Fascioliasis of Ruminants in Japan

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Fascioliasis is one of the most important parasitic disease of the domestic animals in Japan and the economical loss caused by this disease is tremendous. It is most commonly observed among cattle followed by sheep, and it is not rare to find it among pigs and horses.

Recognition of hexachloroethane as an anthelmintic for liver flukes arose public concern on the control of fasciola infestation and at the same time, activated studies on this disease in Japan. As the result of these studies, there have been remarkable improvements in its diagnosis, treatment and prophylaxis.

Liver fluke infestation

The rate of fasciola infestation among cattle in Japan is estimated to be around 20 to 30%. Heavily contaminated areas are scattered all over Japan. Generally speaking, however, the rate of infestation is high in southern districts with mild climate and low in northern districts with cold climate. Shown in the following table are the annual fasciola infestation rates among the animals slaughtered at the abattoirs in Tokyo and other districts.

Table 1. Fasciola infestation of cattle slaughtered in Tokyo abattoir

Fiscal Year	Number slaughtered	Fasciola infestation	Rate (%)	Remarks
1954	53,655	15,322	28.55	
1957	60,083	13,739	22.87	
1958	73,613	20,535	27.76	March excluded
1959	81,026	22,645	25.48	
1960	73,848	20,098	27.35	

Table 2. Fasciola infestation among the animals slaughtered in the abattoirs in Japan

		1954	1955	1956	1957	1958	1959
Cattle	a)	435,514	600,048	680,726	571,321	607,520	696,667
	b)	66,414	102,989	121,142	99,050	190,775	134,126
	c)	15.25%	17.16%	17.80%	17.34%	18.07%	19.25%
Calf	a)	79,624	210,456	191,774	145,438	167,107	181,051
	b)	791	1,846	1,413	702	1,091	1,052
	c)	0.99%	0.88%	0.74%	0.48%	0.65%	0.58%
Sheep	a)	23,631	22,464	33,876	57,792	67,035	114,712
	b)	1,445	528	896	1,979	2,930	5,600
	c)	6.11%	2.35%	2.64%	3.42%	4.37%	4.88%

Goat	a)	44,451	41,879	53.442	95,794	96,547	120,273
	b)	542	1,097	1,061	2,640	2,284	3,124
	c)	1.22%	2.62%	2.00%	2.76%	2.37%	2.60%
Pig	a)	1,432,603	1,659,592	2,149,675	2,673,142	3,130,720	3,562,160
	b)	134,510	73,221	160,375	13,296	10,240	11,119
	c)	9.39%	4.41%	7.46%	0.49%	0.33%	0.31%
Horse	a)	140,002	123,811	145,615	107,019	105,005	134,264
	b)	3,313	1,012	1,599	37	60	88
	c)	2.37%	0.82%	1.09%	0.034%	0.057%	0.06%

a): Total slaughtered, b): Numbers infested, c): Positive ratio.

Specific identification of Japanese common liver flukes

All the liver flukes recovered in Japan have hitherto been identified as *Fasciola hepatica* L., 1758, but they have larger bodies and ova than those of *F. hepatica*. In this connection. the present author has engaged in the morphological and ecological investigations of the Japanese common liver flukes for many years.

Ultimately, however, itwas indispensable to make comparisons with the known species of Fasciola, so that, the author requested and received the supply of the live ova of *F. hepatica* and *F. gigantica* from England and Africa. Together with Japanese species, the author succeeded in artificially growing the three kinds of ova up to the adult worms. The following are the findings of the morphological and ecological observations made in those experiments.

Aside from the flukes recovered from imported cattle, the infestation of liver flukes identifiable as *F. hepatica* does not exist in Japan and the infestation of *F. gigantica* is localized in some districts and small in number. While, the liver flukes prevalent in Japan belong to a form intermediate between the above mentioned two.

Following the reports by Lailliet (1895) and Looss (1896), discussions have frequently been made by numerous investigators on the presence of an intermediate or subspecies in the genus *Fasciola*. Based on the findings of the present investigation, the author supports the opinion to say that there exists an intermediate species and, probably, the author's intermediate species is the same as *F. indica* Varma, 1953.

