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STUDIES ON ECHINOCOCCOSIS XXIV  
AGE DIFFERENCE IN RESISTANCE TO INFECTION  
WITH *ECHINOCOCCUS MULTILOCULARIS*  
IN AKR STRAIN OF MOUSE\*

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Age difference in resistance to infection with larval *Echinococcus multilocularis* LEUCKART, 1863, was investigated in addition to sex difference of AKR strain mice.

- 1) Mice 29-day-old or younger were highly susceptible to larval *Echinococcus multilocularis*.
- 2) Forty-eight-day-old mice showed the highest resistance to this cestode, and 83- and 148-day-old mice were less resistant than 48-day-old mice.
- 3) Sex difference in resistance was not evident.
- 4) Susceptibility was 100% in AKR mice.

INTRODUCTION

About larval *Echinococcus* spp., SCHWABE et al. (1959) reported age resistance to secondary intraperitoneal infection to unilocular echinococcus in white mice.

The present author could not find any report of age difference in resistance to infection with larval *Echinococcus multilocularis* LEUCKART, 1863, in experimental animals. He therefore examined age resistance to infection with this parasite in AKR mice by the oral infection of eggs.

MATERIALS AND METHODS

AKR mice used for the experiment came from the breeding stocks of Experimental Animals Laboratory, Hokkaido University. These mice were bred in a breeding room with a thermostat for keeping the room temperature at 24°C. Suckling mice were weaned at 3 weeks after birth. The mice marked individually were segregated into their sexes and the mice of same age were kept in a cage. A syringe with a cannula was used to inoculate each mouse

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\* This work is a part of Master's thesis by the author at the Graduate School of Veterinary Medicine, Hokkaido University.

with approximately 330 eggs of Alaskan strain of *E. multilocularis* in physiological saline. The eggs were obtained from adult tapeworms of experimental dogs given hepatic foci of mice infected experimentally with larval cestode. The inoculated mice were killed by bleeding 30, 60 and 90 days after the inoculation, echinococcal foci were investigated carefully with naked eyes, the livers were weighed and the diameter of the large cysts was measured. The tissue materials were fixed with 10% formalin solution, and paraffine sections for microscopy were stained with hematoxyline-eosin.

## RESULTS

### 1 Susceptibility

All cases were infected by *E. multilocularis* and all the echinococcal foci were limited to the liver (tab. 1).

TABLE 1 *Susceptibility in AKR mice*

AGE AT INOCULATION (Days)	SUSCEPTIBILITY*		
	Male	Female	Total
22	4/4	5/5	9/9
29	3/3	3/3	6/6
48	4/4	3/3	7/7
83	4/4	4/4	8/8
148	4/4	3/3	7/7
Total	19/19	18/18	37/37

\*: Number of mice positive/examined

### 2 Weight of the liver

In the mice 30 days after the inoculation, the weight of livers did not increase clearly irrespective of ages, but at 60 and 90 days after the inoculation, the young mice, less than 30 days old when inoculated, showed apparent enlargement of the liver (tab. 2).

The analysis of variance presented in table 3 was made between the immature (22- and 29-day-old) and the mature (48- and 83-day-old). In this analysis, the sexes did not show significant difference in weight of the liver, even at 0.05 level of significance. But it was very significant that the weight of livers of the immature mice was considerably greater than those of the mature ( $P < 0.005$ ). And, as expected, the increase of weight of livers as the days passed after the inoculation showed a significant difference ( $P < 0.01$ ).

TABLE 2 *Relationship among weight of liver, age and sex of mice*

AGE AT INOCULATION (Days)	WEIGHT OF LIVER (g)					
	Male			Female		
	30*	60	90	30	60	90
22	1.5	5.2	2.4	1.2	4.7	3.7
"	—	—	3.9	1.5	—	5.7
29	1.6	2.2	4.5	1.6	2.6	4.5
48	1.7	1.5	2.0	1.2	1.2	1.3
"	—	—	2.2	—	—	—
83	1.5	1.8	2.1	1.4	1.5	1.8
"	—	—	2.5	—	—	2.0
148	1.5	1.9	2.3	1.5	2.6	—
"	1.7	—	—	1.5	—	—

\*: Days after the inoculation

TABLE 3 *Analysis of variance in the weight of livers*

SOURCE	SS	DF	MS
Sex	0.3038	1	0.3038
Day <sup>1)</sup>	10.1309	2	5.0655**
Age <sup>2)</sup>	13.3504	1	13.3504***
Sex × Day	0.0474	2	0.0237
Day × Age	5.8108	2	2.9054*
Age × Sex	0.1837	1	0.1837
Sex × Day × Age	0.0176	2	0.0088
Residual	7.7150	12	0.6429
Total	37.5596	23	

<sup>1)</sup>: Days after the inoculation (30, 60 and 90 days)<sup>2)</sup>: Immature or mature (estimated puberty age: 33-day-old)

\*: P&lt;0.05    \*\*: P&lt;0.01    \*\*\*: P&lt;0.005

### 3 Development of larval cestode

YAMASHITA et al. (1958) investigated the susceptibilities of various rodent animals to multilocular echinococcus, and classified the host animals into two types. AKR mice in this study belong to their type 1.

