# ABILITY OF SOME MOSQUITOES TO TRANSMIT *DIROFILARIA IMMITIS* IN FLORIDA[1]

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ABSTRACT. In order to obtain infective third stage larvae of *Dirofilaria immitis* Leidy with which to produce experimental infections in dogs, the following mosquitoes were used: *Aedes taeniorhynchus* Wiedemann, *Culex quinquefasciatus* Wiedemann, *Anopheles quadrimaculatus* Say, and *Aedes aegypti* Linnaeus (three strains). Standard rearing procedures were used unless otherwise noted. An extremely high rate of mosquito mortality occurred.

Control groups of mosquitoes fed on guinea pigs were found to survive for 20–25 days while those blooded on an infected dog experienced 95–97 percent mortality. The majority of the deaths occurred within the first 3 days after the blood meal. Results from all species of mosquitoes used were similar except for the *A. quadrimaculatus* groups in which a few females lived long enough (10–17 days after blooding) to allow development of infective larvae.

INTRODUCTION. A prerequisite for the production of experimental infections of *Dirofilaria immitis* Leidy in dogs is the passage of the parasite larvae through mosquitoes to the $L_3$ or infective stage. Complete larval development of *D. immitis* has been reported by various authors in over 50 species including: *Aedes aegypti* Linnaeus (Del Rosario, 1936; Kartman, 1953), *Aedes taeniorhynchus* Wiedemann (Hu, 1931), *Anopheles quadrimaculatus* Say (Hu, 1931; Phillips, 1939; Kartman, 1953; Newton, 1957), and *Culex quinquefasciatus* Wiedemann (Bancroft, 1901; Del Rosario, 1936; Summers, 1943; Travis, 1947; Kartman, 1953; Rosen, 1954). Yen (1938) compared the relative susceptibility of 12 species of mosquitoes to infection with *D. immitis*. In those species which he classified as refractory to the parasite, the reason given was either immediate elimination of the larvae in the feces or encapsulation of them in a chitinous cyst. In none of these cases did he report any increased mortality rate due to the *D. immitis* larvae. This paper is an account of our experiences at the University of Florida in the production of infective larvae.

MATERIALS AND METHODS. Adult mosquitoes of the following species and strains were used: *A. taeniorhynchus* (Shiloh Strain), *C. quinquefasciatus* (Gainesville Strain), *A. quadrimaculatus* (Gainesville Strain), *A. aegypti* (Mascarensis Thorax, Yellow Body Larvae, Silver [M.Y.S.] Strain, Liverpool Strain, and a "Wild" Strain). The adults were kept either in 7″ lantern chimneys (Dietz Fitzall #852) with gauze-screened ends, or in aluminum-framed gauze-covered cages (12″ x 12″ x 6″). In the former case, they were housed in a modified room at our laboratory and in the latter they were maintained in an insectary at the Insects Affecting Man and Animals Research Laboratory of the U. S. Department of Agriculture, Gainesville. In both cases, the holding room was maintained at a constant 85° F and 85 percent humidity. Except for a time immediately preceding a blood meal, and while actually taking a blood meal, cotton saturated with a solution of 2.5 percent sugar water was available in the cages as nourishment.

Six naturally infected dogs were used as microfilaria donors; microfilarial counts are shown in Table 1. The general procedure for the blood-meal feeding of mosquitoes was to expose each cage to one dog only until a maximum number of females had become engorged (usually about 45 minutes). Blooding was carried out at 3 to 5 days post-emergence depending on the genus and species of mosquitoes being used. Each time groups were

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TABLE 1.—Dogs used in experimental *Dirofilaria immitis* transmission

| Dog | Microfilariae/ml |
|---|---|
| A | 60,000 |
| B | 1,300 |
| C | 1,900 |
| D | 1,600 |
| E | 1,700 |
| F | 5,300 |

fed, one cage of mosquitoes was fed on a guinea pig and maintained in the same location in the holding room as the dog-fed groups. In feeding the first 26 groups, the donor dog was anesthetized with sodium pentobarbital to facilitate handling; this practice was then discontinued and unanesthetized dogs were used thereafter because it was felt that the barbiturate was causing too much mosquito mortality.

Other changes made in the environment during the course of the experiment included placing oviposition cups in the cages after feeding, provision of split raisins soaked in water as an additional nourishment source, and a change to a feeder of a hanging type as opposed to a cup with cotton in it. Also tried was moist cotton batting (12" x 12") over the tops of the cages in order to increase the humidity.

