THE USE OF ANTIBIOTICS AS AN AID IN REARING LARVAE OF *CULEX TARSALIS* COQ.

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INTRODUCTION. One of the most frustrating problems in rearing mosquito larvae, whether involving specimens brought in from the field or in laboratory colonies, is the formation of a scum or pellicle on the water surface. The problem becomes especially acute when larval nutrients are added to the water. The scum, if not removed at regular intervals has a deleterious effect on larval development. If this scum is permitted to develop unchecked, it is almost always associated with very high larval mortality, particularly if one is attempting to rear adults from eggs or early larval instars. In laboratory colonies the usual attempts to control scum development are by aeration, by changing the water, or by "scumming," a procedure in which scum is dipped from the rearing pans with a small screen, brush or a highly absorbent paper. These methods are rarely completely effective. Aeration is often impractical if large numbers of rearing pans are used. Changing the water and scumming are not only very time consuming, but may aid in the spread of contaminants.

The exact nature of the effects of the scum on mosquito larvae is not known. We have assumed that the scum was largely formed by micro-organisms which under the influence of optimum conditions in the laboratory, favorable temperatures, and large amounts of organic food sources had produced large blooms on the surface and throughout the water and inner surfaces as well. The exact causes of larval mortality which are associated with surface scum need investigation. Many workers believe the surface scum or pellicle interferes with larval breathing, particularly in the early instars. If this is so we need to know how laboratory scums differ from the very heavy scums which occur in the field on water from which apparently healthy larvae often are taken. Heavy concentrations of bacteria could result in an accumulation of toxic waste products that may account for mortality in mosquito larvae. Gram negative bacilli (see below) are known to produce powerful endotoxins. It was not the purpose of this study, however, to determine the actual causes of mortality in mosquito larvae during rearing. We were concerned with the possibility of achieving beneficial effects in rearing by controlling the development of the surface scum. We first ran cultures from numerous samples of surface scum. The scum proved to be primarily bacterial in origin, predominately gram negative bacilli. A few colonies of yeast and other fungi also appeared on the plates. In addition a few protozoan and algal forms were present in the scum. No attempt was made to identify the bacterial species or other organisms present. We then attempted to control the surface scum with various common medical antibiotics.

LITERATURE. The literature on mosquito culture techniques is voluminous. Excellent bibliographies on the subject are contained in publications by Horsfall (1955), Trembley (1955) and in the "References to Literature" which appears in each issue of *Mosquito News*.

Williams (1953) used terramycin hydrochloride and a growth factor containing streptomycin and terramycin to determine the growth stimulating effects of food supplements and antibiotics on the development of *Aedes aegypti* larvae. He made no reference to the effects of these antibiotics on contamination caused by microorganisms. We are not aware of any previous work in which antibiotics have been used during rearing of mos-

quito larvae to control the surface scum produced by bacteria or other microorganisms.

METHODS AND MATERIALS. All tests were made in white enamelware (porcelain) pans, 11½" x 7" x 2". One thousand (1,000) ml. of distilled water was constantly maintained in each pan during the experiments. All pans were placed in a water bath. The same series of tests were carried out at two different temperatures, 22° C. and 27° C. with the temperature controlled within the limits of ±2.0° C. for each. For each series of tests 500 first instar larvae of *Culex tarsalis* Coq. were added to each pan. Pure preparations of six antibiotics were used, achromycin, aureomycin, chloromycetin, penicillin, streptomycin and terramycin. The effectiveness of each was tested by adding dosages of 4 micrograms/ml., 10 micrograms/ml., 50 micrograms/ml., 100 micrograms/ml. and 200 micrograms/ml. to separate pans. The concentration of 200 micrograms/ml. was chosen as the maximum concentration as preliminary tests had revealed that this amount of all of the antibiotics produced virtually complete bacterial control in pans containing larvae without nutrients added. One control pan without antibiotic added was used for each series of tests. The surface scum in the control pan was controlled by skimming with a small screen. In the initial series of experiments it was observed that the bacterial scum was suppressed in the test pans for a period of about 5 days and then began to reform unless additional antibiotic was added. Therefore, in all subsequent tests the original dosage of antibiotic was added again to each pan every fifth day. This effectively prevented the characteristic surface scum from appearing in any of the test pans.

