CONTRIBUTION TO THE KNOWLEDGE OF INFECTIVITY OF FASCIOLA HEPATICA ADOLESCARIAE AFTER THEIR PASSAGE THROUGH THE DIGESTIVE TRACT OF WHITE MICE

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Abstract. The study of infective elements of Fasciola hepatica which passed through the digestive tract of white mice revealed that those adolescariae (transitive adolescariae) (Table 1), which were not visibly damaged or possessed a more or less digested outer cyst wall, remained infective. The mice infected with transitive adolescariae harboured higher number of trematodes in the livers than those infected with infective elements which had not previously passed through the digestive tract of the definitive host.

During the experimental studies of mouse fasciollosis it was found that some of the adolescariae from the infective dose were released with the faeces to outer environment (Mitterpák 1973). This was supposed by Dawes (1962a) and Dawes and Hughes (1964) on the basis of the paper by Schumacher (1938) who reported that undamaged cysts of F. hepatica were present in the large intestine of guinea pigs four hours after infection.

The findings of transitive adolescariae in mouse faeces led us to the studies of their infectivity to various host species. The results obtained may contribute to the elucidation of some problems of species, infection process, propagation of this trematode and other questions. In Czechoslovakia, the experiments with transitive elements of parasites were performed by Bejšovec (1965).

MATERIAL AND METHODS

The eggs of F. hepatica were obtained from sexually mature trematodes originating from bovine liver. The eggs were cultivated in tubes wrapped in black paper and kept in a thermostat at the temperatures of 26–27°C for 10–11 days. The intermediate hosts (Galba truncatula) were collected in biotopes in nature. The snails were bred (according to Mitterpák) under artificial "semi-natural" conditions, i.e. on tables adapted to original places of occurrence. Young snails of the length not exceeding 5 mm were used for experimental infection. Each intermediate host received a dose of five miracidia in a Petri dish. Infected snails were transferred to new adapted places and after a period supposed to be sufficient for the development of cercariae (50–60 days) they were left in a dry place for six hours. Then they were again transferred to Petri dishes with water where they started to release cercariae after 10–15 minutes. The encystation took place after a short period directly in Petri dishes. The adolescariae were kept in a refrigerator at 5°C. Before the experiment some of them were tested for the viability using the digestive method of Wikerhauzer (1960).

Albino mice (Mus musculus SPF males, VEL.AZ Prague) and merino lambs (Ovis aries) were used as definitive hosts. Experimental infection was carried out after Mitterpák, putting a small ball of cellulose wadding containing a given number of infective elements of F. hepatica on the root of the tongue of the host. The faeces of mice were examined eight hours after the morning infection and then on the following days in the morning, at noon and in the evening. The excrements were moistened with a tepid water and after three hours they were examined stereoscopically or microscopically. The adolescariae were pipetted to watch-glasses with clear water and then applied for the infection of other hosts using the above-described method.
RESULTS

Two experiments were carried out in order to verify the viability of transitive adolescence. The aim of the first experiment was to obtain transitive adolescence, i.e. those which passed through the digestive tract of the definitive hosts. Ten mice were infected with 200 adolescence each on 20th October 1975. The infected hosts were kept together, only one control mouse was separated. After 24 hours, the faeces of infected mice contained 715 adolescence and 375 of them were apparently undamaged by digestion (Table 1). This number included also those in which no damage of the outer cyst wall was observed under light microscope. These adolescence were not transparent. Further 45 adolescence had a slightly digested outer cyst wall and were therefore visible. A relatively large number of adolescence (295) were destructed. The empty cysts were not studied.

