Disseminated Protothecosis in a Cape Hyrax (*Procavia capensis*)

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ケープハイラックスにおける播種性プロトテカ症の1例

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ABSTRACT. Disseminated protothecosis in a Cape hyrax (*Procavia capensis*) was studied. Severe granulomatous inflammation with myriads of PAS-positive prothothecal organisms, 5 by 5 to 20 by 30 μ m, were seen in the thickened mucosa in the gastrointestinal tract. The kidneys had multifocal granulomas with organisms. Electron microscopy revealed numerous algae-like organisms with obvious cell wall but without chloroplasts in the cytoplasm of macrophages. This case may be the first case of protothecosis in Cape hyraxes.

Key Words: Protothecosis, Dissemination, Cape hyrax, *Procavia capensis*

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Protothecosis is a rare infectious disease caused by species of achlorophyllous genus *Prototheca*. Members of Genus *Prototheca* have ovoid nucleus and the cell wall, and morphologically closely similar to those of the genus *Chlorella* [1,2]. Protothecosis has been reported in a wide variety of animals including mammals and a fish. Of four species of *Prototheca* (*P.*) known, two species, *P. wickerhamii* and *P. zopfii* were reported to have pathogenicity to humans and animals [3]. *Prototheca* spp. cause granulomatous inflammation with various numbers of lymphocytes and plasma cells in various

organs such as skin, eyes, mammary gland and intestine [2]. In domestic animals, protothecosis was first reported in mastitis in cattle [4]. Then, sporadic protothecosis were observed in dogs [5-7] and a cat [8]. In wildlife, protothecosis were observed in deer [9], beaver [10], fruit bat [11] and salmon [12].

This report is concerned with histopathologic features of a necropsy case of Cape hyrax died of disseminated protothecosis in a zoo.

An adult male Cape hyrax (*Procavia capensis*) was found dead without any apparent clinical signs in a zoo. The animal was

bred and reared at the zoo. They were fed commercial diets for rodents and vegetables, and tap water *ad libitum*.

A complete necropsy was done immediately after discovery of death. The lungs, liver, spleen, lymph nodes, kidneys, urinary bladder, adrenals, thyroid, salivary glands, stomach, small intestine, large intestine, pancreas, skeletal muscle and skin were collected and fixed in 10% buffered formalin. After fixation, the tissue blocks were dehydrated and embedded in paraffin wax in the usual manner, sectioned ($5\ \mu\text{m}$) and stained with haematoxylin and eosin (HE). Selected sections of liver and intestine were stained with periodic acid-Schiff (PAS). For electron microscopy, small pieces of formalin-fixed tissues from small intestine were post fixed in 2% phosphate buffered glutaraldehyde and 1% osmium tetroxide and routinely processed for epon embedding. Ultra-thin sections were stained with uranyl acetate and lead citrate, and examined with a transmission electron microscopy (Hitachi; H-800, Japan).

Grossly, the animal was moderately emaciated with minimal subcutaneous fat storages. The body weight at death was

2.3 kg. There were numerous 1 to 5 mm white foci scattered on the surface and in the parenchyma of the both kidneys. The duodenum showed moderately thickened mucosa with multifocal hemorrhagic foci. The cecum had multifocal hemorrhagic foci on the surface of mucosa with slightly bloody stool. The mesenteric lymph nodes showed a marked lymphadenopathy.

Histopathologically, various degrees of granulomatous inflammation as well as myriads of unicellular algae-like organisms were observed in the gastrointestinal tract (Figs. 1 and 2), kidneys, lungs and mesenteric lymph nodes. The organisms were round to oval, and varied in size from 5 by 5 to 20 by 30 μm , which were identical to *Prototheca* spp. (Fig. 2). They possessed one to several round nuclei with a plenty to scarce clear cytoplasm and prominent cell wall. The stomach, small and large intestine showed moderate to severely thickened mucosa that contained myriads of protothecal organisms as well as various degrees of macrophage and lymphoplasmocytic cell infiltrations (Fig. 2). The organisms

Fig. 1 Severe granulomatous enteritis with myriads of patotothecal organisms in the duodenum. The organisms vary in size. HE. $\times 175$.

Fig. 2 The organisms vary in size, and have round to oval nuclei. HE $\times 700$.

