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Studies on *Eurytrema coelomaticum*

III. Development of *Eurytrema coelomaticum* in Mice*¹

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Introduction

The advance of experimental studies on the parasite harbored in large domestic animal is strikingly different depending upon whether the parasite is infective or not for the experimental animals²⁾. In the previous experiment⁵⁾, the authors tested the infectiousness of *Eurytrema coelomaticum* for experimental animals such as various strains of mice, goats, rabbits, and guinea pigs. It is recognized that only heterozygous nude mouse (BALB/c-nu/+) in addition to goats is infected with *E. coelomaticum*, although other strains of mice are not to be infective with.

The authors were much interested in the infectiousness of the heterozygous nude mouse, and tested the difference of the infectiousness and development of the fluke between heterozygous nude mouse and mouse without the recessive nude-gene (BALB/c-+/+). In this paper, the results obtained in the experiment are described.

Materials and Methods

The metacercariae of *Eurytrema coelomaticum* (Giard et Billet, 1892) Looss, 1907 were collected from the hemocoelom of the long-horned meadow grasshoppers, *Conocephalus maculatus* le Guillon. Ten metacercariae were orally given to each of 10 heterozygous nude mice of BALB/c background (BALB/c-nu/+), 5 normal BALB/c mice (BALB/c-+/+), and 10 of SL/QDJ mice (SL), and 20 metacercariae to each of 10 Mongolian gerbils (*Meriones unguiculatus*).

The respective two cases of the BALB/c-nu/+ mice were sacrificed 50, 112, 130, 153 and 265 days after infection. The BALB/c-+/+ mice, SL mice and Mongolian gerbils were dissected 130 days after inoculation.

About 1000 metacercariae of the flukes were orally given to each of 5 goats. The goats were sacrificed 40, 140, 140, 175 and 620 days after inoculation. The adult flukes obtained from the infected goats were used as a standard of comparison for the development of the flukes in the present experiment.

On the other hand, the metacercariae obtained from the long-horned meadow grasshoppers were placed in saline solution containing 1000 u/ml penicillin G, 1000 µg/ml streptomycin sulfate and

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1000 u/ml nystatin at 4°C over night. Twenty of the metacercariae were orally given to each of 7 athymic nude mice of BALB/c background (BALB/c-nu/nu) and 10 of heterozygous nude mice (BALB/c-nu/+). Those mice were dissected 170 days after inoculation.

Results

I. Development of *E. coelomaticum* in heterozygous nude mice (BALB/c-nu/+)

Each of BALB/c-nu/+ mice was orally inoculated with 10 metacercariae of *E. coelomaticum*. At first, two mice were dissected 50 days after inoculation. Two of the remaining mice began to eliminate the eggs of the fluke 112 days after infection, and were dissected on the day. The first elimination of the eggs was detected in the feces from every mice surviving until 118 days after infection. The respective two of the 6 mice surviving were dissected 130, 153 and 265 days after inoculation, respectively. Three to 10 (average 6.5) of the flukes were collected from each of the mice dissected.

1. 50-days-old fluke

The fluke is foliate and immature. The size of the flukes is 2.70~3.16 (mean±standard

Table 1. Development of *Eurytrema coelomaticum* in BALB/c-nu/+. unit of dimensions: mm

