動物園で飼育されているニホンザル(Macaca fuscata)での多包虫集団感染事例

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NOTE *Parasitology*

Outbreak of Larval *Echinococcus multilocularis* Infection in Japanese Monkey (*Macaca fuscata*) in a Zoo, Hokkaido: Western Blotting Patterns in the Infected Monkeys

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ABSTRACT. A high prevalence of larval *Echinococcus multilocularis* (Em) infection was found in zoo primates in Hokkaido, Japan. In October 1997, a Japanese monkey (*Macaca fuscata*) died and histopathologically diagnosed as alveolar hydatidosis. Serum samples were collected from the remaining Japanese monkeys and examined for antibodies against Em by enzyme-linked immunosorbent assay and western blotting. Serological tests showed 12 more animals of the remaining 57 monkeys were possibly infected. Ultrasonography revealed that nine of these 12 animals had a cystic lesion in the liver. The band patterns of western blotting in the monkeys were very similar to those in human.

KEY WORDS: *Echinococcus multilocularis*, Japanese monkey, serodiagnosis.

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Alveolar hydatid disease (AHD) caused by larval *Echinococcus multilocularis* (Em) has been recognized as a serious public health problem in Hokkaido, northern part of Japan [4]. The parasite has already spread all over Hokkaido and infected animals were ubiquitously found in Hokkaido [6]. Human is known as an accidental intermediate host of this parasite. In addition to the human case, primates have been reported to be naturally infected with Em metacestodes in Switzerland [7], Germany [1] as well as in Japan [5].

In this paper we report an AHD outbreak and high seroprevalence in Japanese monkeys (*Macaca fuscata*) raised in a zoo, Hokkaido, Japan.

A Japanese monkey in one zoo died in October 1997 and was histopathologically diagnosed as AHD. The pathological and histopathological findings on this monkey were shown in Fig. 1. An encapsulated abscess-like lesion was identified in the right lobe of the liver (Fig. 1a). Histopathological examination of the sectioned specimen revealed the presence of multiple cysts (Fig. 1b). Granulomatous reaction was prominent in the periphery of the lesion, accompanied by marked inflammatory cell infiltration and many multinucleated giant cells (Fig. 1c). Protoscoleces were recognized in some of these sectioned cysts (indicated by open triangles in Fig. 1b, c). The cysts had typical outer laminated layers (indicated by arrow heads in Fig. 1b, c).

Since the monkey had been raised in a group, the remaining monkeys were investigated for Em infection. A total of 57 serum samples were obtained from them and examined for the antibodies against Em by the methods of western blotting (WB) and enzyme-linked immunosorbent assay (ELISA) which are routinely used for diagnosis of human AHD at the Hokkaido Institute of Public Health. In this out-

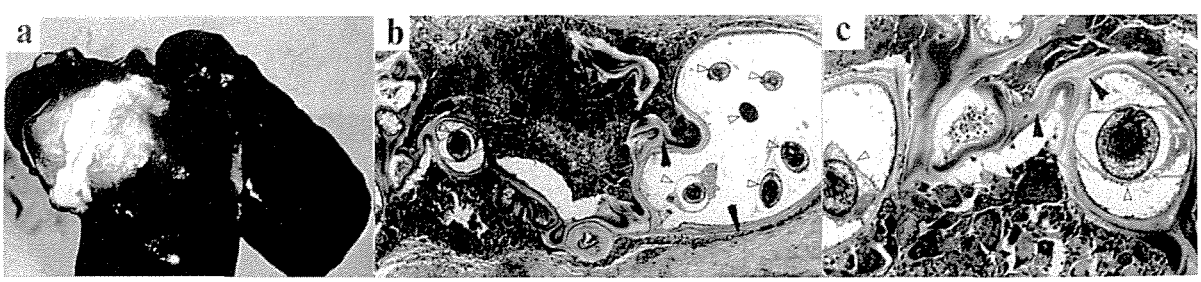

Fig. 1. Pathological findings in the dead monkey. Abscess-like cystic liver (a). Histopathological finding of the liver lesion [laminated cuticular layers (arrow heads) and protoscoleces (open triangles)], HE stain × 100 (b). Higher magnification of the same field (c).

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break, the source and route of transmission to the monkeys were unable to be determined in spite of intensive survey around the zoo.

The antigen for serodiagnosis was prepared from larval Em, European strain, which has been maintained in the cotton rats [3]. WB and ELISA were carried out as described previously [3, 8]. Sera from human AHD patients and healthy adult were used as positive and negative controls, respectively, for both WB and ELISA. Briefly, the WB was carried out on nitrocellulose membrane to which the denatured antigen was blotted after separated with 8% polyacrylamide gel electrophoresis containing SDS. Peroxidase labeled anti-monkey IgG was used as a secondary antibody. The results were regarded as positive when at least one of the three diagnostic bands were seen with significant intensity: 55 kDa, 66 kDa and loosely scattered band of 30–35 kDa [2]. This criterion was the same as those employed in a human case. In human AHD positive cases, the WB patterns were further classified into two types: one is complete type, in which serum samples react with two or three of the three diagnostic bands at 55 kDa, 66 kDa and broad-scattered 30–35 kDa and the other is incomplete type, in which serum samples react only with an antigen of 30–35 kDa [2]. This broad and scattered antigen, which we call C-antigen, has been detected in all serologically positive human cases [4].