Generally speaking, *Fasciola* sp. resembles to *F. gigantica* in the shapes of ova and adult worms, but it resembles to *F. hepatica* in the behavior of miracidia, prepatent period, pathogenicity, etc., and, moreover, its growth in rats is extremely poor. Ova and miracidia of *F. gigantica* have weak cold resistance and the larval stage in the final host is comparatively long showing active migration in the host, and produce marked lesions by their infestation.

Limnea ollula, which is one of the most important intermediate hosts to the liver flukes prevalent in Japan, fails to be an adequate intermediate host to *F. hepatica* and this fact must have been the main cause which is preventing the spread of this species in Japan.

Diagnosis

As measures for the diagnosis of fascioliasis, fecal examination to detect the presence of liver fluke ova and immunological intradermal reaction are most popular in Japan. As fecal examination, Watanabe's method is recommended for clinical use as it is simple in manipulation and has high detectively.

Watanabe's method

Apparatus: a) Beaker, b) Micropipet, c) Metal gauze, d) Siphon or sucker, e) Slide and cover glass.

Technique:

1. Collect 4 g of sample from several parts

Table 3. Identification of Genus *Fasciola*.

		F. hepatica Linnaeus, 1758	*F. gigantica* Cobbold, 1885	*Fasciola* sp. (syn. *F. indica* Varma, 1953 ?)
Measurements of eggs	Cattle, sheep	0.135×0.075 mm (0.120–0.145× 0.067–0.077)	0.166×0.090 mm (0.150–0.190× 0.078–0.100)	0.160×0.084 mm (0.140–0.190× 0.070–0.100)
	Rabbit	0.126×0.072 mm (0.117–0.133× 0.067–0.077)	0.165×0.087 mm (0.150–0.190× 0.087–0.100)	0.146×0.076 mm (0.138–0.154× 0.068–0.090)
	Phototaxis	+	+	+
	Perpendicular distribution in the medium	Superficial layer	Lower layer	Superficial layer
Hatching of eggs (days)	30°C 25°C 20°C 16.8°C	7 9—10 15 20	11—20 17 31	10 14 25 37
Limnea ollula as an intermediate host		Not so adequate	Adequate	Adequate
Prepatent period (days)	Rabbit Sheep, Goat	55—63 60—65	72—110 72—93	55—63 60—65
Adult	Length×breadth	2–3×0.8–1.3 cm	3.5–5.0×0.7–1.0 cm	3.0–5.0×1.2–1.4 cm
	Length : breadth	2.3 : 1	5.0 : 1	3.4 : 1
	General shape	Leaf-shaped	Elongated, side of the body roughly parallel	Intermediate between *F. hepatica* & *F. gigantica*
	Cuticular scales	Fine, elongated and sharply edged	Broader at the base, thicker but easily rent	Much larger, bluntly edged
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	Ovary	Oval branches few and simple (9—20)	Oval branches numerous (26—46)	Oval branches numerous (26—46)
	Testes	Profusely branched, occupy about ⅔ of the body length	Racemose and developed, occupying about half the total body length	Like *F. gigantica* but occupy less than half the total body length
	Intestine	Lateral and median diverticula few and simple	Both lateral and median branches numerous and racemose	Like *F. gigantica*
Distribution		Europe	Africa and Oriental countries	Oriental countries

of feces and suspend it in about 200 ml of water.

2. Filter the suspension through metal gauze of 80 to 100 mesh into another beaker Pour about 300 ml of water through the gauze to wash down filtrable materials.
3. Keep the filtrate still for 10 to 15 minutes so as to make solid matters settled.
4. Decant the supernatant by siphon or sucker, leaving 20 to 25 ml of sediment.
5. Shake up the beaker to make the suspension homogenous. Decline the beaker so that a half of the bottom of beaker appears. Then whitish-gray line will soon be formed on the bottom along the surface of the suspension.
6. Pipet about 0.1 ml of this whitish-gray substance by a micropipette and place it on a slide-glass.
7. Place a cover-glass over this specimen carefully so that it may not be squeezed out.