To insure larval development a food supplement was necessary. The food used during these experiments was Purina Mouse Chow. This was added to each pan in the amounts of either 0.1 g., 0.5 g. or 1.0 g. at 2–3 day intervals.

All tests were repeated five times.

RESULTS AND DISCUSSION. Within a week after the tests were begun the pans containing Purina Mouse Chow in amounts of 0.5 g. and 1.0 g. regardless of antibiotic concentrations became heavily contaminated with bacteria and/or fungi. Heavy scum formed on the surface, the water became cloudy and a very heavy larval mortality occurred. It was determined that 0.1 g. supplied an adequate amount of food if added every 3 days to pans containing first and second instar larvae and every 2 days when larvae were in the third and fourth instars. At these concentrations of food only the control pans developed a heavy scum.

All of the antibiotics at concentrations of 50, 100 and 200 micrograms/ml. gave very unsatisfactory results at the temperatures tested. In every pan at these concentrations a very heavy infestation of fungi appeared within 3–4 days, and this was followed by a 90 percent–100 percent larval mortality. Cultures of these pans revealed very low bacterial counts. It thus appeared that fungi were not only unaffected by the antibiotics used but were actually favored by the removal of bacteria in the culture pans. Figure 1 shows the concentrations of terramycin and larval mortality at 27° C. compared with the control. The other antibiotics did not show significantly different results.

All of the antibiotics, except penicillin and streptomycin gave most effective results at 10 micrograms/ml. and 27° C. ± 2.0°. At this concentration and temperature no surface scum appeared, and a good harvest of pupae was obtained. See Fig. 2.) The antibiotics, achromycin, aureomycin, chloromycetin and terramycin gave remarkably similar results throughout all of the tests. Streptomycin was about as effective as the control where only scumming was used, and penicillin gave inferior results to the control. All of the pans with concentrations of antibiotics at 4 micrograms/ml. developed a very slight surface scum at both temperatures. In no

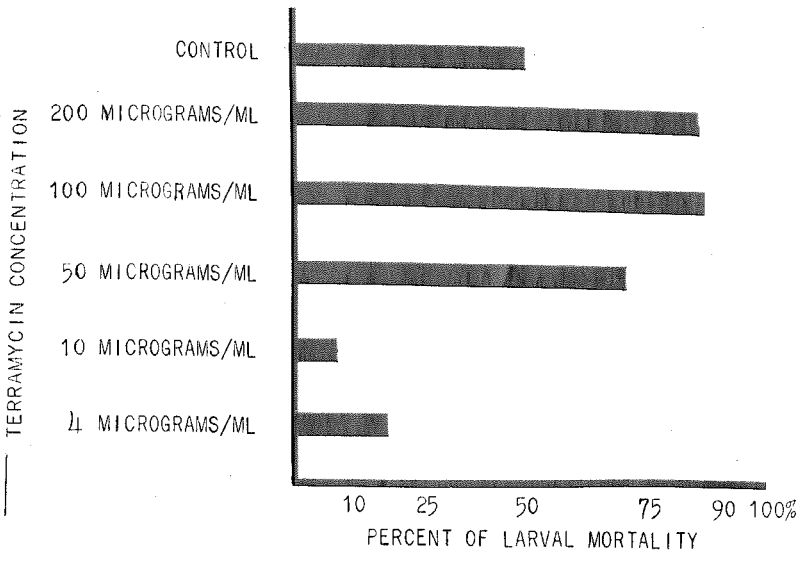

FIG. 1.—Larval mortality at 27° C. ±2.0 in test pans containing terramycin compared with the control.

