FOLIA PARASITOLOGICA (PRAHA) 15: 341 – 349, 1968.

# The Development in the Egg of *Fascioloides magna* (Bassi, 1875) and the Duration of the Ovulation Cycle

B. ERHARDOVÁ-KOTRLÁ

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague

**Abstract.** Our studies on the embryogony of eggs from the trematode *Fascioloides magna* revealed that eggs from trematodes living for more than 22, 29 and 34 months in their hosts are greatly reduced in their capability of development. For example from eggs of trematodes living for more than 22 months in their hosts, 4.5% of miracidia emerged after 53 days; from trematodes living for more than 29 months in the host, 7.4% of miracidia emerged after 51 days. Over half of the eggs died without further development. The remaining eggs developed up to certain stages (the morula stage, the anlage of the miracidium) and then started to degenerate gradually. The development of eggs from these old trematodes was very protracted (more than 50 days) in comparison with the 14—20 days of normal embryogony. This reduced developmental capability was also observed in young trematodes the eggs of which had been used 7 months after the experimental infection. In this instance 12.6% of miracidia emerged from the egg shells after 39 days and one half of the eggs were dead.

Our results about the effect of temperature changes on the shortening or prolonging of egg development in the embryogony of the trematode *F. magna* were published in previous papers (ERHARDOVÁ 1960, 1961). While attempting to produce in a very short time large numbers of miracidia for artificial infections of snails we found that even under the optimal temperature of +28 °C we could often not achieve a massive emergence of the miracidia from the egg shells. Frequently this process was very protracted, the eggs developed at irregular intervals and hatching continued over a long period although the experiments were performed under the same well-tried method, at the same temperature and pH. Even certain changes described under Material and Methods did not accelerate the emergence of the miracidia. Therefore it became necessary to pay more attention to the problem of embryogony.

## MATERIAL AND METHODS

Repeatedly washed eggs recovered from the uterus of trematodes or directly from the faeces of the deer (*Cervus elaphus*) were kept in preboiled water in glass dishes in the incubator at a temperature

of +28 °C. The samples were frequently aerated and regularly examined under the microscope. At the optimal temperature of +28 °C the morula stage started as soon as on the 6th—7th day and the first miracidia were observed in the eggs 8—10 days from the start of the experiment. The whole development ending with the emergence of the miracidia from the egg shells lasted 14 days. This was found to be the shortest possible time for the emergence of the miracidia from the egg shells.

In some cultures, however, no emergence of the miracidia from the eggs occurred even after 30 days and, therefore, the water had to be repeatedly changed or aired. Only then did the miracidia start to emerge from the egg shells. Hatching of the miracidia was also achieved by placing snails into these dishes which ingested the eggs with the developed miracidia. These eggs not only passed undamaged the intestinal tract of the snail, but the activity of the miracidia was greatly increased by this passage and they started to emerge from the egg shells. This activation was also demonstrated on miracidia lying motionless inside the eggs. After ingestion by the snails and the passage through their intestinal tract, the majority of miracidia emerged spontaneously from the egg shells. Sometimes, when almost all egg shells were empty having released their miracidia, we still found some eggs in which the miracidia had only started to develop in spite of all these changes. The hatching was protracted over a long period, sometimes beginning as late as three months after the ontset of the experiment. Miracidia which had not managed to emerge from the egg shells during the fourth month, decayed inside the eggs. These findings suggested the necessity of further studies.

## RESULTS

1. Our investigations were started with the following experiment: eggs, recovered from a closed cavity in the liver of a deer *(Cervus elaphus)* were kept in cultures at a temperature of +28 °C from Nov. 20, 1961. 15 days later miracidia were found in some of the eggs. Hatching started on Dec. 21 and continued until Jan. 13, 1962. A month later, on Feb. 17, new miracidia still swam in the cultures and during examination on March 7, 5% of eggs still contained motile miracidia while on March 21, all miracidia in the eggs were dead.

We repeated these experiments with eggs recovered from infected deer and found this phenomenon to occur mainly in cultures with eggs recovered from the cavity. In these cavities the eggs are usually cemented into large, hard formations; sometimes, many eggs filled with a minute amount of dispersed vitellar substance are dead; sometimes they are normally developed and capable of further development. The balls consisting of millions of dead eggs are dark-brown to black in colour, hard and not crumbling.

