

Alveolar Hydatidosis in a Gorilla and a Ring-Tailed Lemur in Japan

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ABSTRACT. Alveolar hydatidosis by *Echinococcus multilocularis* (Em) infection occurred on a 22-year-old (approx.) male gorilla (*Gorilla gorilla*) and a 4-year-old female ring-tailed lemur (*Lemur catta*) in a zoo, in Hokkaido, Japan. Case 1: The gorilla presented neurologic signs in course of nine months and died. Pathologically, alveolar hydatid lesions were found in the liver, the liver-associated lymph nodes, the cerebrum, and the lungs. A protoscolex was found only in one hepatic cyst. Case 2: In the lemur, large masses of hydatid cysts were found on the liver and at the lung-associated lymph nodes. Cysts contained numerous calcareous corpuscles and protoscolices. The lemur appears a favorable intermediate-host for Em. The identification of Em in both cases were confirmed by PCR. — **KEY WORDS:** *Echinococcus multilocularis*, gorilla, lemur.

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Four species are known in the genus *Echinococcus*. *Echinococcus granulosus* (Eg) has a wide distribution in the Northern and Southern hemispheres, but the others have rather narrow distributions. *Echinococcus multilocularis* (Em) is distributed in the Northern hemisphere, particularly holarctic regions, and *Echinococcus vogeli* and *Echinococcus oligarthrus* in South America. Primates, including humans, are accidental intermediate hosts for these parasites. In non human primates, there have been some reports on hydatidosis by Eg [1, 5, 6, 8], but few by the other species [4, 7], moreover there has been no report of cerebral hydatidosis by any *Echinococcus* spp., to the best of our knowledge.

In this paper, we report alveolar hydatidosis by Em in an approximately 22-year-old male gorilla (*Gorilla gorilla gorilla*) with cerebral involvement and a 4-year-old female ring-tailed lemur (*Lemur catta*) raised in Asahiyama Zoo in Asahikawa, Hokkaido, Japan.

The gorilla and the lemur died in July and August, 1994, respectively. They were necropsied within 12 hr after death, and multiple tissues were examined histopathologically. PCR was performed as by Bretagne *et al.* [2] with some modification to confirm the identification of Em by detection of Em species-specific DNA (U1snRNA gene).

Case 1: The gorilla had been brought to the zoo in 1978 at 6 years of age (approx.), and was in fair health until October 1993. At the time, it presented cerebral ischemic symptoms such as several fainting fits and right-sided hemiparesis, but recovered three months later. In early June 1994, a Jacksonian seizure occurred, and such seizures became frequent late in the month. The gorilla became anorectic and had facial paresis in mid-July. It died on July 19, 1994. Its diet had consisted of leguminous forage harvested from fields adjacent to the zoo, fruits, carrots, and potatoes.

At necropsy, there was a large bladder-like necrotic cavity (14 cm in diameter), which contained yellow-white cloudy fluid, on the visceral surface of the right hepatic lobe. Numerous smaller cystic lesions, often containing yellow-gray necrotic debris and encapsulated with thick connective

tissue, were scattered in the parenchyma and the subserosa. The liver-associated lymph nodes were enlarged and contained cysts which involved 50 to 80% of the nodal parenchyma. In the parenchyma of the lungs, several white miliary nodules (3 to 7 mm in diameter) were found. The brain was edematous though otherwise superficially normal, but on transection of the cerebrum, a cystic lesion (3 cm in diameter) was found in the left front region (Fig. 1).

In histological examination, cystic lesions of the liver and the associated lymph nodes consisted of alveolar cysts and surrounding inflammatory host tissues. The cysts had a very thin laminated layer and a thin germinal layer lining each. Calcareous corpuscles and a protoscolex were found only in one of the hepatic cysts, which had a thick laminated layer. The pericystic surrounding tissues consisted of an inner zone of epithelioid cells containing foreign-body type giant cells, an outer zone of dense fibrous tissue, and an intercystic heavy infiltration of lymphocytes and macrophages. The cysts were often embedded within

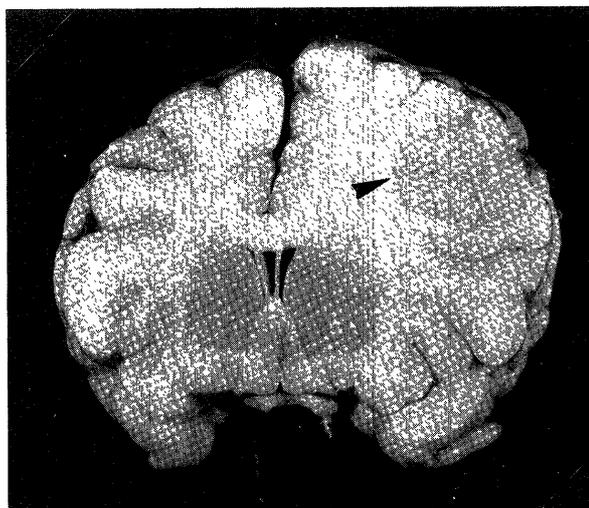


Fig. 1. Cerebrum of a male gorilla. A spherical lesion of the alveolar hydatid (arrow-head) appears on the cut surface of the left front region.