Table 1. Findings of transitive adolescence in the faeces of albino mice after application of 200 infective elements per host

<table>
<thead>
<tr>
<th>Date of infection of mice</th>
<th>Infective dose of non-transitive adolescence</th>
<th>Date of examination of faeces</th>
<th>Findings of transitive adolescence in faeces of 10 mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Visibly undamaged</td>
</tr>
<tr>
<td>20 October 1975 8.00 h</td>
<td>2000 (200 per mouse)</td>
<td>20 October 1975 (18.00 h)</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 October 1975 (8.00 h)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 October 1975 (16.00 h)</td>
<td>85</td>
</tr>
</tbody>
</table>

Fifteen days after infection the mice started to lose weight and took only little food. After 18 days they had swollen abdomens and partly paralysed hind legs. The dissections were carried out starting from day 16 after infection. The highest number of parasites was found in the liver on days 16—18 after infection. After this time the liver was already cirrhotic, the abdominal cavity was filled with brown ascites and the number of trematodes in the liver gradually decreased. Other data are given in Table 2. The decrease in the number of Fasciola hepatica in strongly damaged liver was noted also by Dawes (1961, 1962 a, b, 1963 a, b) and other authors.

In the second experiment we studied the viability (infectibility and other development) of the adolescence which passed through the digestive tract of mice in the first experiment. The studies were carried out on 29th October 1975 using lambs protected against natural infection of F. hepatica and a control group of hosts. Three lambs were infected with visibly undigested adolescence, receiving 65 adolescence each. The fourth lamb served as a control. The results of this experiment are summarized in Table 3.

In order to follow the behaviour of transitive adolescence in further rodent hosts (which in this second experiment served as a control group in relation to the lambs), six mice (Nos. 12—17, Table 4) were fed with 225 transitive adolescence on the same
Table 2. Findings of trematodes in the liver of mice infected with non-transitive adolescraiae

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Date of infection</th>
<th>Infective dose</th>
<th>Date of dissection</th>
<th>Finding of trematodes</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>October 20, 1975</td>
<td>200</td>
<td>November 5, 1975</td>
<td>45  22.5</td>
<td>liver enlarged</td>
</tr>
<tr>
<td>2</td>
<td>October 20, 1975</td>
<td>200</td>
<td>November 6, 1975</td>
<td>19  9.5</td>
<td>liver enlarged</td>
</tr>
<tr>
<td>3</td>
<td>October 20, 1975</td>
<td>200</td>
<td>November 7, 1975</td>
<td>17  8.5</td>
<td>liver enlarged</td>
</tr>
<tr>
<td>4</td>
<td>October 20, 1975</td>
<td>200</td>
<td>November 12, 1975</td>
<td>5   2.5</td>
<td>cirrhosis of liver</td>
</tr>
<tr>
<td>5</td>
<td>October 20, 1975</td>
<td>200</td>
<td>November 13, 1975</td>
<td>4   2.0</td>
<td>cirrhosis of liver</td>
</tr>
<tr>
<td>6</td>
<td>October 20, 1975</td>
<td>200</td>
<td>November 14, 1975</td>
<td>3   1.5</td>
<td>cirrhosis of liver</td>
</tr>
<tr>
<td>7</td>
<td>October 20, 1975</td>
<td>200</td>
<td>November 17, 1975</td>
<td>2   1</td>
<td>cirrhosis of liver</td>
</tr>
<tr>
<td>8</td>
<td>October 20, 1975</td>
<td>200</td>
<td>November 24, 1975</td>
<td>0   0</td>
<td>death — disintegration of liver</td>
</tr>
<tr>
<td>9</td>
<td>October 20, 1975</td>
<td>200</td>
<td>November 24, 1975</td>
<td>0   0</td>
<td>death — disintegration of liver</td>
</tr>
<tr>
<td>10</td>
<td>October 20, 1975</td>
<td>200</td>
<td>November 24, 1975</td>
<td>0   0</td>
<td>death — disintegration of liver</td>
</tr>
<tr>
<td>11</td>
<td>control mouse</td>
<td></td>
<td>November 24, 1975</td>
<td>0   0</td>
<td>liver normal</td>
</tr>
</tbody>
</table>

Table 3. Findings of trematodes in the liver of lambs infected with transitive adolescraiae