These findings suggested that the eggs might stay for over a differently long period in the liver before being washed out and that there may be, in some way, a certain influence of the age of the egg on the loss of its capability for further development.

2. The results of the following experiments led to similar conclusions. We had to produce masses of miracidia to infect snails, because we needed infective larvae for the definitive hosts. Therefore, eggs were recovered from two fallow deer *(Dama dama)* infected $5^1/_2$ month previously. Since there was no difficulty to obtain these animals they seemed most suitable for our experiments. But, contrary to our

assumptions, no miracidia emerged from the egg shells after a fortnight and there were only undeveloped miracidia in the eggs. Although these experiments were repeated several times, the results were always the same.

3. In these experiments we used two Virginian deer *(Odocoileus virginianus)* infected about 22 months previously. There was no possibility of reinfection and still, eggs were continuously present in the faeces of these animals. Although these experiments were performed in exactly the same way as described earlier in this paper, neither the passage through the snails, the aeration of the water in the dishes nor several repetitions of the experiment started egg development.

4. In these experiments we used three different types of eggs:

a) eggs of trematodes recovered from the faeces of deer *(Cervus elaphus)* in nature. Because these animals are constantly exposed to reinfection, the trematodes recovered from their faeces were of different age (we did not identify their exact age) but, having achieved good results with these samples in our previous experiments, we used this type of eggs for control.

b) eggs from experimentally infected fallow deer *(Dama dama)*. These animals were infected 7 months before the start of our experiment and the first eggs were recovered from the faeces $4^1/_2$ months later. This means that the eggs used in our experiments were laid by the young trematodes shortly after the ontset of ovulation.

c) eggs recovered from the faeces of two Virginian deer *(Odocoileus virginianus)* of approximately $1^1/_2$ years of age. Since, at that time, eggs were present in their faeces the infection must have occurred in nature. In these animals we traced the ovulation dynamics of egg production of the trematodes and excluded any possibility of reinfection by keeping them in separate boxes. At the time when these two Virginian deer were used in our experiment for investigating the influence of the age of the trematodes on the capability of egg development, they were under control for 22 months. During this time eggs were found constantly in their faeces.

The development of the eggs in the separate cultures was completely different. In the cultures the samples no. 1 (i.e. eggs recovered from the faeces of deer in nature), development started on the 4th day; on the 6th day the germinal cells in more than half of the eggs had moved to the centre and formed the anlage of the embryo and, in some eggs, even the outline of the future miracidium became visible. In the following 10 days miracidia developed in 10% of eggs and on the 13th day, when motile miracidia were present in the egg shells of 40% of eggs, they started to emerge. Freely swimming miracidia were in the dishes and 10% of egg shells were empty. In the other two cultures the eggs showed no sign of development during the first 10 days. In samples no. 3 (eggs from the artificially infected fallow deer) cleavage started as late as on the 13th day and that only in 10% of eggs. After 20 days the development of the eggs continued in the following way: in samples no. 1, developed miracidia were present in 42.4 % of eggs and 18.2 % of miracidia had left the egg shells; in samples no. 2 the eggs just started to show signs of development and that only in 3.8 % of eggs, which were at the morula stage at this time; in samples no. 3, 15.8 % of eggs had reached the morula stage.

In the following 4 days, almost half of the miracidia had left the egg shells in samples no. 1, while in samples no. 2 only 16 % of eggs were at the morula stage. In samples no. 3 the percentage of developed eggs increased, in 10.5 % of eggs the germinal cells had moved to the centre of the egg and formed the anlage of the embryo.

One month later (i.e. 28 days from the start of the experiment) the first miracidia occurred in samples no. 3, this being 21 days behind samples no. 1. In the following days development was very slow particularly in samples no. 3. After 39 days the first miracidia emerged from the eggs samples no. 3, being 25 days behind the development of eggs in samples no. 1. The first miracidia appeared in samples no. 2 as late as on the 53rd day.

The results indicate that the whole process of embryogony is influenced by the duration of the ovulation cycle. The development was fastest in eggs isolated from the faeces of the infected deer. Miracidia started to emerge as early as on the 13th day and hatching reached its peak on the 24th day. Since the animals were constantly reinfected on the infested sites, the trematodes recovered from their faeces were at different developmental stages. This may be the explanation of the fact that even after 24 days we still found eggs with no signs of development while, on the other hand, we found eggs, with germinal cells accumulated in their centre. After 30 days the percentage of dead eggs increased because these eggs had not started to develop during this time, but there were also dead eggs in which embryogony had started and in which miracidia were formed. These eggs seemed to have been laid by trematodes which had been living for a long time in the liver of their host or by very young trematodes, which had only started to ovulate. These assumptions are supported by the results of our other experiments.

