NOTE *Pathology*

First Case of *Echinococcus multilocularis* Infection in a Zoo-Housed Flying Squirrel (*Pteromys volans orii*)

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ABSTRACT. A 33 month-old male flying squirrel kept in a zoo developed progressive dyspnea and died. Macroscopically, the liver and lung were enlarged with numerous nodular vesicles. Histologically, these organs were replaced by numerous collapsed vesicles demarcated by fibrous tissues. The cysts lined by a cellular, germinal layer contained numerous brood capsules with abundant production of well-developed protoscolices. Protoscolices were about 80–100 μm in diameter, and had hooks being visible as refractive structures. This zoo locates in the east of Hokkaido where is an endemic area of *Echinococcus multilocularis* infection. From epidemiology and pathological findings, this animal was diagnosed as *E.multilocularis* infection. This report describes the pathology of the first case of *E. multilocularis* infection in a flying squirrel.

KEY WORDS: *Echinococcus multilocularis*, flying squirrel, Hokkaido, new host record, pathology.

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Echinococcosis, also known as hydatid disease, is one of the most important zoonotic diseases, causing human morbidity and mortality worldwide [3]. Generally, six species of *Echinococcus* are recognized, and four of them are of public health concern: *Echinococcus granulosus, E. multilocularis, E. vogeli* and *E. oligarthrus* [6, 9–11]. *E.granulosus* is characterized by the presence of unilocular cysts. In contrast, *E.multilocularis* which causes alveolar echinococcosis, and *E.vogeli* and *E.oligarthrus* are associated with the multicystic lesion. While *E.vogeli* and *E.oligarthrus* are restricted to South and Central America [5], the geographical distribution of *E.granulosus* is worldwide, and that of *E. multilocularis* is wide in the northern hemisphere, such as central Europe, Russia, central Asia, China, Alaska and Canada [9–11]. The life cycle of *E. granulosus* involves dogs and other canids as definitive hosts, and domestic and wild ungulates as intermediate hosts. Meanwhile, *E. multilocularis* exploits predator-prey relationships between canids and rodents [2]. In Hokkaido, an endemic area of *E. multilocularis* in Japan, the Ezo red fox (*Vulpes vulpes chrenchki*) and red-backed vole (*Myodes rufocanus bedfordiae*) mainly maintain its life cycle [7]. In foreign countries, the common vole (*Microtus arvalis*), the water vole (*Arvicola terrestris*) and the muskrat (*Ondatra zibethicus*) are important intermediate host for this species [2]. In addition to voles, grounds squirrels (*Citellus daurious*, *C. erythrogenys, C. ungugalatus* and *C. alashanicus*), red squirrel (*Sciurus vulgaris*), bobak marmot (*Marmota bobak*) and Prevost's squirrel belonging to the suborder Sciuromorpha have been reported rarely as the intermediated host [2, 12, 13]. This paper describes the first case of Ezo flying squirrel (*Pteromys volans orii*) affected with larval *E. multilocularis*.

A 33 month-old male Ezo flying squirrel, which was born and kept in the lair at a logged timber soon after birth to one month-old, and then housed in a metal cage enclosure with an outdoor area in Kushiro City Zoo. The animal developed progressive dyspnea and died on three days after the onset of dyspnea, and then dissected by a zoo veterinarian. Main organs including liver, spleen, kidney, heart, lung, colon and testes were fixed in 15% formalin and submitted to our laboratory. Samples were embedded in paraffin wax, and sections (4 μm) were stained with hematoxylin and eosin (HE) or periodic acid-Schiff (PAS). At necropsy, the thoracic cavity was filled with enlarged lung containing vesicular cysts. One third of the liver was replaced by a mass sized head of the thumb consisting of conglomerate cysts (Fig. 1). These cysts showed multilocular vesiculation containing white to yellow gruel-like fluid. Histologically, the liver and lung were replaced by numerous collapsed vesicles demarcated by fibrous tissues (Fig. 2). These fibrous tissues in both organs were not so broad, and thinned especially at the periphery of the vesicles projecting to the parenchyma. In the liver, fibrosis was associated with bile-duct proliferation. The outermost of the vesicles was a homogeneous layer in HE-stained sections, and stained strongly with PAS. This structure showed often fragmentation and convolution. The inner cavity of the vesicles lined by a cellular, germinal layer was composed of plural brood capsules containing numerous