RESULTS AND DISCUSSION. The first phase of the experiment involved *A. taeniorhynchus, C. quinquefasciatus* and *A. quadrimaculatus* (Table 2). Adults of these species were first placed in glass lantern chimneys in the modified room in our laboratory and all died within 2 days post-emergence. Several groups of each species were tried but the result was the same. Since both non-infected and infected mosquitoes died, the cause of mortality was assumed to be external to the experiment. This arrangement, therefore, was judged as unsatisfactory due to unknown factors. Mosquito rearing was then moved to the Insects Affecting Man and Animals Research Laboratory of the U.S.D.A. where a mosquito insectary room had been made available for use.

After feeding the first two groups from the new location on dog "A" (60,000 microfilariae/ml.) and experiencing great mortality, it was decided to feed the mosquitoes on dogs with lower microfilaremia

TABLE 2.—Larval development of *Dirofilaria immitis* in certain mosquitoes

| Mosquitoes & Strains | Mosquitoes Fed on Infected Dogs: Groups & No. | | Dog & mf./ml.* | | Larval Development: L 2 | L 3 |
|---|---|---|---|---|---|---|
| *Aedes taeniorhynchus* (Shiloh Strain) | 2 | 6,000 | A | 60,000 | 1–A | 0 |
| | 2 | 3,000 | B | 1,300 | | |
| *Culex quinquefasciatus* (Gainesville Strain) | 2 | 7,000 | B | 1,300 | 1–D | 1–D |
| | 1 | 3,000 | D | 1,600 | | |
| *Anopheles quadrimaculatus* (Gainesville Strain) | 6 | 18,000 | C | 1,900 | 4: | 4: |
| | 7 | 21,000 | D | 1,600 | 1–C | 1–C |
| | 6 | 18,000 | B | 1,300 | 1–D | 1–D |
| | | | | | 2–B | 2–B |
| *Aedes aegypti* (M.Y.S. Strain) | 1 | 3,000 | F | 5,300 | 0 | 0 |
| | 1 | 4,000 | E | 1,700 | | |
| | 1 | 4,000 | C | 1,900 | | |
| *Aedes aegypti* (Liverpool Strain) | 3 | 3,000 | F | 5,300 | 1–E | 1–E |
| | 4 | 12,000 | E | 1,700 | | |
| | 3 | 3,000 | C | 1,900 | | |
| *Aedes aegypti* (Wild Strain) | 1 | 3,000 | C | 1,900 | 0 | 0 |
| | 1 | 3,000 | E | 1,700 | | |

* Microfilariae per milliliter of blood

since Wharton (1957) had suggested that in *Brugia malayi* Buckley, microfilarial counts of over 3,000 per ml. were likely to cause death of the vector. Despite this change in procedure most groups of mosquitoes continued to present similar characteristics: engorged females began to die on day 2 after blooding, and by day 3–4 there was about 75 percent mortality. A characteristic common to these groups was that the abdomens of the mosquitoes remained distended for 2–3 days, indicating that the blood meal was not digested properly. By the 12th day, only about 6–7 percent of the original number remained and, upon dissection, these contained no larvae except in *A. quadrimaculatus* where mortality was slightly lower (10–15 percent remaining) and a few $L_3$ stages were found (Table 2).

In an effort to determine the cause of this mortality, it was decided to change, one at a time, some environmental conditions on the assumption that a mosquito infected with *D. immitis* larvae was especially subject to environmental stresses, and therefore, susceptible to even slight variation from optimal conditions. The first change was to reduce the numbers of mosquitoes per cage to approximately 1,000 per half cubic foot volume. After handling about 15,000 mosquitoes in this manner, no effect on reducing mortality could be detected and thus, crowding was eliminated as a possible cause of the high mortality rate.

Another change instituted was the feeding of a group of mosquitoes on a guinea pig each time that a group was fed on a dog. These control mosquitoes were maintained in the same rooms and under identical conditions as those fed on the infected dog. In each instance (15 trials), the groups fed on guinea pigs outlived by several days those fed on the dog and, in fact, went through the complete, normal cycle of oviposition.

Another environmental change was the placing of the oviposition cups in the cages of groups of mosquitoes after blood meal feeding. This was to allow the females easy access to a suitable place for depositing and possibly remove another source of stress. The mosquitoes did oviposit in the cups, but the mortality was not reduced.

The next change was the addition of raisins, split and soaked in water, to the cage as an additional source of nourishment after the blood meal. This produced no change in the mortality rate. It had been noted that there were large numbers of adult mosquitoes dead around and in the feeding cups (filled with sugar water-soaked cotton). It was theorized that the sugar water might be crusting just enough to trap them or not allow them to feed, even though the cups were changed each day. To eliminate this possibility, a hanging feeder was used in each cage. This apparatus consisted of a glass vial of 50 ml. capacity with a wick of cotton inserted into the open end. The vial was filled with the 2.5 percent sugar water and suspended in the cage. This alteration eliminated the deaths around the feeders, but did not help reduce the overall mortality.

