Metacercaria Dctecting Buoy (MDB) Method for Estimating Contamination of Rice Fields with Fasciola Metacercariae

By HAKARU UENO

First Research Division, National Institute of Animal Health

Fascioliasis of livestock, particularly of ruminants, occurring everywhere in the world, is regarded as an important helminthiasis from the viewpoint of animal protein resources, because it causes reduced production and quality deterioration of milk and meat.

In Japan, about 20% of cattle are affected by fascioliasis. Unlike the methods of management in other countries, cattle are usually kept in cattle pens in Japan, and the Fasciola infection occurs through the year-round supply of large quantity of rice straw as roughages. Thus, the occurrence of bovine fascioliasis is closely related to the rice cultivation, the mainstay of Japanese agriculture.

Since fresh cattle manure or stable manure containing Fasciola eggs is applied to rice fields a few months before transplanting of rice, and irrigated fields furnish a suitable environment for *Lymnaea ollula*, an intermediate host for Fasciola, rice plants, especially their stems, are contaminated with Fasciola metacercariae to varying extents.

In spite of the existence of such an important inter-relationship between Fasciola infection and rice cultivation, almost nothing has been clarified with regard to the process of emergence of Fasciola cercariae from *L. ollula* in rice fields and the subsequent cyst formation on stems of rice plants.

The present paper deals with the Metacercaria Detecting Buoy (MDB) Method, which was newly deviced to capture Fasciola cercariae at the metacercarial stage in rice fields, as already reported by Ueno et al. (1975), and the field application of this method to find out effective measures for controlling fascioliasis.

Basic idea for developing MDB method

Field surveys on the distribution of *L. ollula* in paddy fields and the infection of this snails with Fasciola have been carried out by Ono et al. (1953), Watanabe et al. (1955), and Akabane et al. (1971). However, these surveys focused on the role of hibernating snails or yearling snails in the Fasciola infection of cattle, and although that problem was clarified to a considerable extent, it was difficult to estimate the time and intensity of emergence of Fasciola cercariae in rice fields.

Ueno et al. (1974), based on studies on ecological behavior of Fasciola cercariae, found out the fact that the cyst formation occurs more abundantly in the shallow layer below water surface, particularly in a range of 2–3 cm deep, and also apt to occur on materials with smooth surface. By taking advantage of these characters, a method to capture Fasciola cercariae at the metacercarial stage was deviced.

Structure of MDB method and its use

As shown in Fig. 1, the buoy is comprised of a disk made of foam styrol (10 cm of diameter × 3 cm thick), a polyethylene sheet (16 cm of diameter), a long bolt with a hole at the tip, rubber band, nylon thread (about

35 cm long) and a lead anchor. The bottom and side of the disk is wrapped with a polyethylene sheet, which is fixed to the disk by rubber bands. A nylon thread which has a lead anchor is connected to the tip of the bolt penetrating the disk.

As shown in Fig. 2, a buoy is installed at each of four corners of a rice field, and numbers of cysted metacercariae counted on each buoy are averaged. The period of stallation depends on the objective of study. For a detailed examination of cercarial emergence during the whole growing season of rice, the period covers about 3 months of a whole irrigation period, starting from immediately after transplanting of rice to the end of irrigation at about late August. During that period, the polyethylene sheet is replaced every 1 week. However, if the purpose is simply to know the occurrence and approximate number of cercariae, it is enough to install 4 buoys for a week from 10 to 20 August.

