain vellow pigment in the round parasitic bodies which were considered as trophozoites (Fig. 1). The larger parasitic bodies which displaced host cell nuclei to one side had many dark granular bodies (Fig. 2). These large parasitic bodies were considered to be the schizonts.

The liver: The lesions in the liver were characterized by multiple small foci of reticuloendothelial cell hyperplasia. The foci of the hyperplasia appeared to have zonal distribution within the hepatic lobule or in the periportal area (Fig. 7). The hyperplastic foci were consisted of reticular cells, macrophages, and lymphoid cells of varying size(Fig. 8). Numerous these cells were vacuolated with parasitization or phagocytosis of pigment(Fig. 8). The schizonts showing varying size were found in the numerous phagocytic cells, predominantly macrophages or Kupffer cells (Fig. 6, 8). The mature schizont was measured up to 14.6 µm in diameter and contained 30 or more merozoites (Fig. 6). Each of schizont contained between 8 and 30 merozoites. The Kupffer cells were found to be pigmented. Parasitized hepatic cells were also observed. Periportal bile duct epithelium appeared to contain brown pigment and the bile canaliculi were frequently distended with bile pigment (Fig. 8).

The spleen: Extensive diffuse proliferation of the reticuloendothelial cells was the distinct feature throughout the splenic pulp(Fig. 4, 9). The white pulp was not discernible clearly from the red pulp and contained few lymphocytes. Numerous sinusoidal endothelial cells and macrophages were vacuolated by parasitization or pigmentation. Mature excerythrocytic schizonts containing merozoites could be found frequently in these cells(Fig. 5).

The lung: The parabronchial area of the affected lung showed distinct capillary congestion and diffuse reticular cell proliferation (Fig. 10). Vacuolated reticular cells and macrophages parasitized with the schizonts were found in the parabronchi.

DISCUSSION

Penguins are highly susceptible to parasitism with plasmodia (Fowler, 1978). Several zoos in Europe and United States have experienced severe losses due to infections by *P. elongatum* or *P. relictum* among penguin flocks.

The authors observed the outbreak of plasmodial infection in the penguin flock imported from Japan in summer. The disease was highly infective among the birds and characterized clinically by acute course and high mortality. The gross lesions noted were pulmonary and epicardial edema and hepato-splenomegaly. The histologic lesions were characterized by reticuloendothelial cell hyperplasia in the affected organs. It appeared that the hepato-splenomegaly observed grossly was due to extensive reticulo-endothelial hyperplasia. The feature of microscopic lesions in the affected penguins were essentially similar to those reported by previous investigators (Griner and Sheridan, 1967; Fleischman et al., 1968).

In the parasitized erythrocytes of the peripheral blood the host cell nuclei appeared to be displaced by mature schizonts. The mature exoerythrocytic schizonts found in the liver and spleen contained 30 or even more merozoites. Upon the morphological feature of the organisms

it was presumed that the plasmodial parasite observed in the disease to be resemble morphologically to *P. relictum*.

The imported penguins had been kept in the glass cage protected from mosquito exposure in Japan. Hence the possibility exists that the penguins might have been transmitted with the disease by mosquitoes from infected wild birds. The authors suggest that the wild birds such as pigeons or sparrows were responsible for transmission of the parasites to the imported penguins. Pigeons and canary are susceptible to infection by P. relictum (Huff, 1963), Herman et al. (1968) commented that P. relictum, P. elongatum and other Plasmodium spp. were relatively nonpathogenic parasites of free living North American birds but penguins had little innate resistance and thus were susceptible to severe epizootics. The authors press that epidemiological survey on infected wild birds with plasmodial species should be performed in this area.

SUMMARY

In June of 1983 authors had investigated the occurrence of the avian malaria in the Humboldt penguins imported from Japan to the Farmland Zoo in central district of Korea. The disease was characterized by acute course and high mortality. The peripheral blood smears from the affected penguins demonstrated different developmental stages of *Plasmodium sp.* in the mature erythrocytes. The predominant gross lesions

noticed were pulmonary and epicardial edema and hepato-splenomegaly. Microscopically the lesions were characterized by extensive reticulo-endothelial cell hyperplasia with striking feature of exoerythrocytic schizogony affecting a variety of tissues. The report also signifies the first description of a disastrous epizootic of avian malaria in Korea.

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=國文抄錄=

自然農園 動物園에서의 펜귄의 말라리아 발생보고

서울大學校 **歌醫**科大學 朴 **應 鍑・朴 宰** 賛

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1983년 6월에 日本으로부터 自然農園 動物園에 수입된 3세령의 Humboldt 펜귄 4수가 입사한지 일주만에 갑자기 식욕절폐, 원기소침하여 4~6일만에 3수가 폐사하였다. 患鳥의 혈액도말표본에서 성숙적혈구내에 발육단계가다른 Plasmodium sp.의 기생체가 확인되었다. 폐사한 동물은 肺臟과 心臟에서 심한 水腫性 變化가 인정되었고 肝臟과 脾臟이 腫脹해 있었다. 각 臟器의 조직학적 검사에서 肝臟, 脾臟 및 肺臟등에서 광범위하게 현저한 細網內皮系細胞의 增殖과 活性化가 관찰되었고 이들 세포에서 여러 발육단계의 schizont가 확인되었다. 이상의 관찰보고는 국내에서 鳥類의 말라리아 유행을 처음으로 기록한 것이다.

EXPLANATIONS FOR FIGURES

- Fig. 1. 2 and 3. The blood films from the sick penguins demonstrate different developmental stages of *Plasmo-dium sp.*, presumably trophozoites (short arrows) and schizonts (long arrows) which displace host cell nuclei to one side in the mature erythrocytes. The affected cells show poikilocytosis and polychromatophilia. Wright stain, ×1,000.
- Fig. 4. The splenic pulp tissue showing extensive hyperplasia of the reticulo-endothelial cells and vacuolated macrophages with parasitization (arrows). Hematoxylin and eosin (HE) stain, ×400.
- Fig. 5. A macrophage containing an exoerythrocytic schizont (arrow) in the splenic sinusoid. HE stain, ×1,000.
- Fig. 6. A mature exoerythrocytic schizont with 30 or more merozoites (arrow) in a Kupffer cell of the liver. HE stain, ×1,000.
- Fig. 7. The hyperplastic foci of reticulo-endothelial cells (r) in the liver tissue. HE stain, ×100.
- Fig. 8. Higher magnification of Fig. 7. A focus presents the lymphoid cell accumulation and the parasitized cells (arrows). HE stain, ×400.
- Fig. 9. The splenic pulp showing distinct reticulo-endothelial hyperplasia and parasitized or pigmented macrophages (arrows). HE stain, ×400.
- Fig.10. The parabronchus area showing diffuse reticulo-endothelial hyperplasia and capillary congestion. HE stain. × 200.