<table>
<thead>
<tr>
<th>Lamb No.</th>
<th>Date of infection</th>
<th>Infective dose</th>
<th>Date of dissection</th>
<th>Finding of trematodes</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>October 29, 1975</td>
<td>65</td>
<td>December 10, 1975</td>
<td>32  49</td>
<td>liver enlarged—haemorrhage</td>
</tr>
<tr>
<td>2</td>
<td>October 29, 1975</td>
<td>65</td>
<td>December 10, 1975</td>
<td>31  47</td>
<td>liver enlarged—haemorrhage</td>
</tr>
<tr>
<td>3</td>
<td>October 29, 1975</td>
<td>65</td>
<td>December 10, 1975</td>
<td>28  40</td>
<td>liver enlarged—haemorrhage</td>
</tr>
<tr>
<td>4</td>
<td>control lamb</td>
<td>0</td>
<td>December 10, 1975</td>
<td>0   0</td>
<td>liver normal</td>
</tr>
</tbody>
</table>

Table 4. Findings of trematodes in the liver of albino mice infected with transitive adolescraiae

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Date of infection</th>
<th>Infective dose</th>
<th>Date of dissection</th>
<th>Finding of trematodes</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>October 29, 1975</td>
<td>36</td>
<td>November 18, 1975</td>
<td>18  50</td>
<td>liver enlarged</td>
</tr>
<tr>
<td>13</td>
<td>October 29, 1975</td>
<td>36</td>
<td>November 19, 1975</td>
<td>13  36</td>
<td>liver enlarged</td>
</tr>
<tr>
<td>14</td>
<td>October 29, 1975</td>
<td>36</td>
<td>November 21, 1975</td>
<td>8   22.2</td>
<td>cirrhosis of liver</td>
</tr>
<tr>
<td>15</td>
<td>October 29, 1975</td>
<td>36</td>
<td>November 24, 1975</td>
<td>5   13.8</td>
<td>cirrhosis of liver</td>
</tr>
<tr>
<td>16</td>
<td>October 29, 1975</td>
<td>36</td>
<td>November 25, 1975</td>
<td>5   13.8</td>
<td>cirrhosis of liver</td>
</tr>
<tr>
<td>17</td>
<td>October 29, 1975</td>
<td>45</td>
<td>November 26, 1975</td>
<td>2   4.4</td>
<td>total cirrhosis of liver</td>
</tr>
<tr>
<td>18</td>
<td>control mouse</td>
<td>0</td>
<td>November 28, 1975</td>
<td>0   0</td>
<td>liver normal</td>
</tr>
</tbody>
</table>
day (29th October 1975). Five of them (Nos. 12—16) received 36 visibly undigested 
adolescariæ each, whereas the sixth one (No. 17) was infected with 45 adolescariæ 
with more or less digested outer cyst wall. The seventh mouse (No. 18) served as 
a control. The results are given in Table 4.

The following conclusions can be drawn from our studies:
1) The cysts of *F. hepatica*, which had passed through the digestive tract of mice 
(Nos. 1—10, Tables 1 and 2) and were released from the host (per anum) without any 
visible damage or with only more or less digested outer cyst wall contained viable 
adolescariæ. Their infectivity and further development were experimentally verified in 
lambs (Nos. 1—3, Table 3) and in the control group of mice (Nos. 12—17, Table 4). 
2) The mice infected with transitive adolescariæ (Nos. 12—17) contained higher 
percentage of trematodes in the liver than those infected with elements which had not 
passed through the digestive tract of the definitive host (Nos. 1—10). For example, the 
mouse infected with 200 non-transitive adolescariæ harboured 17 trematodes, i.e., 
8.5% of the infective dose on 18th day after infection, whereas the mouse infected 
with 36 transitive adolescariæ harboured 18 trematodes, i.e., 50% of the infective 
dose, on 20th day after infection.
3) The course of fasciolosis in mice was almost the same in both experiments. The 
difference seems to be due to smaller number of trematodes in rodent liver in the second 
experiment (compare Tables 2 and 4).