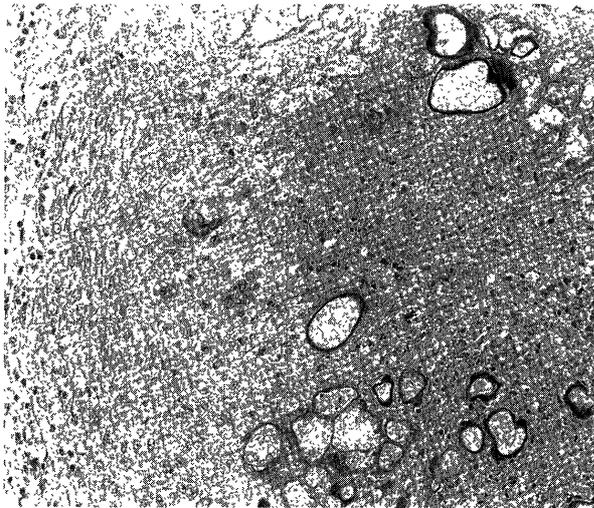


Fig. 2. Hepatic alveolar cysts of a male gorilla. P.A.S. positive laminated layers of alveolar cysts are embedded in necrotic debris and are surrounded by thick granulation tissue with a few giant cells. P.A.S. staining. $\times 122$.

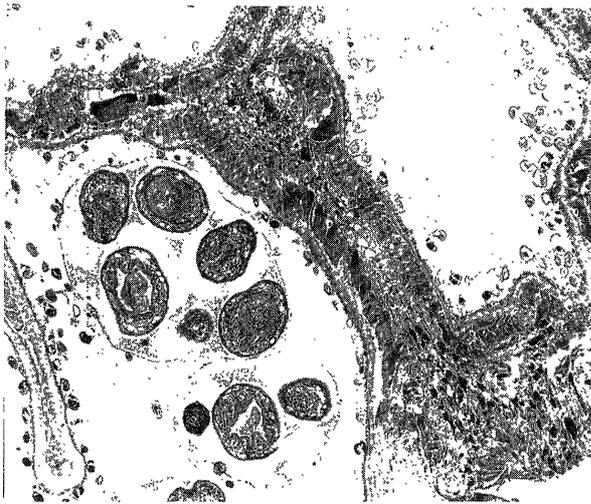


Fig. 4. Hepatic alveolar hydatid cysts of a female lemur consist of an outer laminated layer and a thin inner germinal layer with numerous calcareous corpuscles and brood capsules containing protoscolices. Cysts are surrounded by palisading epithelioid cells and foreign-body-type giant cells. HE. $\times 31$.

homogeneous necrotic debris with granular mineralization, and cysts themselves were often degenerative (Fig. 2).

Pulmonary nodular lesions contained only a few cysts, but their structures were similar to those in the liver. Cerebral lesions were also similar to hepatic ones with a lesser degree of granulomatous reactions. Astrocytosis was prominent in pericystic edematous neuropil. No protoscolex or calcareous corpuscle was found in either the lungs or the brain.

Case 2: The ring-tailed lemur, born in a zoo in Kagoshima, Japan, had been brought to Asahiyama zoo at one year of age, and appeared in good health. Abdominal

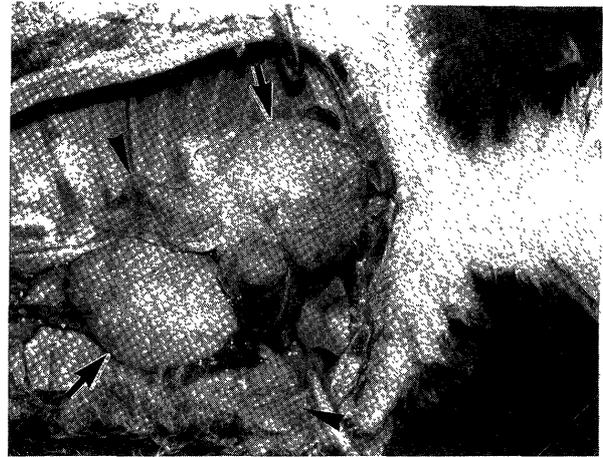


Fig. 3. A female lemur. Large alveolar cystic masses (arrows) in the thoracic cavity compress the lungs (arrow-heads).