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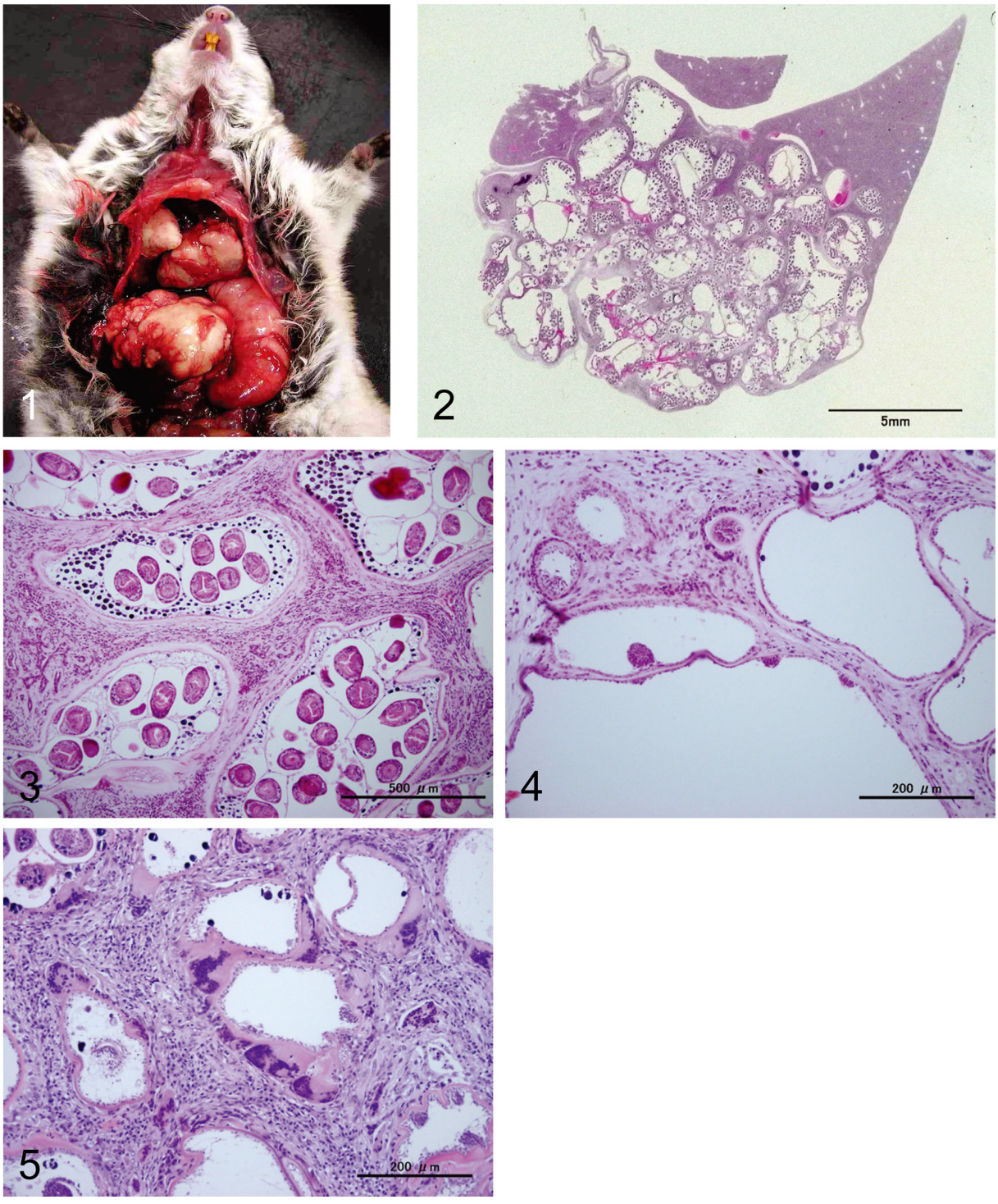