Another environmental factor considered was that of humidity in the microenvironment of the cage. To assure a high degree of humidity, the cages were covered with moist cotton batting. This change failed to produce an improvement in the mosquito survival rate.

All of the trials mentioned thus far had been completed in the same holding room. In this room were thousands of other mosquitoes of like and differing genera and species. It was of interest to note that none of these other groups experienced mass mortality on a scale comparable to that in the *D. immitis*-infected mosquitoes.

Next, arrangements were made to obtain *A. aegypti* (Liverpool Strain). This strain has been used successfully by others in the production of *D. immitis* (Tulloch *et al.*, 1968). The M.Y.S. Strain and a "Wild" Strain of *A. aegypti* maintained at the Insects Affecting Man and Animals Laboratory were also used in this series of trials.

Results of the first trials with the 3

*A. aegypti* strains were no better than those of the trials with other genera. Persistance of the distended abdomens 2–3 days after blood meal engorgement continued to be the main observable abnormality.

Until this time, the dogs used for feeding mosquitoes had been anesthetized with intravenous sodium pentobarbital and it was postulated that this might be the cause of the mortality in the mosquitoes. Therefore, an experiment was devised where two groups of *A. aegypti* (Liverpool Strain) were each fed on guinea pigs, one anesthetized with sodium pentobarbital and the other not. The mosquitoes were then placed in the same insectary as the other groups and observed. There was no difference in the mortality rates of either of the groups, it being quite low in both. In spite of these results, it was decided to stop the use of anesthetized dogs as an added precaution. Nevertheless, there was no difference observed in the reactions to infection with *D. immitis* among the three strains of *A. aegypti*. Table 2 is a summary of all results in this series of experiments.

There is very little information recorded in the literature which would help to explain these negative results. The majority of the problems of mosquito deaths in experimental transmission of filariasis reported occur in the latter days of the larval development period within the mosquito. For instance, Webber and Hawking (1955) state that in cases where the donor dog had a high microfilaremia (approx. 10,000/ml.), great numbers of microfilariae were ingested by the mosquitoes and the development of these larvae led to destruction of the malpighian tubules. This destruction produced a high mortality rate relatively late (10–12 days) in the development period. Kershaw *et al.*, (1953) reported a high mosquito mortality in the first few days post-blood meal and again from the 21st to 25th day in *A. aegypti* infected with *Wuchereria bancrofti* Seurat.

On the other hand, Jordan and Goatly (1962) reported a high mortality rate of *Culex fatigans* Wiedemann infected with *W. bancrofti* within 12 hours of an infective blood meal. This mortality was explained by the high levels of microfilaremia in the donors used (18,000–25,000/ml.). They also reported a low rate of mosquito mortality (less than 1 percent) when the microfilaremia of the donors was from 50–6200/ml.

SUMMARY. The high level of microfilaremia and subsequent ingestion of large numbers of microfilariae by mosquito vectors has been given as one explanation of excessive mosquito mortality rates in experimental transmission on studies on filariasis. However, in the present study, although dogs with *D. immitis* microfilaremia ranging from 1300–5300/ml. were used as the primary sources of infective blood meals for the various genera and species of mosquitoes, excessive early mortality routinely occurred. In an effort to reduce the mortality rate, several adjustments were made in the environment of the engorged mosquitoes, but none of these efforts was successful. Therefore, it is assumed that *A. taeniorhynchus* (Shiloh Strain), *C. quinquefasciatus* (Gainesville Strain), *A. quadrimaculatus* (Gainesville Strain), and *A. aegypti* (M.Y.S. Strain, Liverpool Strain, and the "Wild" Strain used) are not efficient as experimental vectors of *D. immitis* in Florida.

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# SEVEN INSECTICIDES AS RESIDUAL SPRAYS IN BULIDINGS NATURALLY INFESTED WITH *ANOPHELES QUADRIMACULATUS*[1]

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Applications of insecticides that have long residual activity inside buildings have been used extensively throughout the world in programs for control of malaria. However, the resistance to DDT and dieldrin that has developed in mosquitoes has made it important to find alternate materials.

Since 1943, the Entomology Research Division has maintained a testing program at the Insects Affecting Man Investigations Laboratory, now at Gainesville, Florida for the evaluation of new insecticides as residual sprays against disease-carrying mosquitoes. To be useful in programs involving buildings occupied by people, the insecticides must have a favorable mammalian toxicity, remain effective for a considerable period, and be cheap. Fast knockdown also would be a highly desirable characteristic.

The present paper reports the results obtained in tests made in mosquito-infested buildings with a group of insecticides that have been highly effective in laboratory tests (Gahan *et al.* 1967; Wilson *et al.*, 1970) and that have an $LD_{50}$

[1] Mention of a pesticide or a proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the USDA.