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STUDIES ON PARAMPHISTOMIASIS. I. THE PROPAGATION OF *BULINUS TROPICUS* KRAUSS 1848

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INTRODUCTION

Paramphistomiasis of cattle and sheep is becoming a disease of prime economic importance to the animal industry of South Africa and, therefore, there has been inititated an intensive research programme upon the results of which it is hoped to be able to prescribe sound and rational prophylactic and therapeutic measures.

The common amphistome parasites have been found to be *Paramphistomum microbothrium* Fischoeder 1901 and *Calicophoron calicophorum* (Fischoeder 1901) Näsmark 1937, identified and redescribed by Swart (1954). The intermediate host of these trematodes in South Africa is *Bulinus tropicus*.

The first step in any research programme on paramphistomes is the development of a routine technique, whereby large numbers of the intermediate host can be maintained and propagated, so that they are continuously available to produce the large number of infective metacercariae required to set up the clinical disease in domestic animals.

It is the object of this report to describe the technique which has been evolved and which is in routine use today. The method is based upon that used successfully by Dr. J. C. Boray for the propagation of *Lymnaea tomentosa* (Pfeiffer, 1855), the intermediate host of *Fasciola hepatica* Linnaeus 1758, at the McMaster Laboratories, C.S.I.R.O., Sydney, Australia. The underlying principle is to propagate the snails in shallow water aquaria simulating their natural habitat. The whole layout is characterized by great simplicity.

MATERIALS AND METHODS

The snail colonies are housed in a comparatively small room, 15 ft. by 12 ft., the essential requirements being a constant supply of water, adequate drainage facilities and a generous number of electrical points. Provision has been made for heating the room by thermostatically controlled electrical tubular heaters, so that in the winter months the temperature will not drop below 25°C. No provision has been made for controlled reduction of temperatures during the summer months nor has this been found to be necessary.

A plastic head tank (capacity 15·0 gallons) is placed against the one wall above the level of the aquaria to supply ordinary tap water at constant pressure to all the aquaria. In the absence of a ball valve the rate of flow of water into the tank is so adjusted that it is always slightly greater than the outflow; the surplus goes to waste through a by-pass (cf. B, C, D, Fig. 1). The rate of flow of water to all the aquaria is adjustable by means of thumb screws attached to the plastic tubing.

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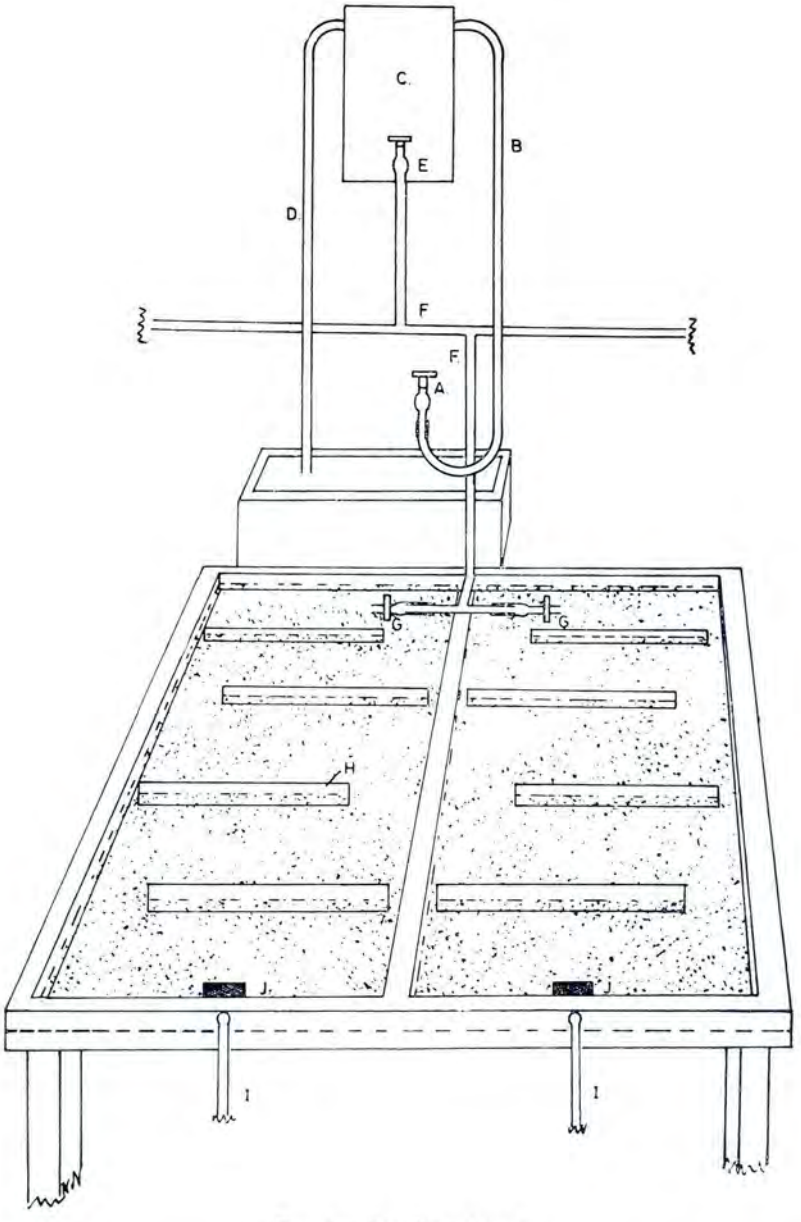