The slowest development occurred in the eggs recovered from the Virginian deer *(Odocoileus virginianus)*. The first miracidia started to emerge from the egg shells after 53 days and a high percentage of these eggs did not start to develop and decayed gradually. At this time we found 41.3 % of dead eggs. This may indicate that these eggs were laid by old trematodes, in which ovulation was still in progress. However, most of these eggs were too degenerate to start development. A similar situation seemed to have occurred in the very young trematodes, in which embryogony had not started in a high percentage of eggs. As shown in Tab. 1, the death rate of eggs was 51 %. Hatching started on the 39th day and the whole process of embryogony was greatly protracted.

The experiments were repeated 7 months after the outset of the first experiment, hence $9^1/_2$ months following the start of ovulation in trematodes from the infected fallow deer. The trematodes recovered from the Virginian deer were 7 months older.

We observed the following development. In trematode eggs from the fallow deer, the cumulation of the germinal cells in the centre of the eggs and the formation of the embryonic anlage occurred in 30 % of eggs at the end of 6 days. 16 days later miracidia were present in 9.8 % of eggs, which started to emerge from the egg shells after 23 days, which is almost half the time observed in the experiment performed 7 months earlier. In the remaining samples embryogony was slower.

**Table 1.** Development of eggs from trematodes laid 2.5 months after the ontset of ovulation (samples no. 3—*Dama dama*) and 22 months after it (samples no. 2, *O. virginianus*). Results in per cents. Control with eggs from *C. elaphus* (samples no. 1).—Start of experiment: June 6.

| Date of control | Developmental stage of eggs | Samples no. 1 *(C. elaphus)* | Samples no. 2 *(O. virginianus)* | Samples no. 3 *(D. dama)* |
|---|---|---|---|---|
| 10. 6. | without develop. | 12.9 | 88.3 | 82.4 |
| | morula | 51.8 | — | — |
| | cumulation of cells | 22.5 | — | — |
| | dead | 12.9 | 11.7 | 17.6 |
| 13. 6. | without develop. | 4.0 | 83.1 | 87.2 |
| | morula | 20.9 | — | — |
| | cumulation of cells | 62.5 | — | — |
| | first sign of mirac. | 5.9 | — | — |
| | dead | 6.9 | 16.9 | 13.0 |
| 17. 6. | without develop. | 12.1 | 74.2 | 72.2 |
| | morula | 25.6 | — | — |
| | cumulation of cells | 44.0 | — | — |
| | miracidia | 10.8 | — | — |
| | dead | 7.3 | 25.7 | 28.0 |
| 20. 6. | without develop. | 7.5 | 84.2 | 59.9 |
| | morula | 15.0 | — | 10.8 |
| | cumulation of cells | 25.0 | — | — |
| | miracidia | 40.0 | — | — |
| | dead | 2.0 | 15.8 | 28.5 |
| | empty shells | 10.0 | | |
| 26. 6. | without develop. | 6.3 | 71.5 | 59.9 |
| | morula | 7.1 | 3.8 | 15.8 |
| | cumulation of cells | 19.3 | | |
| | miracidia | 42.4 | — | — |
| | empty | 18.2 | | |
| | dead | 6.3 | 23.7 | 23.2 |
| 30. 6. | without develop. | 6.2 | 68.2 | 37.9 |
| | morula | 2.5 | 16.0 | 25.3 |
| | cumulation of cells | 6.2 | — | 10.5 |
| | miracidia | 33.3 | — | — |
| | empty | 45.5 | — | — |
| | dead | 6.2 | 15.8 | 26.3 |
| 8. 7. | without develop. | 11.6 | 52.5 | 5.1 |
| | morula | 16.1 | 28.9 | 53.5 |
| | cumulation of cells | 9.2 | 2.0 | — |
| | miracidia | 11.6 | — | 23.2 |
| | empty | 34.8 | | |
| | dead | 16.1 | 16.6 | 18.3 |
| 15. 7. | without develop. | 9.0 | 62.6 | 9.0 |
| | morula | 12.1 | 24.0 | 15.1 |
| | cumulation of cells | 6.0 | 6.2 | 15.1 |
| | miracidia | 21.2 | — | 20.4 |
| | empty | 40.4 | — | 12.6 |
| | dead | 12.1 | 7.2 | 27.8 |
| 20. 7. | without develop. | — | 30.0 | 6.6 |
| | morula | — | 16.6 | 20.5 |
| | cumulation of cells | 5.2 | 13.3 | 13.9 |
| | miracidia | 21.9 | 13.3 | 8.3 |
| | empty | 52.6 | — | 23.4 |
| | dead | 21.9 | 26.6 | 27.3 |
| 29. 7. | without develop. | | 27.2 | 6.8 |
| | morula | | 13.5 | — |
| | cumulation of cells | | 9.0 | 7.9 |
| | miracidia | | 4.5 | 6.8 |
| | empty | | 4.5 | 27.4 |
| | dead | | 41.3 | 51.1 |

