MASTER

RESEARCH NOTE

"A SIMPLE METHOD OF MASS PRODUCTION OF FASCIOLA HEPATICA MIRACIDIA"

As part of a current study on the immunogenic effect of irradiated metacercariae in rats, and its potential use in cattle and other species, a simple and inexpensive method was devised for mass production of fluke miracidia used for infecting large number of snails for metacercariae production.

Fasciola hepatica eggs may be obtained at the slaughterhouse from the gall-bladder of cattle with fascioliasis. The gall-bladders are slit open and the egg containing bile collected in a gallon beaker. The gallon beaker is filled with tap water and allowed to stand for 15 minutes during which time the fluke-eggs and bile debris settle to the bottom. The supernatant then is carefully decanted so as to save the bottom half-inch. These steps are repeated until the supernatant fluid appears clear.

The eggs, with some liver debris, are resuspended in glass-distilled water and filtered through a 100-gauge (149 M) sieve. The "clean" eggs are then spread on a 21 cm. finger bowl which, covered with aluminum foil, is placed in a refrigerator at 5°- 6°C. The eggs are viable for a year in this state, but it is advisable to use them within the first sixth months.

To induce embryonation, transfer some eggs to sterile Erlenmayer flasks (125 ml.), add about 125 cc of glass distilled water, shake gently, and let stand for 10 to 15 minutes. The supernatant fluid is then siphoned off until about 50cc are left in the flasks. An antibiotic-antifungal solution is added so that the final concentration per ml. of the water contains penicillin 5×10^4 units, streptomycin 5×10^4 Mcg. and amphoterecin B "Fungizone" 5×10^2 Mcg.

Cap the flask with aluminum foil; punch pin-holes in the foil-cap. Wrap each flask completely in carbon-copy paper so as to exclude light while not making it air-tight and place upright under a lighted 60W. electric bulb set about 12 to 14 inches above the surface upon which the flasks are placed. This arrangement will raise the temperature of the water in the flasks to about 30°C.

Gently shake the flasks daily to prevent clumping of the eggs. On the eighth or ninth day, the miracidia are well developed inside the eggs and are ready to hatch. At such time, remove the flasks to a drawer or a box where they should be kept at room temperature. Shake the flasks carefully thrice a week. When miracidia are needed, hatching can be stimulated by transferring embryonated eggs to a watch glass with some dechlorinated tap water. Within 5 to 10 minutes mass hatching of the embryonated eggs follows.

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In our experience, the embryonated eggs have lasted more than 105 days in this condition. During such time, the antibiotic-antifungal solution prevented the growth of bacteria and fungi in the water, and the clumping of eggs. Eggs refrigerated at 5°C. for 5 months, subsequently treated with the antibiotic-antifungal solution and incubated, released a higher number of miracidia (53%), than the control eggs (9%). The miracidia from the treated eggs had 100% ability to penetrate Lymnaea cubensis.

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