FIG. 1.—*Breeding Aquaria*

A. Water tap.
B. Inlet pipe.
C. Plastic reservoir tank.
D. Overflow pipe.
E. Plastic tap.
F. Plastic tubing with T joints.
G. Screw clips.
H. Plastic strips.
I. Drainage pipes.
J. Fibre glass gauze traps.

A. *The snail or breeding aquaria*

A table in the centre of the room is used. The table top, 8 ft by 4 ft, is converted into two rectangular compartments by nailing strips of wooden planks to the sides, ends and down the centre to project 3 inches above the table level. Each compartment is converted into a water-tight shallow tray by covering it with a single sheet of " Polyvinyl " plastic sheeting. An outlet for the water is provided by fixing a tube, inserted through the " Polyvinyl " sheet (with any suitable cement such as " Pliobond ") at a point $1\frac{1}{2}$ inches above the table top, distal to the water inlet, to maintain the level of the water at a constant depth of $1\frac{1}{2}$ inches. To prevent escape of any snails, or invasion up the outgoing pipe, the water outlet is guarded by a cup of fibreglass or nylon gauze (1 mm mesh). The water outlet is connected to the main drain. Water is supplied from the steady head tank, the rate of flow being about 100 ml per minute, which has been found adequate to flush out the aquarium as well as to replace any loss of water by evaporation.

The floor of the trays is covered by about half an inch clean, coarse river sand banked up round the edges to a level about 1 inch above the surface of the water. The flow of water is interrupted by a series of perspex strips about 18 inches long and 2 inches wide which are pressed on edge, in zig-zag fashion, into the sand so as to project 1 inch above the level of the water.

To provide the light necessary to stimulate the growth of algae necessary for food for the snails it has been found adequate to install four tubular mercury vapour lamps (two " day " and two " night " tubes) of the type in common use in any office or laboratory. Should supplementary feeding be necessary the provision of lettuce leaves, either fresh, dried or powdered, has been found satisfactory.

Breeding snails.—The colony was started with 28 *B. tropicus* collected in the field.

Fortunately the snails do not lay their egg masses on the sand. Banking the sand round the sides to a level above that of the surface of the water prevents oviposition on the " Polyvinyl " sheet, from where the eggs could be collected only with some difficulty. The snails deposit their eggs in yellowish gelatinous packets at or just below the surface of the water, chiefly on the perspex strips, but also on the shells of other snails and on lettuce leaves. On an average each egg packet contains 17 eggs. The eggs start hatching in about seven days time; these snails in turn will start laying eggs from the age of 14 days.

The snail colony multiplied so rapidly that within a period of six months more than 3,500 were counted, by which time it was found necessary to eliminate surplus snails to reduce the number to about 50 adult and 100 young snails per square foot which is the density, found quite empirically, at which they thrive best.