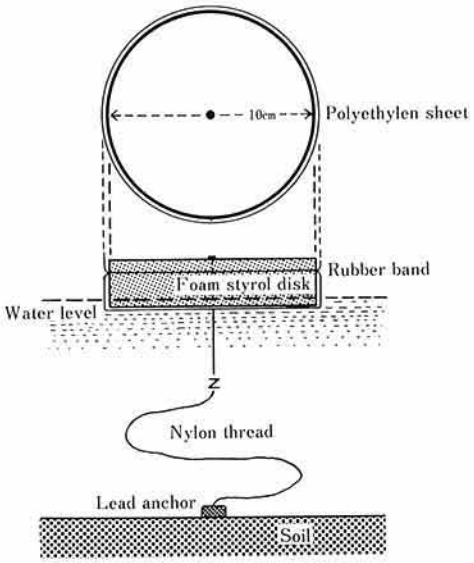

Fig. 1. Metacercariae detecting buoy floating on irrigation water of rice field

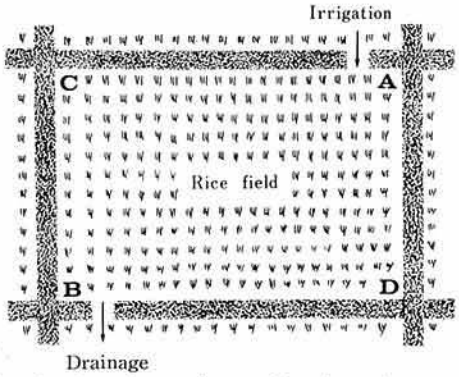

Fig. 2. Locations, A to D, of metacercaria detecting buoys installed in a plot of rice field

Fasciola metacercariae can be identified by naked eye, but if it is confusing with metacercariae of other kinds of trematoda parasites, a dissection microscope is used. Distinction between Fasciola metacercariae and metacercariae of stomach flukes which often encyst on the polyethylene sheet can easily be done according to Table 1.

Table 1. Distinction between Fasciola Metacercariae and Paramphistoma

Genus	Size	Color	Black spots	Outer cyst
Fasciola	Large	Light browh	Absent	Present
Paramphistoma	Small	Dark brown to black	Present	Absent

Examples of the application of MDB method

1) Seasonal pattern of emergence of Fasciola metacercariae

Result obtained in the flucky area of Gifu Prefecture in 1969 with 4 plots of rice field to which cattle manure was applied is given in Fig. 3. As illustrated by the histogram, Fasciola metacercariae was first detected on 27 July, 6–7 weeks after rice transplanting. The number of metacercariae increased and reached a peak 1–2 weeks later, and then began to decrease at middle August. The seasonal patterns at 4 fields under a similar condition were much alike, showing one-peak patterns, although the intensity was different each other.

An example obtained in two fields of Nara Prefecture in the same year as above is given in Fig. 4. Transplanting of rice was done

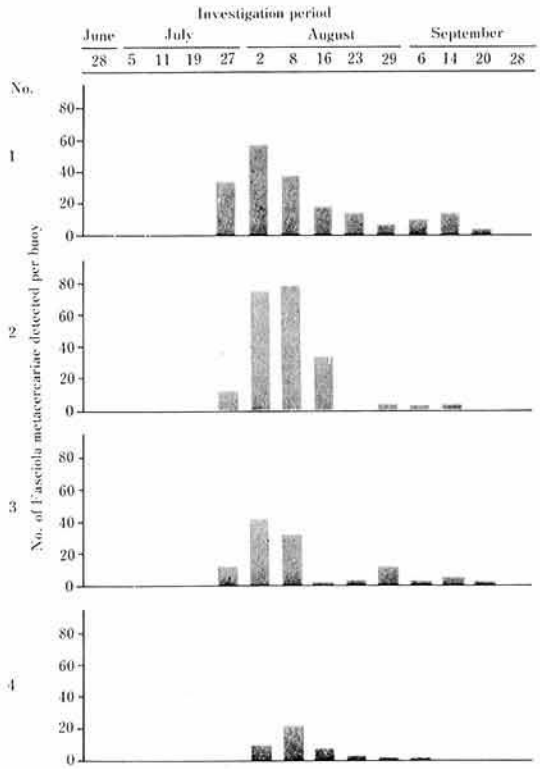

Fig. 3. Seasonal emergence of Fasciola cercariae in rice fields in suburbs of Gifu City

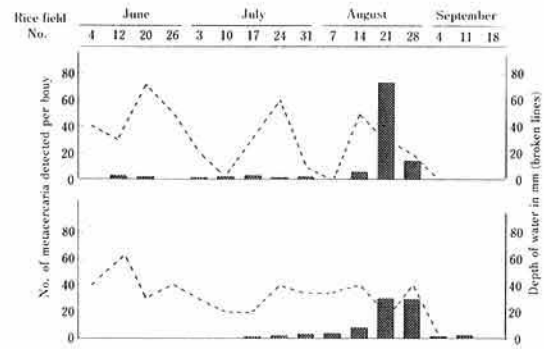

Fig. 4. Seasonal emergence of Fasciola cercariae in rice fields in Nara Prefecture

on 4 June in both fields, and metacercariae was detected 8 days after the transplanting in field No. 5, whereas it was 43 days after the transplanting in field No. 6. In spite of such a big difference the peak occurrence of metacercariae was found at the middle August in both fields.

