

Studies on Infectious Disease, Pathology,  
Clinical Workup and Basic Biology  
of Zoo Animals

(動物園動物の感染症、病理学、臨床処置および基礎生物学に関する研究)

A Summary of Ph. D. Thesis

Miya Ueda, D. V. M.

Graduate School of Veterinary Medicine and Life Science  
Nippon Veterinary and Life Science University

(Doctoral Supervisor : Shinpei Wada, Professor)

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Yokohama Zoological Gardens (Zoorasia; a nickname for the Gardens) opened in 1999. Yokohama City has entrusted Yokohama Greenery Foundation with the management of Zoorasia from the opening period. The total area of Zoorasia is 53.3ha which is divided into eight special areas. In total 750 animals of 100 species including 87 rare species on the IUCN red list are kept in the area at present.

The animal hospital is not open to the public and has a total area of approximately 2,000m<sup>2</sup>, with a dissection room, a medicine room, an examination room, an operating room, a clinical workup room, and a hospitalization facility. Four veterinarians are employed by the hospital. They perform clinical practice, clinical examination, dissection, etc., and some special examinations including histopathology are outsourced to professional institutions.

The author has been working at Zoorasia since the opening preparation period in 1997. This paper summarizes the studies on the diseases and their pathology that the author had experienced in Zoorasia keeping animals so far.

In chapter two, the author described nontuberculous mycobacteriosis in northern carmine bee-eaters. Avian non-tuberculous mycobacteriosis is a chronic infection in birds, and clinical symptoms are nonspecific. Four northern carmine bee-eaters (*Merops nubicus*) kept in the same indoor exhibition died in a row. Three dead birds were diagnosed as systemic mycobacteriosis through histopathological examination. In addition, acid-fast long-rods were detected from the contents of alimentary tracts of other dead individuals by direct microscopy. From these features, it was suspected that all of the dead birds might possess the similar pathological changes caused by acid-fast microorganisms. From sequencing analysis of 16S rRNA gene and *hsp65* gene using DNA samples extracted from the livers and spleens of two dead birds, it was revealed that the obtained base sequences of these house-keeping genes showed 100% homologies with *Mycobacterium genavense* which is a kind of non-tuberculous mycobacteria. As a result, it was concluded that the present serial mortalities were caused by endemic infection of *M. genavense*, non-tuberculous mycobacteria.

The indoor exhibition where the four dead birds were kept was occupied more than half by fake-rocks. These fake-rocks could not be washed with water, because they were not waterproof. While the primary route of exposure to the first victim, and the source of causative agent are still unclear, it was suspected that the first victim might shed feces containing considerable amount of *M. genavense* on the fake-rocks, and the soil, food and drinking water might be secondary contaminated by the feces. It was also suspected that some amount of the feces on fake-rocks might aerosolize. Other three victims, whose immune status decreased due to some causes, could be infected by the organism through ingesting the contaminated food and/or water, inhaling the contaminated dusty soil and aerosolized feces. No mortality with similar features has been recorded after exhibition refurbishment.

In chapter three, the author described the cases of neoplasms observed in the rearing animals of Zoorasia, from the opening period to 2017. Neoplastic conditions in zoo mammals have been recorded in total 45 animals of 24 species from 1999, the opening of Zoorasia, to 2017. In the most cases, neoplasms were detected at necropsy performances, however, 23 cases were diagnosed using biopsies. The biopsy of zoo animals has performed by using hand holding or under anesthesia. Collected tissue samples were fixed and stored in 10% phosphate-buffered formalin solution, and fixed samples were processed routinely to prepare the paraffin sections for histology. The sections were stained with hematoxylin and eosin stain. Some selected sections were also stained with tumor markers, such as cytokeratin, chromogranin A, synaptophysin, calcitonin, lysozyme etc., by using indirect technique for immunohistochemistry. These sections were observed under the light microscopy.

However, the total rearing number of animals of Carnivora was the largest in Zoorasia, the neoplastic conditions were also the most frequently recorded in Carnivora (9.2%). This result has supported the previous reports from other zoos. Especially in Carnivora, the highest occurring number of neoplastic conditions, nine cases were recorded in dhole (*Cuon alpinus*). Eight out of nine cases of dhole had blood relationship, therefore, the high incidence

of neoplastic conditions in these animals might be a natural consequence of close breeding as genetic background.

In the present survey, squamous cell carcinoma of oral cavity was more common in nonhuman-primates (three cases) and Diprotodonia (two cases). As for nonhuman-primates, the same tendency was reported in the previous record. Although there were many reports of squamous cell carcinoma of oral cavities in red kangaroos (*Macropus rufus*), there was no report of the tumor in goodfellow's tree kangaroo (*Dendrolagus goodfellowi*), just like the present case. This feature might indicate that squamous cell carcinoma is likely to develop also in Diprotodonia as in nonhuman-primates.

In chapter four, the author described immune-mediated disorder observed in a spectacled bear. A 5-year-old female spectacled bear (=Andean bear, *Tremarctos ornatus*) had pruritic eczema all over the body surface. Clinical signs were eczema with severe pruritus, epiphora, blepharodema and sneeze. Hematological test showed increase WBC and eosinophils in number. Biochemical test showed significantly high value of LDH. Antihistamines had no effect for the symptoms. The bear was treated with prednisolone, and the symptoms were improved. After the bear moved from Yokohama to Hokkaido, these symptoms were disappeared. This skin disease was considered atopic dermatitis because of the periodicity of symptoms according to seasons, severe pruritus, eosinophilia, effect of corticosteroid and disappearance of clinical signs due to the change of living place. Recent publications regarding a specific dermatological condition in female spectacled bears have been proposing the name of novel immune-mediated skin disorder, Andean bear alopecia syndrome (ABAS). From the clinical features and blood biochemistry, it was suggested that the present case might be diagnosed as ABAS.

In chapter five, the author described cellular susceptibility of proboscis monkey's lymphocytes to Epstein-Barr virus (EBV). EBV is one of the human herpes viruses (human herpesvirus 4). Although it is well known that the natural host of EBV is human, other nonhuman-primates could be infected with EBV. It has been known that EBV infects B-lymphocytes of the apes and New World monkeys, and let them immortalize. On the other

hand, susceptibility of B-lymphocytes from genus *Colobus* and genus *Trachypithecus* of Colobinae in old world monkeys to EBV was confirmed in the 1980's. Therefore, it has been interested in the relationship between susceptibility to EBV and phylogenetic classification of nonhuman primates. However, there was no report on susceptibility of genus *Rhinopithecus* and genus *Nasalis* of Colobinae to EBV.

In this chapter, susceptibility of proboscis monkeys (*Nasalis larvatus*) to EBV has been determined. Lymphocytes collected from peripheral blood of four proboscis monkeys (two males and two females) reared at Zoorasia was infected with EBV in vitro. Blastogenic transformation of the lymphocytes occurred by sensitization with EBV, and proliferation of the lymphocytes became stable at one month after the sensitization. From expression of EBV related protein and presence of virus in the proliferating cells, it was confirmed that proboscis monkey's lymphocytes were susceptible to EBV. This results strongly suggest that the receptor motif of CD21, which is also a receptor of EBV, is universally conserved in Colobinae.