**DISCUSSION**

The attention paid to the parasite-host relationship between *F. hepatica* and the 
range of its hosts is proportional to the damage caused by this infective disease. A brief 
survey of the occurrence of fasciolosis starting from the first half of the sixties was 
published by Dawes and Hughes (1964). The present state of research was outlined in 
the papers read in 1968 at the International Symposium on Fasciolosis in Warszaw 
and at the Third International Congress of Parasitology in Munich in 1974. These 
papers were published in Wiadomości parazytologiczne No. 5/6, 1968 and in Proceedings 
The trend of the parasitological research, which followed from the reports read at this 
Congress, was mentioned by Piekarski (1974). This paper dealt also with the questions 
of fasciolosis and we assume therefore that our results need not be confronted with those 
known from the literature (cf. also Odening 1971).

The purpose of our experiments was to complement the known data about the fasci- 
olosis. They were intended to show whether the genotypical and phenotypical structure 
of helminths can be determined on the basis of the helminth-host relations. The species 
*F. hepatica* served as a model for a wider investigation of intraspecific relations of 
parasitic worms. The results obtained suggest that the methods used in the solution 
of this problem may also serve for the study of the distribution of this trematode which 
can be performed, to some extent, by the dissemination of transitive adolescariæ by 
the definitive hosts. After the experimental verification of this phenomenon there 
remains to study this way of *F. hepatica* distribution in the nature.

It was also found that the transitive infective elements of fasciolosis caused higher 
infestation in mice than those which did not come into contact with their digestive tract. 
This phenomenon should be verified, since the doses of adolescariæ used for the infection 
of mice were not identical in both experiments.

It was not possible to ascertain whether the transitive adolescariæ cause higher
infection also in sheep, since these hosts were not infected by non-transitive adolescariae. According to the literature (Montgomerie 1928, Schumacher 1938, Kendall and Parfitt 1962, Sinclair 1962, Hughes 1963, Furmagas and Gundlach 1966), approximately 30—40% infection was obtained in sheep fed with non-transitive infective elements of F. hepatica. In our experiments with transitive adolescariae the percentage was somewhat higher, 45% on the average (Table 3). On the other hand, Boray (1967) obtained up to 46.84% of mean infection in sheep infected with 200—1,000 adolescariae, the range of infection being 20.5—76.5%. Considering that our methods (cultivation of adolescariae, their storage and collection from liver etc.) were not as specialized as those used by Boray (1967), it may be assumed that the transitive adolescariae caused higher infection also in sheep in our experiments. If this increased infectivity is confirmed by other experiments, this fact will be important not only from the epizootological view, but also in the study of microevolution of this species.

Since the problem of viability of transitive infective elements of helminths deserves more attention, it is dealt with elsewhere, both from the theoretical and practical views.

Acknowledgement. The authors wish to thank Dr. M. Breza, C.Sc. for his criticism and comments.

К ИЗУЧЕНИЮ ИНВАЗИОННОСТИ АДОЛЕСКАРИЙ FASCIOLA HEPATICA ПРОШЕДШИХ ПИЩЕВАРИТЕЛЬНЫМ ТРАКТОМ БЕЛЫХ МЫШЕЙ

И. Машко и И. Басанда

Резюме. При изучении инвазионных элементов Faschiola hepatica пропишедших пищеварительным трактом белых мышей было обнаружено, что ацолескарии (табл. 1) без видимых повреждений или с более или менее поврежденной переварением внешней оболочкой были способны инвазии. В печени мышей зараженных этими ацолескариями было найдено большее количество трематод, чем у мышей зараженных инвазионными элементами, которые не были в контакте с пищеварительным трактом дефинитивного хозяина.

REFERENCES


—, The migration of juvenile forms of Faschiola hepatica L. through the wall of the intestines in the mouse, with some observations on food and feeding. Parasitology 53: 109—122, 1963 a. —, Some observations of Faschiola hepatica L. during feeding operations in the hepatic parenchyma of the mouse, with notes on the nature of liver damage in this host. Parasitology 53: 135—143, 1963 b.


MONTGOMERIE F. R., Observations on artificial infestation of sheep with Fasciola hepatica and on a phase in the development of the parasite. J. Helminthol. 6: 167—174, 1928.

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