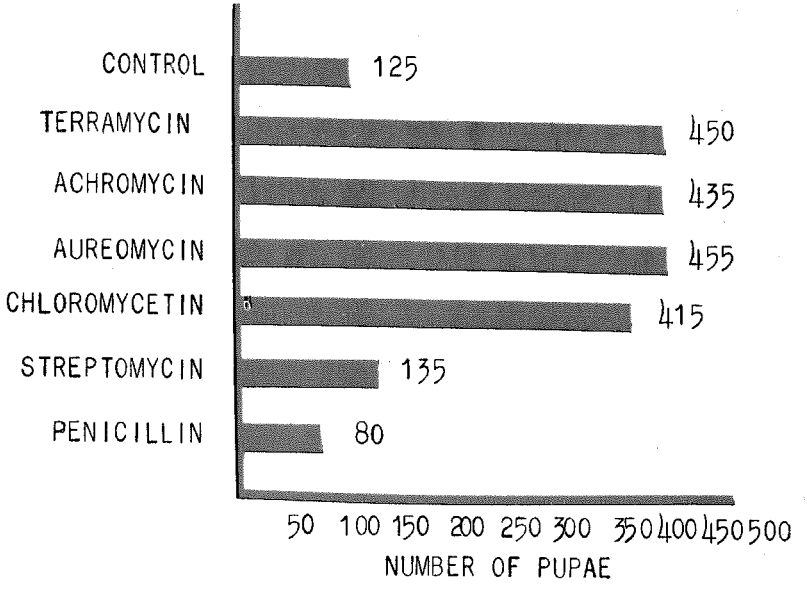

FIG. 2.—Number of pupae harvested from 500 first instar larvae in control pans and in test pans containing various antibiotics at concentrations of 10 micrograms/ml. at 27° C. ±2.0. Totals represent averages of all tests.

instance was it as heavy as the scum which appeared in the control pans.

The greatest difference in the rate of larval development between the test and control pans occurred at 27° C. At the lower temperature, 20° C., the period required from first instar to the pupal stage averaged 12 days in both test and control pans, with a range of from 10 to 14 days. At 27° C. this period required an average of 7 days in the test pans, with a range of 6½ to 8 days; in the control pan the average was 9 days, with a range of 8½ to 10½ days. It is interesting to note that although larval mortality rates varied with the concentration of antibiotic the rate of development did not appear to be influenced significantly.

No adverse effect on the pupae obtained during this study was noted. Emergence of normal adults occurred without delay.

All of the antibiotics tested appeared to be strongly algacidal. In some of our control pans heavy algal contamination occurred, and a high larval mortality resulted. None of the test pans contained algal growths during the study. Our attempts to infect pans containing antibiotics with algae were unsuccessful even at concentrations of 4 micrograms/ml.

An interesting result of this study was the conspicuous increase in the size of the larvae which developed in the test pans. It is well known that antibiotics added to feed of domestic animals can stimulate growth. Williams (*op. cit.*) by using antibiotics as food supplements, was able to produce larvae of *Aedes aegypti* that were 4–13 percent larger than the largest larvae normally occurring in nature. Most of the larvae which we reared in the six antibiotics used in this study were at least 10 percent larger than those reared in the control pans; some showed a size increase of as much as 17 percent.

SUMMARY. 1. Six antibiotics, achromycin, aureomycin, chloromycetin, penicillin, streptomycin and terramycin were tested as a means of controlling the surface scum or pellicle which often is the cause of high larval mortality during rearing. Various concentrations of each antibiotic were used in pans containing larvae of *Culex tarsalis* Coq. Tests were run at temperatures of 22° C. and 27° C. Purina Mouse Chow was added as the food supplement.

2. Cultures were run on numerous samples of surface scum obtained during larval rearing; gram negative bacilli were the predominant organisms present in these samples.

