Characteristics of a Hepatozoonosis in Lungs of Japanese Black Bears (Ursus thibetanus japonicus)

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ABSTRACT. In a survey of pathologic agents in the wild animals of central Japan, we found a Hepatozoon sp. in the lungs of Japanese black bears in Fukui, Shiga, and Gifu Prefectures, Japan. Histopathologic examination of organs and tissues from the 18 bears inspected showed hepatozoonosis in all. Immature and mature meronts were found in all lobules and between alveoli, with a few found between pleura and in connective tissue. In the lungs, inflammatory cells were not found around meronts, merozoites, or tubercles made of macrophages including zoites, but inflammatory cells were found around degenerating cells, zoites, and tubercles. A Hepatozoon sp. has not been reported as being detected in bears of any species before.

KEY WORDS: Hepatozoon sp., Japanese black bear, lung.

Species of the genus Hepatozoon infect various vertebrates (amphibia, reptiles, birds, and mammals) that are their intermediate hosts, and cause hepatozoonosis in these animals [15]. The life cycle of these organisms may begin with the ingestion of mites, ticks, or insects that contain sporulated oocysts. The sporozoites in the oocysts are released within the intestinal tract to penetrate the gut wall and travel via the blood or lymph to the spleen, bone marrow, lungs, liver, or muscles, where cystic and merogonic development occurs [5, 15].

Latent cysts are formed in the internal organs, especially the liver and lungs, of some vertebrate hosts infected with Hepatozoon species [15]. The lesion in infected dogs is of pyogranulomatous myositis, which results in muscular hyperesthesia and a distinct periosteal reaction [17]. However, the site(s) preferred by species of Hepatozoon depends on the animal species and may differ geographically for a single host species. Skeletal muscle tissue of infected dogs has been examined in detail [5], but there is little histopathologic information about other organs of infected mammals.

Some Hepatozoon species found in many vertebrate groups have low host specificity for both the definitive and intermediate hosts (according to a list of Hepatozoon species) [15]. Infections by the parasite have been found in many families of carnivores [3, 6, 8, 18], but the extent of host specificity for the cystic stage in the intermediate hosts is not known. In Japan, few reports about Hepatozoon species have been published, but such species have been found in wild rats [11], wild foxes [12], dogs [14], and wild martens [18]. No Hepatozoon species has been reported as being found in Japanese black bears.

This research was an epizootiologic and histopathologic examination of a Hepatozoon sp. in the internal organs of Japanese black bears. During our survey of Fukui, Shiga, and Gifu, infections by this Hepatozoon sp. were found in all 18 of the wild-caught bears examined.

A survey for the purpose of conservation and control of Japanese black bears (Ursus thibetanus japonicus) was done in 1991 and 1992. Eighteen bears were examined in Fukui (9 animals), Shiga (5 animals), and Gifu Prefectures (4 animals). The bears were killed in accordance with the policies of the Ministry of the Environment, Japan, concerning the conservation of Japanese bears. Organs and tissues collected were the liver, kidneys, heart, lungs, spleen, mesenteric lymph nodes, and skin (this from two animals only), and the specimens were fixed in 10% buffered formalin. Paraffin sections from each organ and tissue were cut 3 µm thick and stained with hematoxylin and eosin. These samples were examined microscopically. For observation by transmission electron microscopy (TEM), samples were postfixed with 1% osmium tetroxide. Samples were stained with uranyl acetate and lead citrate and examined (Hitachi H-800).

Meronts, merozoites, and tubercles containing zoites of a Hepatozoon sp. were found in the lungs of all 18 bears examined by light microscopy, but not in other organs. Figure 1 shows a zoite next to the large nucleus of the macrophage in the cytoplasm; this is the early cystic stage. The zoites within macrophages were round to oval and such macrophages were generally not found in association with an inflammatory response.

Immature and mature meronts found in all lobules were large and were round to oval. Both kinds of meronts surrounded by a membrane were found between alveoli, and a few were found between pleura and in connective tissue. The nuclei within immature meronts were lined up at the margin (Fig. 2). No inflammatory response was found around immature meronts surrounded by a membrane. Mature meronts (mean size, 52.9 ± 40.1 µm, n = 10) were filled with many merozoites; in Fig. 3, infiltrating eosinophils and neutrophils are not seen around the mature meront of normal appearance. However, such infiltration was
found around other meronts that had collapsed. Merozoites (mean size, 4.9 × 2.0 µm, n = 13) that had been released were observed inside capillaries and between cells of the lung.