2) Effect of cattle manure applied on the occurrence of Fasciola cercariae

In 1969, a study was carried out in Gifu Prefecture for a week from 5 to 12 August with 6 rice fields to which stable manure was applied before transplanting of rice and another 6 fields to which no stable manure was applied but fresh excreta was flowing in. As shown in Table 2, both groups of field were contaminated, but the fields adjacent to cattle stall were more contaminated than the other. Number of metaceriae by location of the buoy (A–D) was so variable that a definite trend was not recognized.

Recently in epidemiological studies of fascioliasis, the MDB method has become to be used in the fluky areas differing in geographical and climatic environment. Results

Table 2. Effect of cattle manure application on contamination of rice fields with Fasciole cercariae

a. Rice fields with cattle manure applied in the previous year

Rice field No.	Area in are	Fasciola metacercariae detected				
		Total number	Number at each location of buoy (%)			
			A	B	C	D
1	20	147	13.6	41.5	34.0	10.9
2	22	36	8.3	19.4	30.6	41.7
3	15	42	0	19.0	28.6	52.4
4	10	0	0	0	0	0
5	6	155	6.5	20.6	32.9	40.0
6	10	69	17.4	27.5	33.3	21.7

b. Rice fields adjascent to cattle pens, but without cattle manure applied

Rice field No.	Area in are	Fasciola metacercariae detected				
		Total number	Number at each location of buoy (%)			
			A	B	C	D
7	20	34	17.6	35.3	14.7	32.4
8	15	128	16.4	11.7	48.4	23.4
9	8	45	66.7	17.8	11.1	4.4
10	5	350	30.3	19.4	26.3	24.3
11	15	186	44.6	9.7	15.6	30.1
12	3	83	15.7	25.3	36.1	22.9

obtained so far are summarized as follows: Although the number of metacercariae detected in rice fields differs very much with different fields, the seasonal pattern of the occurrence is grouped into two types, A and B, as shown in Fig. 5. In the type A, small number of cercariae begins to emerge 1–2 weeks after rice transplanting and the number does not increase rapidly until the end of July when it reaches the peak. In the type B, time of cercarial emergence is 1–1.5 month later than that of type A, but within a short period, the number reaches its maximum, which occurs about 0.5 month later than that of type A. The cercariae which emerges soon after the transplanting is originated from hibernated snails infected with Fasciola and the peak of emergence in both types is undoubtedly caused by the emergence from yearling snails.

Cercarial emergence from the hibernated snails was recorded in temperate areas with scarce snow, whereas the emergence from only yearling snails was recorded in cold areas with harsh climatic conditions. As to the relative importance of hibernating snails and yearling snails, these results indicate that the infection of cattle with Fasciola coming from hibernated snails is almost negligible.

Discussion

The MDB method is proved to be very useful to determine fairly in detail the emergence of Fasciola cercariae in rice fields that has not been disclosed yet, although the method still remains to be improved.

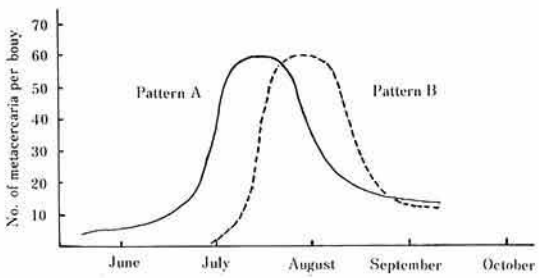

Fig. 5. Patterns of the emergence of Fasciola cercariae in rice fields

Time for beginning and end of contamination of rice plants with metacercariae and the intensity of the contamination may vary according to the distribution of *L. ollula*, an intermediate host of Fasciola, regional difference in rice growing season and other complicated factors. However, because the development of larval stages of Fasciola in an intermediate host snails and the emergence of cercariae as the final process is determined predominantly by temperature among many factors, data obtained at a given site may not show a great variation from year to year, unless a great change in the natural world occurs.

By applying this method to rice fields in the fluky areas it is made possible to examine not only the extent and intensity of the contamination but also factors related to the contamination of rice straw with Fasciola metacercariae. The final objective is to remove these factors but the method may give a useful data in determining the optimum time of applying molluscides.

References

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