In samples with the faeces of red deer, variations were observed in the development of the eggs. In 5.5 % of eggs the first signs of miracidia became conceivable on the 9th day, on the 16th day miracidia were present in 21.6 % of eggs. In the first experiment, by this time almost half of the miracidia had left their egg shells. Hatching started on the 23rd day. In eggs recovered from the faeces of Virginian deer, cleavage started only three days later than in the other samples and reached its peak on the 12th day, but after this day further development was arrested and we found more eggs with a disturbed vitellar substance. A month later, in a few eggs the embryonic substance started to move to the centre of the egg and 5 days later miracidia were found in 4.8% of eggs. 51 days later very few miracidia were observed to swim freely in the culture. This indicates that more than one half of eggs had died before reaching a certain developmental stage. Although development continued in the remaining eggs, miracidia were formed only in a few of them and also very few miracidia emerged from the egg shells. For results see Tab. 2.

There remained one more question to be solved; whether there were some living trematodes present in the liver of one of the two infected Virginian deer at the end of 34 months, because even at this time we recovered continuously a few eggs from the faeces of this animal. However, these eggs showed no sign of development when placed in the incubator. There is the possibility that living trematodes were no longer present in the liver and that these eggs may have been washed out by chance from an accumulation of eggs in the liver cavity. Because the structure of these eggs was disturbed, they were not capable of further development. This assumption, however, was contradicted by the results of the post mortem of the killed Virginian deer. In its liver we found two sexually mature trematodes measuring 56—63 by 32—34 mm. No more trematodes or cysts containing eggs were present. Hence it follows that these eggs with a greatly reduced developmental capability must have been laid by old trematodes.

## DISCUSSION

The results derived from our observations may be also of practical importance because there is little indication that the phenomenon had occurred by chance. However, the question about the origin of these observed variations in the development is still unsolved. There are several possibilities:

1. Could the restriction or direct arrest of development in the eggs be caused by the specific differentiation of the host animals?

The following reasons, however, contradict this train of thought: *Odocoileus virginianus* and *Cervus canadensis* are the original hosts of this trematode species and by them this infection was distributed to other animals. Therefore, their trematodes or the eggs of these trematodes should be capable of maximum development. In spite of that in our experiments only a few eggs developed in these original hosts; contrary to that we obtained a maximum number of miracidia from

**Table 2.** Experiments repeated in the same hosts after 7 months. Control samples recovered from faeces of infected deer in nature. Start of experiment: Jan. 18