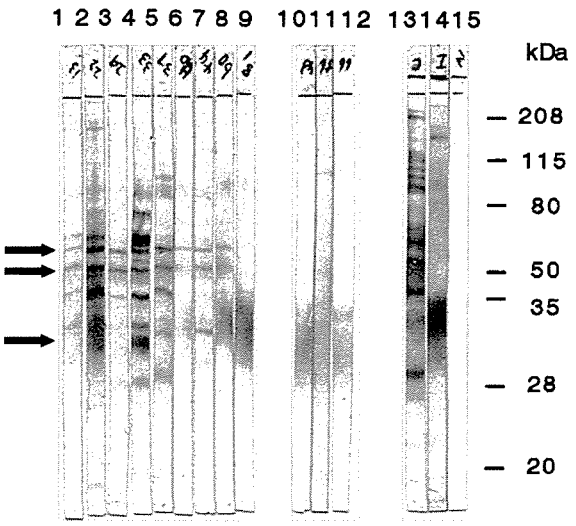

Fig. 2. Western blotting patterns of serum samples from Japanese monkeys in comparison with those of human AHD sera. Lanes 1 to 9; sera from Japanese monkeys showing complete type, lane 10 to 12; sera from Japanese monkeys showing incomplete type, lane 13; human AHD case judged as complete type, lane 14; human AHD case judged as incomplete type, lane 15; healthy adult volunteer. Arrow heads show diagnostic bands, 30–35 kDa, 55 kDa and 66 kDa. Molecular size is noted at right-hand.

In ELISA, we could not determine the positive-negative threshold line because serum samples of normal monkeys in Em-free area were not available. Therefore, the reaction conditions for ELISA were determined as giving the positive serum (human AHD) ELISA OD value of 1.00.

Out of 57 Japanese monkey serum samples, 12 sera showed positive reaction in WB (Fig. 2). In this study WB patterns of the Japanese monkeys were also found to be classified into two types like a human case. Nine of 12 WB positive sera, showed the complete type and three were the incomplete type. These 9 complete type sera had relatively high ELISA OD value (more than 0.7, average 1.00) and

Table 1. Results of WB, ELISA and ultrasonography performed in Japanese monkeys infected with the larval *Echinococcus multilocularis*

Subject number[a)]	Age (years)	WB patternt[b)]	ELISA	Ultrasonography[c)] (lesion size; mm)
Japanese monkey				
1	16	C	0.71	+ (80 × 70)
2	14	C	1.41	2+ (97 × 74; 23 × 22)
3	11	C	0.98	+ (59 × 46)
4	10	C	1.03	+ (93 × 74)
5	9	C	1.06	+ (121 × 86)
6	8	C	1.08	2+ (41 × 40; 28 × 30)
7	7	C	0.79	+ (75 × 70)
8	5	C	0.95	+ (40 × 20)
9	3	C	1.03	–
10	7	IC	0.61	+ (20 × 28)
11	5	IC	0.43	–
12	4	IC	0.33	–
Human				
13		C	0.97	+ (90 × 85)
14		IC	0.75	–
15		–	0.08	ND

a) The numbers correlate those of lanes in the Fig. 2.
b) C; complete type, IC; incomplete type.
c) +; one locus observed, 2+; two loci observed.
ND: Not done.

three incomplete type sera fell in equivocal zone between 0.33 and 0.61 (average 0.46, Table 1). The remaining WB negative sera showed low ELISA OD value of 0.28 or less with an average of 0.07 (data not shown).

The positive results in serological tests, ELISA and WB, were unable to exclude a possibility of the reaction to parasites other than Em. For confirmation of these serological results each Japanese monkey with possible infection with Em was further examined by ultrasonography as shown in Table 1. Foci of the liver were verified in the eight animals with the complete type in WB and in one of three animals with the incomplete type in WB. However, no clear foci were seen in two incomplete type animals and in one complete type animal; in the former case, it is conceivable that two incomplete type animals might have very small lesion in the liver unable to be detected by ultrasonography, on the other hand, for the one complete type animal a possibility cannot be excluded that the larval Em infiltrated into organs other than liver. Although these 12 monkeys were administered with albendazole (0.175 g/day), 9 animals died from a wound or in old age until the end of 2003 and three living animals (number 4, 6 and 7) were all the complete type. One (animal number 12) of three monkeys in which no cysts had been observed by ultrasonography was confirmed to be infected with Em by a post-mortem examination (data not shown). These data suggest that the results of serological tests are reinforced by ultrasonography.

In this study, it was shown that WB patterns of the Japanese monkeys are very similar to those of human. Moreover, the complete type was seen in the older monkeys (average is 9.2 years old), in contrast, the incomplete type in younger monkeys (average is 5.3 years old). This result coincides with the suggestion given by Furuya *et al.* [4] that the complete type may be due to the immunological stimulation to metacestodes of Em over a long period.

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