3. All antibiotics were effective in controlling surface scum. Achromycin, aureomycin, chloromycetin and terramycin gave remarkably similar results in all of the tests. Best results with these antibiotics were obtained at 27° C. ± 2.0 at concentrations of 10 micrograms/ml. with 0.1 g. of Purina Mouse Chow added every 2–3 days as a food supplement. Under these conditions the pupal harvest generally exceeded 85 percent. Pupal harvest in the control pan and in the test pans containing penicillin and streptomycin did not exceed 30 percent.

4. Best results with the Purina Mouse Chow food supplement were obtained by adding 0.1 g. at 2–3 day intervals. Concentrations of 0.5 g. and 1.0 g. regardless of antibiotic concentration became heavily contaminated with bacteria and/or fungi, and high larval mortality resulted.

5. All of the antibiotics at concentrations of 50 micrograms/ml. and higher gave very unsatisfactory results at the temperatures tested due to the appearance of abnormally high populations of fungi. Low numbers of bacteria appeared to favor fungus growth.

6. At 27° C. ±2.0 development from first instar larvae to pupae in pans containing antibiotic averaged 7 days; in the control pans 9 days. The same development required an average of 12 days in both tests and control pans at 22° C. ±2.0.

7. All of the antibiotics were strongly algacidal at all of the concentrations tested. This would seem to preclude the use of

antibiotics in rearing larvae of species requiring algae as food.

8. Larvae reared in pans containing antibiotics were significantly larger than those in the control pans; most showed a size increase of 10–17 percent.

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THE EFFECT OF TEMPERATURE ON HATCHING OF EGGS OF THE MOSQUITO, *CULEX PIPIENS QUINQUEFASCIATUS* SAY.[1]

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INTRODUCTION. According to Bates (1949), culicine mosquito eggs laid in rafts normally hatch as soon as the embryo is fully developed. Unlike the eggs of *Aedes* species there is no special stimulus required for hatching, and it has been generally understood that development of the embryo and hatching are a direct function of temperature. It appears that Kirkpatrick (1925) and Boissezon (1930) are the only workers who have reported on studies of the duration of the egg stage of *Culex pipiens*. Kirkpatrick (1925) found that eggs of the Egyptian form of the species would hatch at 10.5° C. with an incubation period of 216 hours. He further reported hatching at the following temperatures and respective incubation periods: 13° C., 144 hours; 18.5° C., 72 hours; 21° C., 36 hours; 28° C., 32 hours; 30.5° C., 26 hours; and 34° C., 21 hours. Embryos survived 34° C. but were killed at 35° C. Boissezon (1930) obtained essentially the same results with one of the French forms when he found at 10° C. incubation required 216–264 hours, at 15° C. incubation required 48–72 hours, and at 20°–25° C. about 24 hours were required.

The main objective of this study was to determine whether the hatching time of the eggs of the mosquito, *Culex pipiens quinquefasciatus* Say is directly related to the temperature of the water upon which they are placed.

MATERIALS AND METHODS. A stock of *Culex pipiens quinquefasciatus* Say was established from five egg rafts from a colony which had been maintained in the Department of Entomology, University of Maryland for about 7 months. Mosquitoes in this colony descended from the colony at the Walter Reed Army Institute of Research which has been identified as the Malayan strain. The stock colony was reared in a cage heated with a Westinghouse heating pad (Model WP-29) controlled by a hermetically sealed Chromalox thermostat (Model WR-66). The cage measured 18 x 24 x 31 inches. The humidity was maintained at a fairly constant level by placing an enamel pan measuring 7½ x 12 x 2 inches containing five cellu-

[1] Condensation of a thesis submitted by the first author to the Faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Master of Science in 1963. Scientific Article No. A1106, Contribution No. 3548 of the Maryland Agricultural Experiment Station, Department of Entomology.

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