#### A) Number of foci and appearance of large cysts

Number of foci in 48-day-old mice were fewer than in other mice. At 30

days after the inoculation, some cysts showed an enlargement only in 22-day-old male and female mice. At 60 days, almost all cases except both sexes of the 48-day-old and 83-day-old female had large cysts more than 4 mm in diameter. At 90 days, large cysts appeared in all cases except in a 48-day-old female, and cysts of young mice increased in size as compared with those in others (figs. 1~4).

#### B) Brood capsule and scolex formations

1) 22-day-old mice Brood capsules had already been detected in both sexes 30 days after the inoculation, and brood capsules in a male manifested scolex formation. At 60 days, matured scolices appeared in both sexes. At 90 days, multilocular cysts were fully developed (figs. 5~10).

2) 29-day-old mice Brood capsules and scolices appeared in both sexes 60 days after the inoculation. At 90 days, brood capsules and immature scolices increased in number and size in the male, although a few matured scolex could be found in the female.

3) 48-day-old mice Development of multilocular cysts was slower than in the other cases. At 90 days, initial brood capsules without scolex were recognized in a male (figs. 11~16).

4) 83-day-old mice Brood capsules with immature scolices were detected 60 days after the inoculation in males, although brood capsule formation did not appear in a female. At 90 days, brood capsule and scolex formations were recognized in males and a female, but other female showed no scolex formation. Mature scolices did not appear.

5) 148-day-old mice In cases at 60 days after the inoculation, brood

TABLE 4 *Relationship among brood capsule formation, scolex formation, age and sex of the host*

AGE AT INOCULATION (Days)	MALE						FEMALE					
	30		60		90		30		60		90	
	B	S	B	S	B	S	B	S	B	S	B	S
22	1/1	1/1	1/1	1/1	2/2	2/2	2/2	0/2	1/1	1/1	2/2	2/2*
29	0/1	0/1	1/1	1/1	1/1	1/1	0/1	0/1	1/1	1/1	1/1	1/1
48	0/1	0/1	0/1	0/1	1/2	0/2	0/1	0/1	0/1	0/1	0/1	0/1
83	0/1	0/1	1/1	1/1	2/2	2/2	0/1	0/1	0/1	0/1	2/2	1/2
148	0/2	0/2	0/1	0/1	1/1	1/1	0/2	0/2	1/1	1/1	—	—

\*: No. of cases showing brood capsule and scolex formations/examined

B: Brood capsule formation

S: Scolex formation

capsules and scolices were detected in a female. At 90 days, immature scolices appeared in a male but mature scolices were not recognized.

Brood capsule formation took place in parallel with scolex formation in all cases, except the 48-day-old, and mature scolices were found for the first time in 22-day-old mice 60 days after the inoculation. Up to 90 days, mature scolices appeared only in young mice.

Relationships among brood capsule formation, scolex formation, age and sex of the hosts are summarized in table 4.

#### DISCUSSION

The multilocular echinococcus is the larval stage of *E. multilocularis* LEUCKART, 1863, its intermediate host is Rodentia and this species is different from *E. granulosus* (BATSCH, 1786). These facts have been clarified since the middle of 1950 by RAUSCH & SCHILLER (1951, 1956), VOGEL (1955), YAMASHITA et al. (1956, 1958) and others. Histogenetic study of larval *E. multilocularis* in rodents has been carried out by several authors. OHBAYASHI (1960) clarified that the development of this larval cestode became evident with progress of days after the inoculation. Consequently, it became easy to study multilocular echinococcosis in experimental animals.

YAMASHITA et al. (1958) investigated the susceptibilities of various rodent animals and two types were classified by them according to combination of morphology of the larva and host tissue reactions. In type 1, the larva develops rapidly, individual cysts are large in size, scolices are recognizable 1.5~2.5 months and host tissue reactions are slight in degree. Strain AKR mice examined in this study belong to this type.

