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Notes on the Hatchability and Infectivity of Refrigerated Eggs of Fasciola hepatica Linn

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NOTES ON THE HATCHABILITY AND INFECTIVITY OF REFRIGERATED EGGS OF *FASCIOLA HEPATICA* LINN.

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The problem of the longevity of trematode eggs, particularly of those which have been kept for varying periods of time at low temperatures to retard development is, apparently, one which has not been investigated to any great extent. It is, nevertheless, admitted that literature on such a subject is difficult to find and it may be far more extensive than the following survey would indicate.

Thomas (1883) stated that when eggs of *Fasciola hepatica* were kept at an average temperature of 16°C., the miracidia developed in 2 or 3 months; under winter conditions no development took place. Ross and McKay (1929) kept eggs of *F. hepatica* at a temperature of 6° to 11°C. for 5 months and at this temperature no noticeable development took place; however, the eggs later developed and hatched normally when kept at room temperature.

Shaw (1931) stated that fluke eggs are capable of lying dormant, even under circumstances which, apparently, are favorable for hatching. Furthermore, he stated that eggs have been known to hatch over a period of 13 months.

The writer, in a somewhat more extensive experiment with *F. hepatica* eggs, has obtained results which are in accordance with those of the above-mentioned writers. In this experiment it is shown that eggs of *F. hepatica* may be kept for long periods of time in a refrigerator, after which they are viable and hatch normally, and the liberated miracidia are infective. Furthermore, it is shown by experiments that the duration of the intramolluscan phase of development may vary greatly in a single host species as well as in different species, temperature being, apparently, one of the important controlling factors.

Temperature as related to hatching may have a bearing on control measures for the fluke, at least in certain localities.

The eggs of *F. hepatica* used in these experiments were collected in New Orleans, Louisiana, April 15, 1931, and were received April 20, 1931. After the water was changed on the eggs, the

bottles containing them were recorked and transferred to an electric refrigerator regulated to maintain a temperature of approximately 2° to 10°C. On October 27, 1933, after a refrigeration period of 2 years, 6 months and 7 days, the eggs were removed from the refrigerator. Examination of the eggs at the time of removal showed that they were still undeveloped. The eggs were then kept at room temperature and in 18 days they began to hatch in large numbers. Hatching continued for approximately 14 days, the miracidia in the majority of the eggs having escaped during that time. The failure of the eggs to develop at the refrigerator temperature is in accordance with the results of Ross and MacKay (1929).

INFECTING SNAILS WITH MIRACIDIA FROM REFRIGERATED EGGS

Some of the miracidia which hatched from the refrigerated eggs were used in infecting snails, *Pseudosuccinea columella*, which had already been shown by Krull (1933) to serve as hosts for *F. hepatica*. The snails were laboratory-raised and descendents of a stock which had produced a number of generations during the several years that this stock had been kept in the laboratory.

Experiment 1. A number of immature snails were subjected to the attack of many miracidia by transferring the latter to the snail habitat with a pipette on November 14, 1933, the water temperature being 74°F. The first cysts were observed in the aquarium 38 days later; many more cercariae were shed by these snails subsequently.

Experiment 2. A number of large snails, most of them mature, were subjected to attack by miracidia November 26, 1933, the water temperature being 75°F. The first cercariae were shed 66 days later and many cercariae were shed subsequently.

Another lot of eggs collected at New Orleans, Louisiana, March 23, 1932, and kept in the same refrigerator for 1 year, 7 months, and 4 days, gave practically the same results as those described above. The eggs began to hatch 18 days after they were removed from the refrigerator. A number of immature snails, *Pseudosuccinea columella*, began to shed cercariae 42 days after being subjected to infection. A number of mature snails of the same species were also exposed to infection and one of these began to shed cercariae in 78 days.

It will be noted from the above discussion that when immature and mature snails were infected and kept under practically identical conditions, twice as much time was required between the time of subjection to infection and production of cercariae in mature

snails as in immature snails. Repeated infection experiments with *Fasciola hepatica* as well as with *Fascioloides magna*, during all seasons of the year and using the above species of snail, indicate that the temperature of the water and the age of the snails are factors which influence the subsequent development of the infections in the snail. There may be still other factors operative in the intramolluscan development of trematodes which may explain some of the peculiarities of development which have been observed by the writer; however, data are not available at present to warrant further conclusions.

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