Disseminated Protothecosis in a Cape Hyrax

Fig. 3 Electron micrograph of protothecal organisms with prominent cell wall, engulfed in a macrophage in the duodenum. $\times 10,000$.

were arranged in closely packed aggregates or cords enmeshed in connective tissues. Frequently these organisms were spreading into the submucosa and muscle layer. The duodenum and jejunum were mostly affected with severe stenosis of the lumen. In the kidneys, there were multifocal small granulomas with central flocks of protothecal organisms and surrounding infiltrations of macrophages, and lymphocytes and plasma cells. The lungs had marked infiltrations of macrophages admixed with lymphocytes and plasma cells in the alveolar wall, as well as frequent protothecal organisms. With PAS reaction, the cell wall of the protothecal organisms showed intensely positive reaction.

Electron microscopy revealed macrophages containing a few to several organisms with an appearance of algae (Figs. 3 and 4). The organisms were round to ovoid in shape (6 to 8

μm in diameter), containing a few to several daughter cells with distinct electron-dense cell walls. Mitochondria were prominent and located in the periphery of the cytoplasm. There were occasional electron-dense bodies, 0.2 to 0.5 μm in diameter, but no chloroplasts were seen in the cytoplasm of the organisms. Occasional macrophages were bearing empty cell wall or collapsed cell wall (Fig. 4). There were numerous protothecal organisms invading into lymphatic or blood vessels in the lamina propria.

Protothecosis is a rare and sporadic infectious disease occurred in various kinds of animals including wildlife. There has been no reported case of protothecosis in Cape hyrax (*Procavia capensis*). To our knowledge, this is the first reported case in this species maintained in zoos.

Protothecosis occurs in two clinical forms, cutaneous and

systemic. The disease in humans has been of the cutaneous type, whereas the systemic form has been inclined to be dominant in animals [2]. As clinical symptoms of protothecosis in animals, mastitis was observed in cattle [13], and systemic protothecosis with dermatitis [7], enterocolitis with bloody diarrhea [5], retinitis [14] and neurological disorder [15] were also reported in dogs. The present hyrax showed no apparent clinical symptoms including bloody stool before death, but there were multifocal hemorrhagic foci in the mucosa of small intestine at necropsy, which was suggestive of gastrointestinal infection of protothecal organisms in hyrax like dogs. Although the present case showed disseminated infection in various organs, severe enteritis associated with protothecosis might be the major cause of death because gastrointestinal tract had severe destruction with myriads of organisms. In this study, it

was difficult to determine the sub-species of *Prototheca* without isolation by culture.

Granulomatous enteritis due to green algal infection was reported in a dromedary [16]. It is quite difficult to differentiate protothecosis from green algae infection in tissue with only HE stain. Ultrastructurally, the true chloroplasts were never found in the *Prototheca* species, but electron-dense cytoplasmic granules were present in some of the *Prototheca* cells as well as green algal cells [17]. Based on ultrastructural features that the present organisms lacked chloroplasts, they were diagnosed as *Protothecal* spp.

Prototheca spp. is the saproplant, and it was isolated from sap, lake, seawater, mud water, sterco and cutis of animals [18]. As the hyrax was bred and reared in a herd in the zoo, it is probable that the environment of animal room including soil

Fig. 4 Electron micrograph of protothecal organisms. A organism (left) contained nuclei (N) and vacuoles with cells wall. Other two organisms (right) are collapsing $\times 18,500$.

Disseminated Protothecosis in a Cape Hyrax

and water was polluted by *prothotheca* spp. Zoo maintained animals have possible opportunity to contact humans directly or indirectly via water, soil and dust. It was uncertain that the present organism might bring disease to humans, especially immunocompromised patients like AIDS patients. Besides, anti-fungal agents are not so effective to *prototheca* spp. The present case warns the necessity for zoo veterinarians to make attention to *prototheca* spp. in zoo animals.

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要 約

ケープハイラックス (*Procavia capensis*) に認められた播種性のプロトテカ症について調べた。著しく肥厚した胃腸粘膜では、大きさ 5×5 から $20 \times 30 \mu\text{m}$ で PAS 反応に陽性を示すプロトテカ菌体の多数の出現を伴った高度な肉芽腫性炎が認められた。腎臓では、プロトテカ菌体を容れた多発性の肉芽腫が認められた。電顕的には、明瞭な細胞壁を有しクロロプラストを欠く algae 様の菌体が多数大食細胞内に認められた。本例はケープハイラックスにおけるプロトテカ症の最初の報告である。

キーワード: プロトテカ, 播種, ケープハイラックス, *Procavia capensis*

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