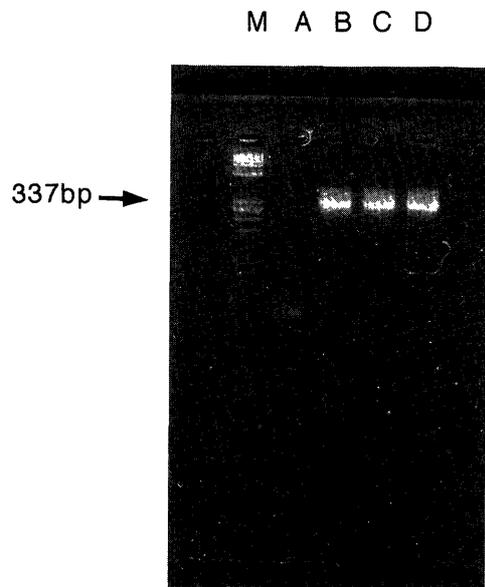


Fig. 5. PCR amplification of cyst DNA. M: Size markers (ϕ X174/HaeIII). A: Negative control. B: Alveolar hydatid from a gorilla. C: Alveolar hydatid from a lemur. D: Positive control DNA (10 μ g of *Echinococcus multilocularis* DNA of protoscolices from an experimentally infected cotton rat). B and C show the same major band of 337 bp as D (Ethidium bromide staining of 5 μ l of PCR products on agarose gel electrophoresis).

distention was noticed with no accompanying disorder in mid-August 1994. It had tachypnea one week later, and was found dead the following morning, August 23. The diet was similar to that of the gorilla.

At necropsy, marked changes were large alveolar cystic masses, which occupied more than half of the thoracic and abdominal cavities, and abdominal hemorrhage. There were 3 large masses (8 to 13 cm in diameter) in the parenchyma and on the serosal surface of the hepatic intermediate lobe

with marked subserosal hemorrhage. There were 3 thoracic masses (4 to 6 cm in diameter) located at the sites of lung-associated lymph nodes (Fig. 3). The lungs were congestive but had no cystic lesions. On transection, all masses were thin-walled alveolar cysts filled with minute yellow-white vesicles. Microscopy of a wet mount preparation demonstrated that the vesicles were micro-cysts containing protoscolices. The central portions of the large masses on the liver were dense connective tissues. No other organs had obvious macroscopic abnormalities.

In histological examination, cystic lesions were found only in and on the liver and the associated lymph nodes of the liver and the lungs. The cysts had a thick laminated layer and a thin germinal layer each, and contained numerous calcareous corpuscles and brood capsules containing protoscolices with well-developed hooks. Cysts were surrounded by palisading epithelioid cells with numerous bizarre foreign-body-type multinucleated giant cells, and intercystic fibrous connective tissue (Fig. 4). In many portions, the surrounding tissue was diffusely necrotizing with light to moderate mineralization. The intercytic connective tissues and adventitial fibrous capsules of the lesions were thin. Hemorrhage was marked at the periphery of lesions in the liver.

PCR: DNA isolates from hepatic alveolar cysts of both cases showed the same pattern upon amplification with a major band of the expected size (337 bp) (Fig. 5). The results confirmed the identification of Em in both cases.

Em mainly uses the fox as the definitive host, and metacestodes occur in small rodents. Em infection was found in 24% of 38 red foxes from the area including Asahikawa (data compiled by the Hokkaido Echinococcosis Conference, Hokkaido Government), and the wild fox is thriving in the region. In the present cases, the source of the infection was considered to be either leguminous forage contaminated by fox feces, or feces defecated in close proximity to the animal cages, although the primate's exposure to fox feces was not confirmed.

The neurologic signs for the gorilla were attributed to the intracerebral hydatid cysts. It is considered that the hydatid cysts developed in nine months. Because the ischemic cerebral syndrome is considered a sign of parasitic embolization, after which movement of the embolus allowed temporal recovery from it, but Jacksonian seizures due to the compression of the brain tissue occurred with the development of the hydatid cyst. Intracerebral hydatid cysts

are reported to grow in at a similarly fast rate in man [10]. Case1 provided a clue for the understanding of the development of cerebral hydatid cysts by Em, the cerebral involvement of which has been rare in human cases (2/152) in Hokkaido [9].

Our two cases of Em hydatidosis presented a striking contrast to each other in terms of the larval development and the diversity of the host-parasite relationship among primates. In the gorilla, except for one fertile cyst in our sections, most cysts were sterile, i.e. they had neither calcareous corpuscles nor protoscolices, and the fibrous capsules were very thick. These findings are similar to those in man [3, 7]. On the other hand, in the ring-tailed lemur, cysts were fertile, i.e. the cysts contained numerous calcareous corpuscles and protoscolices. Therefore, it appears that the lemur is a much more favorable intermediate-host for Em than the human or the gorilla.

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