		Number of days after inoculation				
		50 days	112 days	130 days	153 days	265 days
Body length	*1	2.70 ~3.16 (2.94 ±0.16)	2.78 ~4.74 (3.97 ±0.57)	3.96 ~4.82 (4.50 ±0.26)	4.80 ~6.44 (5.69 ±0.63)	6.55 ~10.20 (8.62 ± 1.19)
Body width	*1	1.12 ~1.26 (1.20 ±0.05)	1.15 ~1.88 (1.71 ±0.23)	1.60 ~1.90 (1.78 ±0.09)	2.90 ~3.50 (3.22 ±0.23)	2.45 ~ 3.46 (3.11 ± 0.31)
Oral sucker	*2	0.30 ~0.34 (0.32 ±0.01)	0.30 ~0.45 (0.36 ±0.04)	0.38 ~0.44 (0.41 ±0.02)	0.55 ~0.62 (0.59 ±0.02)	0.47 ~ 0.71 (0.62 ± 0.08)
Ventral sucker	*2	0.35 ~0.36 (0.36 ±0.003)	0.36 ~0.44 (0.39 ±0.03)	0.41 ~0.45 (0.43 ±0.01)	0.65 ~0.74 (0.69 ±0.03)	0.58 ~ 0.79 (0.71 ± 0.08)
Sucker length ratio	*3	1.058~1.125 (1.119±0.046)	0.97 ~1.20 (1.102±0.075)	1.023~1.079 (1.045±0.023)	1.155~1.193 (1.178±0.013)	1.092~ 1.231 (1.138± 0.048)
Pharynx	*2	0.12 ~0.14 (0.12 ±0.007)	0.12 ~0.14 (0.12 ±0.006)	0.14 ~0.16 (0.15 ±0.007)	0.19 ~0.24 (0.21 ±0.02)	0.22 ~ 0.31 (0.28 ± 0.029)
Cirrus pouch-length		0.52 ~0.64 (0.58 ±0.04)	0.64 ~0.85 (0.77 ±0.06)	0.76 ~0.94 (0.84 ±0.06)	0.80 ~1.24 (1.08 ±0.15)	1.25 ~ 1.67 (1.44 ± 0.13)
Cirrus pouch-width		0.14 ~0.16 (0.15 ±0.009)	0.15 ~0.22 (0.18 ±0.022)	0.16 ~0.28 (0.21 ±0.04)	0.22 ~0.34 (0.27 ±0.04)	0.25 ~ 0.75 (0.49 ± 0.14)
Right testis	*2	0.15 ~0.20 (0.18 ±0.02)	0.22 ~0.30 (0.27 ±0.03)	0.26 ~0.30 (0.28 ±0.013)	0.32 ~0.48 (0.39 ±0.05)	0.40 ~ 0.63 (0.52 ± 0.08)
Left testis	*2	0.18 ~0.33 (0.22 ±0.05)	0.23 ~0.30 (0.27 ±0.02)	0.27 ~0.31 (0.29 ±0.014)	0.31 ~0.46 (0.41 ±0.05)	0.40 ~ 0.77 (0.55 ± 0.12)
Ovary	*2	0.07 ~0.14 (0.11 ±0.02)	0.18 ~0.24 (0.21 ±0.02)	0.18 ~0.23 (0.21 ±0.02)	0.25 ~0.35 (0.30 ±0.03)	0.22 ~ 0.37 (0.32 ± 0.04)

*1 Numbers parenthesized show the mean and standard error

*2 Average of cross diameters of sucker, pharynx, testis or ovary

*3 Sucker length ratio: $\frac{\text{Average of cross diameters of ventral sucker}}{\text{Average of cross diameters of oral sucker}}$

error: 2.94 ± 0.16) \times $1.12 \sim 1.26$ (1.20 ± 0.05) mm. The cross diameters of oral sucker are $0.30 \sim 0.34$ (0.32 ± 0.01) \times $0.30 \sim 0.34$ (0.32 ± 0.01) mm, and those of ventral sucker are $0.34 \sim 0.36$ (0.35 ± 0.01) \times $0.36 \sim 0.38$ (0.37 ± 0.01) mm. The ratio of the average of cross diameters of ventral sucker to that of oral sucker (sucker length ratio) is $1.059 \sim 1.125$ (1.119 ± 0.046). Cirrus pouch, testes, ovary and ootype are considerably developed, and the sizes of those organs are shown at Table 1. The vitelline glands and uterus are poorly developed. No egg is contained in the uterus.

2. 112-days-old fluke

The fluke is mature and has a uterus filled with a number of eggs. The body-size is $2.78 \sim 4.74$ (3.97 ± 0.57) \times $1.15 \sim 1.88$ (1.71 ± 0.23) mm. The cross diameters of oral sucker ($0.30 \sim 0.45$ (0.37 ± 0.04) \times $0.30 \sim 0.45$ (0.35 ± 0.05) mm) are approximately equal or smaller than those of ventral sucker ($0.36 \sim 0.44$ (0.40 ± 0.03) \times $0.33 \sim 0.44$ (0.39 ± 0.04) mm). Only one fluke in which the oral sucker is slightly larger than in case of ventral one is recognized, and its sucker length ratio is 0.98. The dimensions of several structures of the flukes are shown in Table 1. Testes are smooth or slightly lobed. Cirrus pouch is prominent. Ovary and seminal vesicle are approximately of the same size, and are smooth and round. Vitelline gland consists of several groups composed of several follicles.