| Date of control | Developmental stage of eggs | Samples no. 1 *(C. elaphus)* | Samples no. 2 *(O. virginianus)* | Samples no. 3 *(D. dama)* |
|---|---|---|---|---|
| 24. 1. | without develop. | 47.3 | 100 | 21.6 |
| | morula | 52.6 | — | 35.2 |
| | cumulation of cells | — | — | 30.0 |
| | miracidia | — | — | — |
| | dead | — | — | 13.0 |
| 27. 1. | without develop. | 6.6 | 77.7 | 15.0 |
| | morula | 26.6 | 16.6 | 40.0 |
| | cumulation of cells | 46.6 | — | 25.5 |
| | miracidia | 14.0 | — | 5.5 |
| | dead | 6.6 | 5.5 | 15.0 |
| 30. 1. | without develop. | 5.2 | 33.3 | 4.7 |
| | morula | 21.0 | 66.6 | 40.0 |
| | cumulation of cells | 47.3 | — | 38.5 |
| | miracidia | 16.0 | — | 6.3 |
| | dead | 10.5 | — | 10.5 |
| 3. 2. | without develop. | 17.2 | 48.4 | 7.3 |
| | morula | 17.2 | 36.3 | 36.6 |
| | cumulation of cells | 27.5 | — | 39.0 |
| | miracidia | 21.6 | — | 9,8 |
| | dead | 17.2 | 15.1 | 15.1 |
| 10. 2. | without develop. | 4.8 | 18.7 | 9.6 |
| | morula | 4.8 | 75.5 | 26.9 |
| | cumulation of cells | 28.5 | — | 42.2 |
| | miracidia | 28.5 | — | 7.6 |
| | empty | 18.9 | — | 7.6 |
| | dead | 14.4 | 6.2 | 9.5 |
| 15. 2. | without develop. | 11.6 | 18.7 | 19.3 |
| | morula | 2.3 | 53.1 | 17.5 |
| | comulation of cells | 11.6 | 15.6 | 21.0 |
| | miracidia | 16.2 | — | 21.0 |
| | empty | 48.8 | — | 5.0 |
| | dead | 9.3 | 12.5 | 15.7 |
| 20. 2. | without develop. | 2.1 | 12.0 | 4.2 |
| | morula | 4.3 | 32.0 | 12.6 |
| | cumulation | 10.8 | 28.0 | 25.5 |
| | of cells miracidia | 13.0 | 4.8 | 27.6 |
| | empty | 63.0 | — | 19.1 |
| | dead | 6.5 | 24.0 | 10.6 |
| 28. 2. | without develop. | — | 19.1 | 12.6 |
| | morula | 4.9 | 17.0 | 17.6 |
| | miracidia | 8.1 | 7.4 | 17.6 |
| | empty | 80.4 | — | 27.6 |
| | dead | 4.9 | 56.5 | 24.6 |
| 10. 3. | without develop. | — | 12.4 | — |
| | morula | — | 3.6 | 8.3 |
| | miracidia | 4.7 | — | 8.3 |
| | empty | 79.3 | 7.4 | 50.0 |
| | dead | 12.6 | 76.6 | 33.3 |

trematodes parasitic in *Cervus elaphus*. This is in fact a new host and the trematodes had to become adapted to the new conditions of Europe.

2. Could the developmental capabilities of the egg be influenced by the new conditions in the Old World?

The fact that a high percentage of trematode eggs from *Cervus elaphus* (40 %) developed in the shortest possible time (within the first 14—20 days, although development continued even in the following month) suggests this assumption to be incorrect. According to various writers, in North America, the original homeland of this trematode, the development in the egg lasts under laboratory conditions 29 days (SWALES 1935), 33—44 days (SINITSIN 1934) and 42 days (WU and KINGSCOTE 1953). None of these reports contained data about the temperature.

The results of egg development in the species *Dama dama*, to which this trematode had to become adapted in Europe, show that the eggs from young trematodes laid 2 $^1/_2$ months after the ontset of ovulation died in numbers exceeding one half of the total output, while in older trematodes 33 % of the eggs died 9 $^1/_2$ months after the start of ovulation.

Concluding we may suggest that in both experimental hosts living together with *Cervus elaphus* in the Old World, the duration of embryogony depends on the age of the trematode and also that this age plays an important role in the development of the egg.

## CONCLUSIONS

Our experiments with the trematode species *Fascioloides magna* show that not only egg masses accumulated in the cavities become incapable of further development, but also the eggs of trematodes, which had ovulated for a long time (22, 29 and even 34 months). Young trematodes, which started to ovulate soon after reaching sexual maturity, produce eggs with a restricted capability of development.

In our opinion this phenomenon should be confirmed on other trematode species to establish the reliability of the data given by various writers who calculated from an incidental number of eggs the production of parasitic embryos in relation to the length of their life, thus indicating innumerable possibilities for new infections. (For example WEILAND and BRAND 1926 estimate that a single trematode of the species *Fasciola hepatica* produces during its life [4—11 years] about two million eggs; according to PANOVA 1951, this number of eggs is produced by a single trematode in two weeks.) It still remains to be clarified how many of these eggs are capable of further development and how many are incapable of development or develop only up to the earliest stages.