Tubercles formed of macrophages were found in all lobules of the lungs, surrounded by squamous alveolar epithelial cells and large alveolar epithelial cells. Zoites within the cytoplasm of the host cells had pushed the host nuclei to the margin of the cells, and chromatin of host cell nuclei was scattered near the nuclear membrane (Fig. 4). TEM of the tubercles of macrophages showed large zoites within the macrophages; the zoites were similar in size to the nucleus of the cell (Fig. 5). Macrophages had tail-like appendages and lysosomes in the cytoplasm. Rough endoplasmic reticulum and micronemes were found in some zoites.

In a lung, zoites were found in an alveolar wall, and an inflammatory lesion was found nearby (Fig. 6). Collagenous fiber, reticular fiber, and elastic fiber were found between squamous alveolar epithelial cells and also between large alveolar epithelial cells around tubercles. Infiltration of inflammatory cells was found around degenerating tubercules and necrotic lesions in the lungs (asterisk in Fig. 7). Zoites (probably merozoites) were found around the inflammatory lesion (Fig. 7, arrows). Thus, cells infected with zoites had collapsed and pneumonia lesions had appeared. Hyperemia, congestion, and disturbances in circulation were not seen.

This parasite did not react to antiserum against Toxo-
plasma gondii or Neospora caninum by the fluorescent antibody method. We identified species of three ticks, *Ixodes ovatus*, *I. nipponensis*, and *Haemaphysalis flava*, on the infected bears. *Hepatozoon* oocysts were not found in the ticks examined.

On the basis of morphologic characteristics of the parasites and evidence that the parasites were found in all 18 bears examined, we concluded that a *Hepatozoon* sp.
infected black bears in central Japan with a high prevalence. Hepatozoonosis of the bears involved formation of tubercles containing macrophages in the lungs only, and also the development of inflammatory lesions. The pyogranulomatous myositis found in other animals infected by a Hepatozoon sp. [17] was not found in the infected bears examined here. In other hosts, there are diverse sites of infection by Hepatozoon species in the same animals [15], but in Japanese black bears, only the lungs were infected. No parasitic stages of the Hepatozoon sp. were found in other organs examined. There was no inflammatory response around mature meronts and merozoites, as a rule, and zoites were found within macrophages. The findings coincided with those of hepatozoonosis of other infected hosts [17]. The structure of Hepatozoon sp. meronts was characterized by peripherally arranged nuclei of the zoite and numerous merozoites forming rosettes: Coccidea) observed by TEM [16].

Meronts are located in the endothelial cells of capillaries in the lungs in vipers [2] and gray squirrels [4]. In our study, histologic examination showed meronts in the walls of alveoli. However, the kind of lung cells infected was not identified. The classification of Hepatozoon species was mainly based on the morphologic characteristics of the parasites, the clinical signs including histopathologic features of the lesions, tissue tropism, and the difference in vectors [15]. Of four papers on cases of hepatozoonosis found in Japan, histopathologic features were reported only for wild martens [18]. In infected martens, Hepatozoon parasites were found in not only the lungs; nodular lesions were found in the heart and other organs. There are no reports about tubercles formed from phagocytes that contained Hepatozoon zoites in the lungs of martens, although tubercles were found in the cardiac muscle tissue [18]. Thus, the species we found in black bears in this examination seems to be different from the other species reported earlier. The nucleotide sequence of the internal transcribed spacer region (ITS-1) of H. clamaeae and H. catesbianae was analyzed and differences in the two species have been reported [10]. The 18S small subunit rRNA gene was used to differentiate H. canis and H. americanum found in dogs [1]. A Hepatozoon species detected from dogs in Japan was suggested to be a strain of H. canis and different species from H. americanum by sequence analysis of the 18S rRNA gene [9]. If we can obtain fresh peripheral blood or tissue samples from Japanese black bears, this Hepatozoon sp. may be identified by the morphologic characteristics of the gamonts and the sequences of such genes of the parasites.

Different tick species are known to be the vector for H. canis and H. americanum [17]. In Japan, oocysts of H. canis have been found in ticks (H. longicornis and H. flava) removed from dogs with hepatozoonosis [13]. The ticks we found may be the vector for this Hepatozoon sp. from bears. The high prevalence of the Hepatozoon sp. in the Japanese black bears we examined suggests that the virulence of this parasite is low and that exposure is frequent in the wild.

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