Concerning sex differences, OHBAYASHI & SAKAMOTO (1966) reported that females of two mouse strains exhibited remarkable resistance to this cestode in varied degrees. AKR mice in this study showed a susceptibility of 100%. In male and female mice at same age, the appearance of scolices in the males was slightly earlier than in the females, but sex difference in resistance was not evident.

YAMASHITA et al. (1958) reported that immature scolices were recognized 2 months after the inoculation in 4-month-old AKR mice and the development at 3 months was still similar to that at 2 months, and that numerous fully developed brood capsules and scolices were found in cases at 5 months after the inoculation. Comparing the results of YAMASHITA et al. (1958) with the results of this study (tabs. 2~4 & figs. 1~16), it is evident that age differences in resistance do exist in liver enlargement, appearance of large cysts, formation of brood capsules and subsequent formation of scolices.

About age difference in resistance to infection with larval *Echinococcus* spp., SCHWABE et al. (1959) reported that white mice of unknown sex, which were inoculated at 48 days of age or younger, were highly susceptible to intraperitoneal infection with scolices of *E. granulosus*. In the present study, however, 48-day-old mice showed the highest resistance to infection with the multilocular larva. OGLE (1934) discussed the relationship between sexual maturity of female mice and their environment, especially the influence of temperature. He reported that the mice kept in 22~25°C reached sexual maturity about 33 days after the birth. On the other hand, the mice kept in 27.5~29°C required about 46 days to reach the sexual maturity. So, the difference between the results of SCHWABE et al. (1959) and those of the present author might be caused by the temperature of breeding room.

As for other larval cestodes, GREENFIELD (1942) suggested age resistance in the albino rat to *Cysticercus fasciolaris*, and DOW & JARRETT (1960) reported age, sex and strain differences in susceptibility to this parasite in mice.

Judging from the results obtained in this study, the phenomenon influenced by the age of host, as same as the strain and sex of the host, must be also taken into consideration in future experiments with multilocular echinococcosis.

#### ACKNOWLEDGEMENTS

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## EXPLANATION OF PLATES

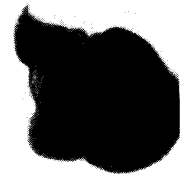
PLATE I Livers at 60 and 90 days after the inoculation  $\times 7/10$   
The number under the photograph shows age at inoculation by days.

Fig. 1 Livers of males at 60 days

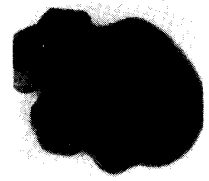
Fig. 2 Livers of females at 60 days

Fig. 3 Livers of males at 90 days

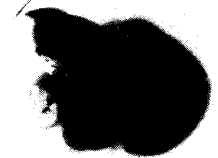
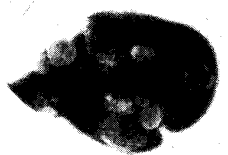
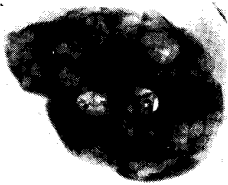
Fig. 4 Livers of females at 90 days



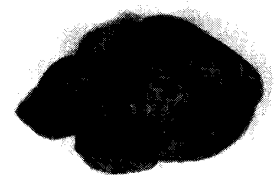
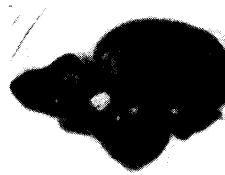
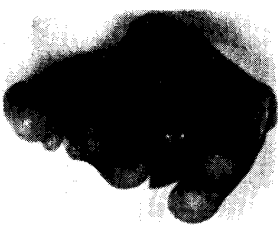
1



2



3



22

29

48

83

148

4

PLATE II Twenty-two-day-old cases at 30 to 90 days after the inoculation  
H.-E. stain

- Fig. 5 Scolex formation in the male at 30 days × 70  
Fig. 6 Brood capsule formation in the female at 30 days × 70  
Fig. 7 A few mature scolex in the male at 60 days × 50  
Fig. 8 A few mature scolex in the female at 60 days × 50  
Fig. 9 Fully developed hydatid cysts in the male at 90 days × 50  
Fig. 10 Fully developed hydatid cyst in the female at 90 days × 50