3. 130-days-old fluke

The fluke reveals almost the same morphological structure as those of the 112-days-old fluke. The body-size is $3.96 \sim 4.82$ (4.50 ± 0.26) \times $1.60 \sim 1.90$ (1.78 ± 0.09) mm. The cross diameters of oral sucker ($0.30 \sim 0.46$ (0.39 ± 0.05) \times $0.40 \sim 0.46$ (0.43 ± 0.02) mm) are slightly smaller than those of ventral sucker ($0.43 \sim 0.46$ (0.45 ± 0.01) \times $0.36 \sim 0.44$ (0.42 ± 0.03) mm).

4. 153-days-old fluke

The structures of the fluke exhibit seems to be similar to those of the 112- and 130-days-old flukes. The body-size is $4.80 \sim 6.44$ (5.69 ± 0.63) \times $2.90 \sim 3.50$ (3.22 ± 0.23) mm. The cross diameters of oral sucker ($0.52 \sim 0.64$ (0.59 ± 0.04) \times $0.56 \sim 0.60$ (0.58 ± 0.01) mm) are slightly smaller than those of ventral one ($0.64 \sim 0.76$ (0.70 ± 0.04) \times $0.66 \sim 0.72$ (0.68 ± 0.02) mm).

5. 265-days-old fluke

The body-size of the flukes ($6.55 \sim 10.20$ (8.62 ± 1.19) \times $2.45 \sim 3.46$ (3.11 ± 0.31) mm) is remarkably larger in comparison with that of the flukes described above. Oral sucker ($0.38 \sim 0.65$ (0.53 ± 0.12) \times $0.55 \sim 0.81$ (0.71 ± 0.09) mm in cross diameters) is generally smaller than ventral one ($0.55 \sim 0.83$ (0.72 ± 0.09) \times $0.55 \sim 0.76$ (0.69 ± 0.07) mm).

II. Infectiousness of the fluke to normal BALB/c mice (BALB/c-+/+)

Five of BALB/c-+/+ mice were orally given 10 metacercariae. The mice were dissected 130 days after inoculation. Only one fluke was found from the pancreatic duct of one of the mice inoculated. Compared with the 130-days-old flukes obtained from BALB/c-nu/+ mice, the body-size of the fluke (2.66×1.20 mm) is distinctly smaller. Oral sucker (0.30×0.24 mm) is smaller than ventral one (0.26×0.26 mm). The average diameters of right and left testes, of ovary and pharynx are 0.13, 0.15, 0.13 and 0.11 mm, respectively. The size of cirrus pouch is 0.56 mm in length and 0.16 mm in width. The morphological findings of organs of the fluke appear to be normal. Considerable number of eggs were seen in their uterus.

III. Infectiousness of the fluke to SL mice and Mongolian gerbils

Each of 10 SL mice and 10 Mongolian gerbils were orally given 10 and 20 of metacercariae, respectively. The mice and gerbils were dissected 130 days after inoculation. No fluke was free from all of the animals inoculated.

IV. Development of the fluke in goats

About 1000 metacercariae were orally given to each of 5 goats. The eggs of the fluke were detected to be eliminated in feces of all of the goats from 98~118 days after inoculation. Each of the animals was dissected 40, 140, 140, 180 and 620 days after inoculation, respectively. The body-

