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STUDIES ON THE SURVIVAL AND INFECTIVITY OF *FASCIOLA GIGANTICA* METACERCARIAE

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Abstract

The infectivity of *Fasciola gigantica* metacercariae was tested in rabbits after kept in water over a period from July to March of the next year. The viability of metacercariae on grasses usually decreased in autumn and winter with the lapse of time, and they were barely viable fore at least 6 months after encystment. Accordingly, metacercariae attached to grasses in water harvested about July remained infective until the middle of January of the next year.

It is well known that in many parts of the world a principal route of infection of liver flukes is grazing swampy pastures of flooded areas contaminated with metacercariae. In Japan, however, metacercariae are quite frequently found on grasses growing on the water-side and rice stems. Therefore, it is very important to clarify the relationship between different adverse environmental conditions and the survival of metacercariae sticking to grasses on water-side and rice stems.

Up to date, some reports have been made on the long survival of metacercariae at different temperatures after encystment[1-3,7-9]. There have been few papers, however, published on the survival of metacercariae on roughage kept in experimental conditions similar to those in nature[4-6]. In the present report, the infectivity of *Fasciola gigantica* metacercariae sticking to grasses and kept in water was evaluated by the number of liver flukes surviving and lesions detected in the liver of rabbits at the postmortem examination.

Materials and Methods

Metacercariae were obtained from *Lymnaea ollula*, an intermediate host, that had been maintained in this laboratory. Snails of this species had been exposed experimentally to miracidia hatched from ova collected from the gallbladder of cattle infected with *F. gigantica*. All the metacercariae on grasses were kept in water after encystment (Table 1).

Twenty-two rabbits were used for experimental infection, weighing from 1.8 to 2.8 kg. All of them had been confirmed to be free from liver flukes by means of fecal examination prior to experimental infection. Each of them was orally given 20 metacercariae of liver flukes. The infected rabbits were sacrificed 58 to 76 days after infection.

Results

The experiment was carried out over a nine-month period from July to March of the next year. Its results are summarized in Table 2. Three rabbits, Nos. 1, 2 and 3, were infected with metacercariae 9 days after encystment. The recovery rate of flukes was 85.0% in No. 1, 100% in No. 2 and 80.0% in No. 3. All the juvenile flukes recovered showed normal viability. It was recognized that the rate of infection began to decrease abruptly 60 days after encystment. When four groups of rabbits were given metacercariae 60, 90, 120 and 180 days after encystment, respectively, they each showed an average rate of infection of 25.0, 8.3, 6.7 and 5.0%. No infection was seen in rabbits given metacercariae 150 or 250 days after encystment. Consequently, the viability of meta-

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Table 1. Temperature in the experimental water used (°C)

	Range	Mean
Summer (Middle of July to end of August)	22.0-30.5	27.1
Autumn (Beginning of September to end of November)	4.2-28.3	16.9
Winter (Beginning of December to end of February)	0 -13.7	4.7
Spring (March)	1.8-14.9	7.2

Table 2. Viability of *F. gigantica* metacercariae kept in water

Rabbit number	Date of infection	No. of metacercariae given	Days after encystment	Days from infection to autopsy	No. of worms recovered	Infection* rate (%)	Mean infection rate (%)	Liver lesion
1	Jul. 27	20	9	72	17	85.0	88.3	++
2	Jul. 27	20	9	72	20	100		###
3	Jul. 27	20	9	72	16	80.0		###
4	Sep. 16	20	60	76	5	25.0	25.0	###
5	Sep. 16	20	60	76	7	35.0		###
6	Sep. 16	20	60	76	7	35.0		++
7	Sep. 16	20	60	76	1	5.0		+
8	Oct. 16	20	90	67	2	10.0	8.3	+
9	Oct. 16	20	90	67	1	5.0		+
10	Oct. 16	20	90	67	2	10.0		+
11	Nov. 15	20	120	71	0	0	6.7	-
12	Nov. 15	20	120	71	4	20.0		+
13	Nov. 15	20	120	71	0	0		-
14	Dec. 15	20	150	69	0	0	0	-
15	Dec. 15	20	150	69	0	0		-
16	Dec. 15	20	150	69	0	0		-
17	Jan. 14	20	180	69	2	10.0	5.0	+
18	Jan. 14	20	180	69	0	0		-
19	Jan. 14	20	180	69	1	5.0		+
20	Mar. 25	20	250	68	0	0	0	-
21	Mar. 25	20	250	68	0	0		-
22	Mar. 25	20	250	68	0	0		-

Remarks.
*: Number of worms recovered/Number of metacercariae given.
+: Slight changes. ++: Middle changes. ###: Serious changes.

cercariae on grasses usually decreased in autumn and winter with the lapse of time.

Discussion

Metacercariae are organisms in the developmental phase with a considerable potentiality for survival and average life which can be expected under different environmental conditions. They are of very great importance both in the study of the life history of the parasite and in the epizootiology of fascioliasis.

MAREK[5] found that metacercariae survived for 8 months in moist hay which had been collected during the rainy period. NÖLLER and SCHMID[6] stated that only mild infection was induced in animals fed heavily contaminated hay that had been dried incompletely and stored under the roof of a stable for 1 to 3 weeks, but that no infection was noticed among animals fed the same hay stored for 5 to 6 months. SHIRAI[9] also reported that metacercariae survived in water frequently renewed at a room temperature of 22 to 31°C for 80 days and in water at 32°C for 30 days

when exposed to sunlight. They survived 24-hour exposure in shade at a room temperature of 25 to 32°C, but died after 72-hour exposure. ONO *et al.*[7] found that all the metacercariae died in 10 days on grass dried at 25 to 32°C. BORAY and ENIGK[2] reported the infectivity of metacercariae of *F. hepatica* and *F. gigantica* after exposure to different temperature and relative humidities in controlled climate chambers. The metacercariae were resistant to freezing at −2 to −10°C, although they lost their infectivity at −20°C. Recently, KIMURA and SHIMIZU[4] showed that metacercariae, attached to rice stems, lost their infectivity, as proved by most test, when exposed to room temperature over a period from October to March of the next year, and no infection took place 5 months after exposure.

It was shown in the present experiment that metacercariae were infective at least for 180 days after encystment when kept in water at room temperature. It is conjectured, therefore, that fascioliasis may occur among cattle fed grasses in water contaminated with metacercariae over a period from the end of July to the middle of January of the next year.

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肝蛭メタセルカリアの生存力に関する研究

木村　重・清水　晃

要　約

夏期に被嚢した肝蛭メタセルカリアを水中に保存し，感染力の持続期間について検索した。

被嚢後9日目（7月下旬）のメタセルカリアを家兎に投与したところ，感染率は80.0〜100％であった。その後虫体の回収率は低下し，被嚢後180日目（1月下旬）のものの投与では，感染率0〜10.0%，平均感染率5.0%であった。そして，この肝蛭感染家兎が最終の感染例であった。また，被嚢後150日および250日を経過したメタセルカリアを投与した家兎には，肝蛭の寄生ならびに肝病変はみとめられなかった。

以上のことから，夏期より冬期にわたって水中に保存したメタセルカリアのうちには，約6カ月間（7月下旬〜翌年1月中旬）生存するものもあった。この生存期間を，野外での肝蛭感染に想定してみると，つぎのようなこともいえる。すなわち，湿地または畦畔のメタセルカリア付着緑草を給与する場合，感染期間は，夏期より秋期，ときに冬期にわたる。