8. Eggs will be demonstrated mostly at the outer zone of the heavy materials in the center.

Intradermal reaction

Following the appearance of a commercial diagnostic antigen (Y. Ono, 1953) which had satisfied national assay, diagnosis by the intradermal injection of similar antigenic preparations has begun to be employed extensively. It is a boiled antigen prepared from the saline soluble fractions of liver fluke worms and 0.2ml of this preparation is injected intradermally into the skin at the caudal fold. Reading of the reaction is made 15—30 minutes after injection by the size of induration and swelling. Reactions with induration and swelling 15 mm and over in diameter are taken as positive and those below 10 mm without accompanying induration as negative.

Studies were also made for the improvement and purification of this diagnostic antigen. Masu and Tsubaki (1955) succeeded in the production of ammonium sulfate antigen.

M. Ono *et al.* (1954—1961) carried out basic studies on the polysaccharides and proteins derived from liver flukes as effective agents for eliciting the dermal reaction. As for proteins, they devised a special method for their elution, investigated their chemical characters, and succeeded in experimental demonstration of the role played by the free SH radical in producing the dermal reaction.

Ono's antigen and protein antigen are as follows:

Ono's antigen

1. Harvest complete whole bodies of matured liver fluke at a slaughter house.
2. Wash them well with running water while they are fresh.
3. Further, wash the same with sterilized physiological saline solution repeatedly (4—5 times).
4. Weigh them.
5. Homogenize the above with a homogenize to the finest possible extent.
6. Emulsify the above (A) with a physiological saline solution (B). Proportion of (A):(B) is to be 3:100.
7. Heat the above at a temperature of 100 °C for 30 minutes.
8. Exclude coagulated protein from the above, and use the remaining emulsion.
9. Add 1/10,000 or less ethyl-mercury-thiosalicylate to the above.

Protein antigen

1. Harvest matured liver flukes.
2. Wash them well with running water.
3. Further, wash them 4—5 times with sterilized physiological saline solution.
4. Weigh them.
5. Homogenize them with homogenizer as fine the particles as possible.
6. Emulsify the above with physiological saline solution to get a saline emulsion, 20% of which will be the previousely weighed liver fluke particles.
7. Centrifuge the above at 3,000 rpm for 20—30 minutes and take the limpid part.
8. Harfly saturate the above with ammonium sulfate.
9. Put it into a refrigerator for overnight in order to have precipitation of protein.
10. Harvest the protein sediment by means of centrifuge.
11. Suspend and disolve the above in saline solution.
12. Dialyze the above thru a cellophane membrane with running water for 3 days.
13. Repeat once again the same process of sedimentation with ammonium sulfate as in the process (8) above.
14. Dialyze the above with a cellophane membrane as in the process (12) above until the same shows negative reaction to the Nessler reagent, as to exclude ammonium sulfate completely from the above.
15. Dilute the above with phosphoric acid buffer saline solution of about 8.0 pH value to the extent of 10 times of the resultant sediment previously causedby the am- monium sulfate in the process No. 13 above.

This is equivalent to a emulsion which

contain 40% of liver fluke bodies, and its Nitrogen content is about 0.3 mg/ml.

320—640 times dilution of the above hydrous protein sediment precipitated by the effect of ammonium surfate as mentioned above has a protein density of 0.01 mg—0.02 mg/ml.

Thus you have completed manufacturing the antigen. Maintain it under a temperature of 4°C.

Treatment

Following the report on the effectiveness of hexachloroethane in the treatment of fascioliasis, this durg has begun to be used extensively as anthelmintic for bovine liver flukes. In Japan too, this drug has been in use since the year 1951. In early days, however, it caused the development of severe side reactions. Accordingly, efforts were made to eliminate these side effects and there have been a considerable improvement in moderating them, but the product is still for from satisfaction. Appearance of a better preparation is strongly desired.