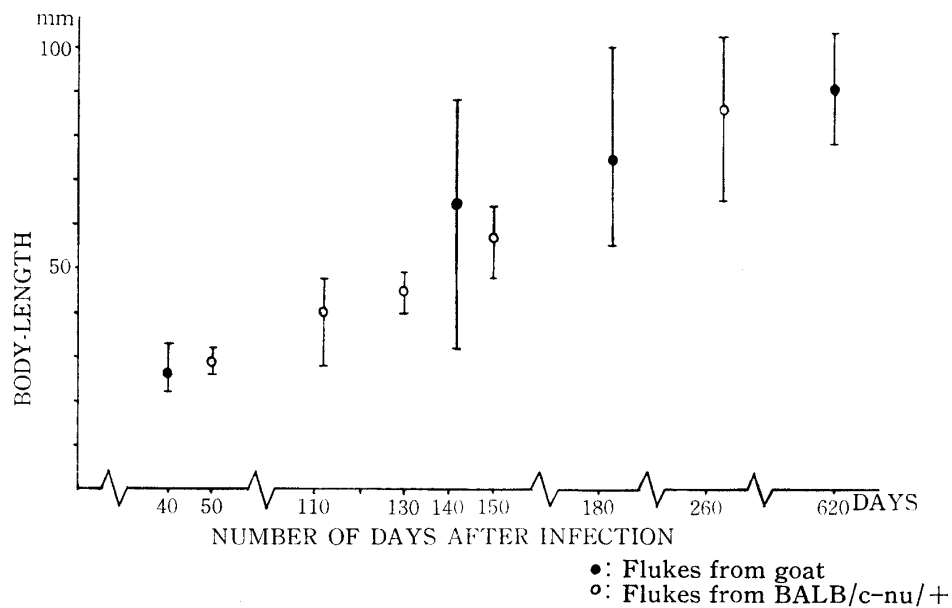


Fig. 1. Development of *Eurytrema coelomaticum* in goats and BALB/c-nu/+ mice.

sizes of flukes obtained from the goats 40, 140, 180 and 620 days after infection, are 2.20~3.24 (2.64 ± 0.32) \times 0.86~1.34 (1.06 ± 0.14) mm, 2.70~8.90 (6.49 ± 1.72) \times 1.50~3.80 (2.92 ± 0.74) mm, 5.40~9.80 (7.53 ± 1.00) \times 1.50~2.60 (2.07 ± 0.32) mm and 7.80~10.3 (9.08 ± 0.75) \times 2.80~4.40 (3.21 ± 0.36) mm, respectively. The average diameter of oral sucker of the flukes is generally smaller than that of the ventral sucker. Those flukes have well-developed organs. It is observed that the body-size of 140-days-old flukes from goats is larger than that of the 130-days-old flukes from BALB/c-nu/+ mice, and some of the 140-days-old flukes is larger than the 153-days-old flukes from the BALB/c-nu/+ mice. Each of the body-sizes of the 153- and 265-days-old flukes from BALB/c-nu/+ mice is smaller than that of the 180- and 620-days-old flukes from goats, respectively. The morphological structures of the flukes from the goats would be reported in another paper in future.

V. Infectiousness of the sterilized metacercariae of the fluke to athymic nude mice (BALB/c-nu/nuJCL)

The metacercariae soaked in saline solution containing 1000 u/ml penicillin G, 1000 μ g/ml

streptomycin sulfate and 1000 µg/ml nystatin for 3 hours. Twenty of the sterilized metacercariae were orally given to each of 7 BALB/c-nu/nu mice and 10 BALB/c-nu/+ mice. No eggs were detected by fecal examination of the inoculated animals for the period of experiment. At autopsy 150 days after inoculation, it was confirmed that all of the mice were not infected with the fluke.

Discussion

Up to the present, it has been reported⁸⁾ that *Eurytrema coelomaticum* is infective to *Bos taurus*, *Capra hircus*, *Ovis aries*, and *Camelus bactranus*. Within the authors' information, however, there is no paper dealing with the experimental eurytreemiasis of rodents. In the previous paper⁵⁾, we reported the first case of heterozygous nude mice experimentally infected *E. coelomaticum*. The results obtained in the present experiment are considered to be an extension of the previous study. We are much interested in the fact that *E. coelomaticum* was infectible to the heterozygous nude mice which have normal immunological capacity, notwithstanding the fluke's non-infectiveness to the strain mice other than these. The present experiment exhibited that one of 5 normal BALB/c mice (BALB/c-+/+) was harbored with only one small sized worm, although 100% of the heterozygous nude mice were infested with the flukes. This fact suggests that the heterozygous nude mice has the higher susceptibility for the flukes than the normal mice. We have no explanatory data as to the cause of the difference of susceptibility for the parasite between the heterozygous nude mouse and the normal mouse. We consider that the difference biological reactions of mice accompanied with nude-gene should be examined in future.