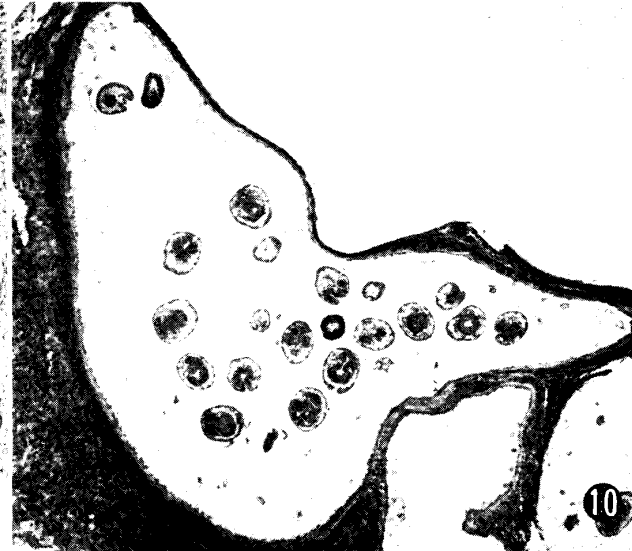
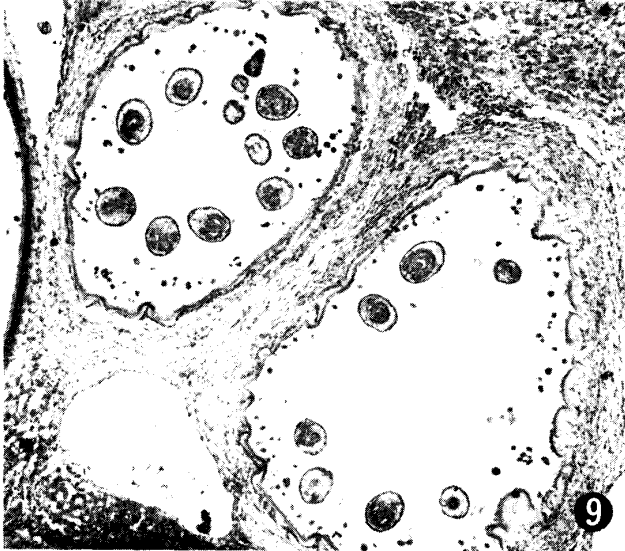
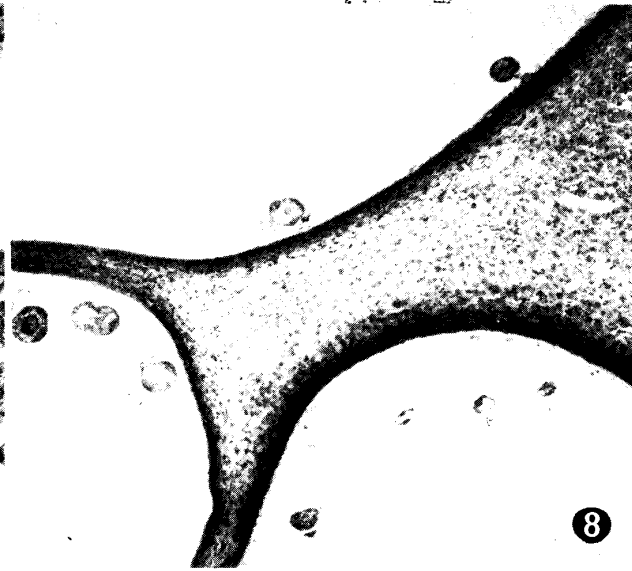
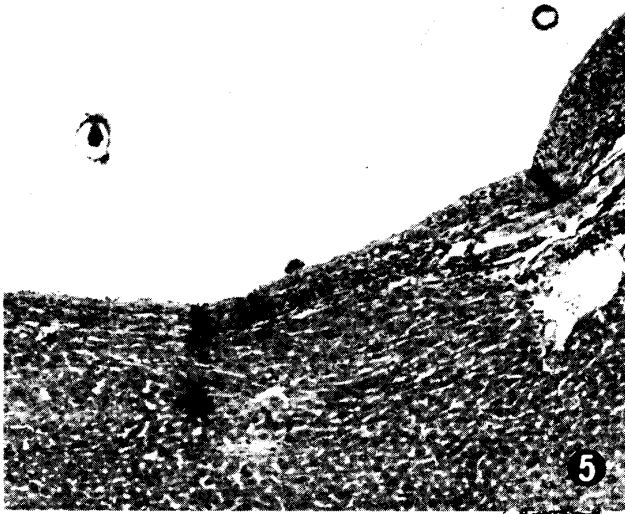


PLATE III Forty-eight-day-old cases at 30 to 90 days after the inoculation  
H.-E. stain

- Fig. 11 The male case at 30 days × 70  
Fig. 12 The female case at 30 days × 70  
Fig. 13 The male case at 60 days × 50  
Fig. 14 The female case at 60 days × 50  
Fig. 15 Initial brood capsule formation in the male case at 90 days  
× 70  
Fig. 16 The female case at 90 days × 50