Fig. 1. The liver and lung have a mass sized head of the thump associated with numerous nodular vesicles.
Fig. 2. The greater part of the liver is replaced by numerous collapsed vesicles demarcated by fibrous tissues. HE stain.
Fig. 3. Liver. A vesicles lined by the homogenous laminated layer and the germinal layer contains numerous protoscolices and calcareous corpuscles. HE stain.
Fig. 4. Liver. Budding of the cells from the germinal layer. The cells of the germinal layer focally proliferate and form internal, small, protruding bud. HE stain.
Fig. 5. Lung. Numerous multinucleated ginat cells are reactive to the vesicles. Interstitial tissues are infiltrated by numerous lymphocytes and plasma cells, associated with fibrosis. HE stain.

protoscolices. Protoscolices were about 80–100 μm in diameter, and had hooks being visible as refractive structures. Numerous calcareous corpuscles were observed within the germinal layer as well as protoscolices (Fig. 3). A dense accumulation of germinal cells, showing budding form, were sometimes observed (Fig. 4). Many multinucleated giant cells being reactive to the vesicles were observed, associated with numerous lymphocytes and foamy macrophages (Fig. 5). No pathological lesions were observed in other organs.

In this study, alveolar echinococcosis was diagnosed in a flying squirrel at postmortem examination. In Japan, Hokkaido, the northern island, is the only region with documented endemic occurrence of *E. multilocularis* [2]. Red foxes (*Vulpues vulpes*), domestic dogs and raccoon-dogs (*Nyctereutes procyonoides*) have been identified as definitive host in Hokkaido [7]. Macroscopical finding showed marked enlargement of the liver and lung characterized by polycystic or multiloculated-cystic structures, and were consistent with alveolar echinococcosis. Histologically, metacestode origin of these cysts showing multivesicular, infiltrating structures consisted of a bladder with numerous protoscoleces. In addition to the epidemiology, these morphological criteria confirm the diagnosis of *E. multilocularis* infection.

E. multilocularis lesion is characterized by a granulomatous host reaction that includes infiltration of cells including lymphocytes, epithelioid cells and multinucleated giant cells and fibrosis [4, 13]. These periparasitic reactions surrounding the metacestode vesicles were not markedly different in the small mammal intermediate hosts including the experimental murine model and our flying squirrel case [2, 8]. The metacestode evades immune responses and proliferates progressively, and is capable to produce large numbers of protoscoleces. The periparasitic granuloma and fibrosis protect the host against the parasite's growth. In experimental murine model and human cases [4, 13], the correlation between resistance and development of fibrosis suggests that fibrogenesis is an important component of the immunologically mediated effector mechanisms that limit larval growth. Since thin fibrosis in the periphery of the metacestode expanding growth does not seem to inhibit the infiltrative growth in this case, the flying squirrel may have high susceptibility to infection. Although lung involvement is rare in *E. multilocularis* infections in human [1], pulmonary lesions with metacestode tissue are sometimes observed in the intermediate animals [2]. In this animal, the pulmonary lesions caused by hematogenous metastasizing spread from primary hepatic lesions induced clinically breathing difficulty and cyanosis, and finally death.

Infection of intermediate hosts occurs by the ingestion of eggs. The zoo maintaining a flying squirrel in this study is surrounded by a fox-tight perimeter fence, and direct contact with foxes can be excluded. Generally, metacestodes of *E. multilocularis* develop rapidly in intermediate host animals, and death of the intermediate host can occur, usually around five months after infection [2]. From these findings, the infection route in this animal may be ingestion of feeds or some branches from wild trees or shrubs contaminated by *E.multilocularis*.

Based on the histological findings, flying squirrel may be one of the most suitable intermediate hosts for *E. multilocularis* as red-backed voles, and could contribute to the transmission of this disease by chance.

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