In the present experiment, the sterilized metacercariae revealed none of the infectiveness to both of the nude mice and the heterozygous nude mice. It is conceivably possible that the infectibility of the metacercariae was lost by the sterilization using antibiotics. It is thought that the re-examination should be made to clarify the reason of this negative result.

Looss and Cuffey⁴⁾ reported that the body-size of the younger (immature) specimens of *E. coelomaticum* is 7.5×3.5~4.74 mm. Bhalerao¹⁾ described that the size of the flukes ranges 7~10×4~5.5 mm. Eduardo *et al.*³⁾ stated that the flukes obtained from cattle in Philippine measured 5.92~10.34×1.85~4.278 (average: 3.209) mm. Travassos *et al.*⁷⁾ reported that the body-size of the fluke from cattle in Brazil is 10~13×6~8 mm. Tang and Tang⁶⁾ described that the bodies of the flukes from a goat is 3.913~6.171×2.077~3.206 mm in size. In the present experiment, it is observed that the body-sizes of the flukes obtained from the heterozygous nude mice and goats were considerably varied with the course of time in growth. Therefore, each of the body-sizes of the present flukes in various ages agrees with one of the measurements reported by the workers as mentioned above.

Summary

Ten metacercariae of *Eurytrema coelomaticum* (Giard et Billet, 1892) Looss, 1907 were orally given to each of 10 heterozygous nude mice (BALB/c-nu/+), 5 normal BALB/c mice (BALB/c-+/+), and 10 SL mice and 20 metacercariae to each of 10 Mongolian gerbil (*Meriones unguiculatus*). Three to 10 of the flukes were found in the pancreatic duct of all of the heterozygous nude mice. Only one worm was found from one of the normal BALB/c mice. No parasite was found from the SL mice and Mongolian gerbils. The body-sizes of 112-, 130-, 153- and 265-days-old flukes obtained from BALB/c-nu/+ mice are 2.70~3.16, 2.78~4.74, 3.96~4.82, 4.80~6.44 and 6.55~10.20 mm,

respectively. The size of 130-days-old fluke from BALB/c-+/+ mouse is small, 2.66×1.20 mm. The dimensions of the flukes collected from the experimentally infected mice were morphologically compared with those of the flukes from the infected goats. Twenty of the metacercariae which were sterilized by being soaked in the saline solution containing antibiotics, were orally given to each of 7 athymic nude mice (BALB/c-nu/nu) and 10 heterozygous nude mice (BALB/c-nu/+). All of the mice were not infected with the fluke. It may be conceived that the metacercariae were made to become infertile through the process of sterilization.

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Explanation of figures

Figures 2~7 are photographs of specimens of *Eurytrema coelomaticum* stained with hematoxylin at the same magnification ($\times 10$).

Fig. 2. 130-days-old fluke obtained from BALB/c-+/+ mouse.

Fig. 3. 50-days-old fluke from BALB/c-nu/+ mouse.

Fig. 4. 112-days-old fluke from BALB/c-nu/+ mouse.

Fig. 5. 130-days-old fluke from BALB/c-nu/+ mouse.

Fig. 6. 153-days-old fluke from BALB/c-nu/+ mouse.

Fig. 7. 265-days-old fluke from BALB/c-nu/+ mouse.

Fig. 8. Pancreas of BALB/c-nu/+ mouse 130 days after infection. Dilated pancreatic ducts containing the adult flukes. $\times 2.9$

Fig. 9. Internal organs of BALB/c-nu/+ mouse 153 days after infection. Pancreatic ducts were dilated by the flukes. $\times 2.3$

Fig. 10. Pancreas of BALB/c-nu/+ mouse 153 days after infection. Adult flukes are seen in the dilated pancreatic duct. $\times 3$

Fig. 11. Pancreas of BALB/c-nu/+ mouse 265 days after infection. Ten flukes are seen in the pancreatic ducts. $\times 2$