In actual present practice the perspex strips are removed once every seven days, and all the eggs deposited on them carefully scraped off, to be used in other experiments. In spite of this weekly removal of some of the eggs the numbers that hatch before the end of the next seven day period, together with those deposited on other objects from which they were not removed, are adequate to maintain the required density for breeding.

B. *Small experimental aquaria*

The walls of the room are fitted with shelving, divided into cubicles 2 ft by 2 ft by 2 ft. Each cubicle is provided with a light socket on its ceiling. A roller blind hung across the front of each cubicle prevents the light from any one cell interfering with the lighting of its neighbours.

Plastic trays 20 inches by 18 inches by 4 inches, fitted with a plastic water exit pipe $1\frac{1}{2}$ inches from the bottom of the tray, lined either with coarse sand or fibreglass gauze, are used. Water is admitted to the tray at the rate of a few drops a minute to maintain a constant depth, the excess water flowing via the outlet pipe to the drain. Escape of any snails is prevented by covering the opening of the exit pipe with 1 mm mesh fibreglass gauze or with nylon.

To encourage an accurate maximum hatchability two methods were devised.

(*a*) *Partial immersion.*—The breeding unit is a plastic cylinder, about 4 inches in diameter by 2 inches high, closed at the bottom by a fine nylon gauze, of a mesh sufficiently small to prevent the escape of any snails or eggs. This cylinder rests on plastic supports, so that the gauze bottom is just below water surface. Snail eggs placed in these containers hatch readily and can be handled without difficulty.

(*b*) *Aeration method.*—Sand placed in a tray is moulded by hand to form a crater about 12 inches in diameter with a $1\frac{1}{2}$ inch flat rim at water level. An aquarium aerating block, attached by plastic tubing to an air pump, is placed at the bottom of the centre of the crater. A circular piece of fibreglass gauze (1 mm mesh) is placed over the crater resting on the rim so that its upper surface is flush with the water. The snail eggs are spread evenly over the gauze. The flow of air through the aerating block is so adjusted that it bubbles very gently causing the gauze to rise and fall in a sort of pulsating movement, whereby the eggs are alternately submerged and exposed to air.

This aeration has proved to be the more uniformly successful of the two methods.

Discussion

The report by the World Health Organization (1957) states that *Bulinus* spp. thrive at a pH varying over a wide range from 4·8 to 9·8. The pH of the tap water used in this work was constant at 7·5.

Boray (personal communication) used deionised water with added minerals and trace elements for breeding *L. tomentosa*. Standen (1951) and Claugher (1960) advocate allowing the water to mature, i.e. to stand for some time, before use. Neither procedure was found necessary, but it is believed that it is important to allow the water that enters to drip in so as to obtain maximum aeration, as also to provide a source of light, so as to stimulate the multiplication of the algae.

In addition to *B. tropicus* the following snails have been bred successfully:—

Bulinus forskalii Ehrenberg 1931,

Biomphalaria pfeifferi Krauss 1848,

Lymnaea natalensis Krauss 1848.

At one stage *Tubifex* spp., collected in the field, were introduced into the snail aquaria as a means of removing snail excreta. Apparently *Physa mocambiquensis* eggs were introduced with the *Tubifex* accidently, for shortly afterwards this snail appeared and started to multiply at an alarming rate. It was found necessary to replace all tubing and waterproof sheeting, to sterilize fresh sand and to remove all adherent egg masses from the shells of the *B. tropicus* snails retained for breeding, before this unwanted snail was eliminated. From time to time *P. mocambiquensis* has been found in the drainage pipes attempting to migrate back into the aquaria, hence the necessity of guarding all exit pipes with fine mesh nylon or fibreglass gauze is emphasized.

It need hardly be pointed out that care must be taken to insulate all electric wires and to waterproof electrical connection wherever possible since the atmosphere is continuously saturated with water vapour.

Summary

1. A system for the mass propagation of *B. tropicus* in shallow water aquaria is described.

2. A system of maintaining numbers of small shallow water aquaria suitable for varied experimental purposes is outlined.

3. Both systems are inexpensive and characterized by simplicity of design, construction and maintenance.

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