In this connection, the author carried out screening tests on many kinds of bisphenol derivatives with an expectation of finding out a powerful and safe drug. As the result of these screening tests, a diphenyl sulfide compound, bithionol and a diphenylmethane compound known as G 11, were found to be effective in eradicating liver flukes. G 11 was quite powerful in eliminating liver flukes, but it was not satisfactory because of its side effects. As for bithionol, *per os* administration of 35 mg per kg body weight for cattle and a single dose of 75mg for sheep and goats could eliminate more than 80% of the adult liver flukes infested. Addition of this drug to the feed showed no difference in the appetite of cattle and its side effects were extremely mild in contrast to hexachloroethane. It, however, has an excellent effect in removing tape worms. In Japan, this product is on the market an anthelmintic for liver flukes and is used extensively in the field. Recently, bithionol was reported by M. Yokogawa and I. Miyazaki (1960) to have an excellent effect in the control of human paragonimiasis for which there had been no effective treatment.

As there are a number of compounds similar to diphenyl sulfide and diphenyl methane, further studies are in progress on the rabbits experimentally infested with liver flukes expecting to find out a compound still better than bithionol. Out of the 33 kinds of compounds examined up to date, Bis-(-2-hydroxy-3-nitro-5-chlorophenyl) sulfide and Bis-(2-hydroxy-3, 5-dichlorophenyl) sulfoxide were found to have a stronger antifasciola effect than bithionol with lesser side effects and field experiments are in progress on cattle.

Prophylaxis

As prophylactic measures for the infestation of liver flukes, following items are considered:

1. Disposition of fasciola ova excreted into feces.
2. Eradication of the intermediate host, *Limnea ollula*.
3. Prevention of the eating of metacercaria.

As most cattle are fed in doors in Japan, improvement of the nature heap storage, prevention of the purchase of rice straw and green grass from the contaminated districts, etc. are recommended for the items 1 and 3. But, it is extremely difficult to observe these recommendations perfectly. A measure which has been found to be quite efficient is the eradication of water snails by the use of a molluscacide.

Popularization of penta-chlor-phenol as a weeding chemical for rice paddies has done a great contribution in reducing the number of water snails as the dose required for weeding twice as much as that required for killing the snails.

Use of small ploughing machines in the place of cattle and popularization of anthelmintics have contributed in the control of fascioliasis which is declining year after year.

There are reports by M. Isoda (1957–1961) on the use of P. C. P. as molluscacides.

Experiments were carried out in the rice paddies of a certain heavily contaminated district in Kanagawa Prefecture covering a period of several years. Summarized in the following are the results obtained thereof.

1. Optimal season for the spray of P.C.P. is the later half of June, when the rice planting season is over. In this season, the largest number of water snails can be found and the snails are most susceptible to the drug.
2. Concentration of P. C. P. recommended is 10 ppm.
3. Snails reduced remarkably in number by the spray continued for three years.

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Germination Test Seeder for Brassica Crops

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In the germination test, a given number of seeds is counted and arranged on a germination bed. The present garmination test seeder reported herein can pick up a given number of seeds and arrrange them in a regular manner on a seed bed. This tool is handy and cheap. It was, at first, devised for *Cruciferous* crop seeds, but it can be applied for other kinds of seeds by changing the attachment.

Structures

The germination test seeder is made of two main parts, the home electric cleaner as a sucking apparatus and the attachment for placing seeds on a seed bed as shown in Fig. 1-a. Any of the commercial cleaners can be used for this purpose if its suction power is sufficient. The attachment for placing seeds is put into the hose of a cleaner. It consists of three important parts, A, B and C as in Fig. 1-b.

A is a hole of plastic tube bundled by a thin iron band. This part functions as a controller of the suction power by changing the hole size. B is a swich cap which operates to drop seeds stuck to C. The cap at B is closed when seeds are being picked up, but it is opened at the time of placing seeds. C is an adhesion vessel with the plate made of a hundred small perforations. It can be exchanged at D with others corresponding to the seed size of different crops. The diameter of this adhesion vessel C used herein is 8.5 cm which fits well to a Petri dish.