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INFECTION OF ANOPHELES BACHMANNI, PETROCCHI, WITH PLASMODIUM VIVAX, GRASSI AND FELETTI, AND OBSERVATIONS ON THE BIONOMICS OF THE MOSQUITO

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INTRODUCTION

Anopheles bachmanni is one of the white hind-footed Anophelines that have been found in Panama in recent years, and is a mosquito that was not included in the studies of Dr. Darling (1910), in which *Anopheles albimanus* was proven to be the important vector of malaria on the Isthmus. The purpose of this paper is to report the infection of *A. bachmanni* with *Plasmodium vivax*.

EXPERIMENTAL

The mosquitoes used were collected as larvae, and positively identified as to species in this stage. The adults were obtained from the known larvae.

The gametocyte carriers were chosen from among the natives in several Chagres River villages that were being surveyed for malaria once a month by H. C. Clark and W. H. W. Komp.[1] Due to the trouble and expense involved, it was not possible to bring the carriers into Panama City for mosquito feedings; therefore, as soon as a carrier was found, the mosquitoes were packed in ice and taken up to the villages for their infective blood meals. Because the biting had to be done at considerable distances away from laboratory facilities, Boyd's and Stratman-

[1] The writer is indebted to Dr. Clark and Mr. Komp for their aid in locating gametocyte carriers, and to Mr. Komp for his many helpful suggestions and criticisms during the course of the work.

Thomas' (1933) technique of waiting for at least 2 micro- and 2 macro-gametocytes per 100 leucocytes before allowing the mosquitoes to feed could not be followed. Blood smears were taken at the time of feeding in order to determine the intensity of infection of the blood taken in by the mosquitoes, but of course this determination could be made only after a return to the laboratory. As the experiment numbers indicate, only occasionally was the

TABLE 1

EXPERIMENT NUMBER	TYPE OF MALARIA	INTENSITY OF INFECTION	SPECIES OF MOSQUITO	NUMBER OF MOSQUITOES DISSECTED	NUMBER OF MOSQUITOES POSITIVE FOR OÖCYSTS	NUMBER OF MOSQUITOES POSITIVE FOR SPOROZOITES	PERCENTAGE OF INFECTED MOSQUITOES
							per cent
5	Estivo-autumnal	One plus (one parasite per field in thick film)	*A. bachmanni* (?)	13	2	0	15.4
			A. albimanus (control)	14	7	0	50.0
7	Tertian	One plus	*A. bachmanni*	7	3	0	42.9
			A. albimanus (control)	5	5	0	100.0
11	Tertian	3 macro- and 6 microgametocytes per 200 leucocytes in thin film	*A. bachmanni*	11	2	1	27.3
			A. albimanus (control)	1	1	0	100.0

writer fortunate enough to feed mosquitoes at a time when sufficient gametocytes were present in the peripheral circulation to infect the insects. The results of the three experiments which were successful are summarized in table 1.

In experiment 5, 2 out of 13 *A. bachmanni*, which had fed on an eight-year-old boy with a one plus infection (at least one parasite per microscopic field in a thick film) of *Plasmodium falciparum*, were found to have oöcysts in the stomach. Each

mosquito had 3 oöcysts. At the same time, 7 out of 14 *A. albimanus* controls became positive with from 2 to 17 oöcysts. Unfortunately, the *A. bachmanni* females used in this experiment were bred from pupae, and as the characters of the adult females are unreliable, we can not be definitely certain that the infected mosquitoes actually were *A. bachmanni*. The pupae were collected from a patch of *Pistia stratiotes*, and although hundreds of larvae have been taken from this patch of water lettuce and not a single specimen of *A. albimanus* has ever been found among them, the evidence from this experiment can only be used as supplementary to that of experiment 7 and 11.

Experiment 7

Thirteen *A. bachmanni*, and 6 *A. albimanus* controls, were allowed to feed on a six-year-old girl, who had a one plus infection of tertian malaria. Several *A. bachmanni* died at a time when they could not be dissected. Two died on the third day; they were not infected. On the fifth day, 5 *A. bachmanni* were left, and 4 were so weak that it was decided to dissect them immediately. One of them was negative, but one had 7 oöcysts in the stomach, another had 2, and the fourth, 1. The remaining mosquito lived for two days more; both its stomach and glands were negative. Thus of the 7 *A. bachmanni* that were dissected, 3, or 42.9 per cent, were positive for oöcysts.

One of the *A. albimanus* controls died at a time when it could not be dissected, but all of the remaining 5 were infected, the first with 2 oöcysts, the second with 5, the third with 7, the fourth with 1, and the last with 3 oöcysts.

Experiment 11

The mosquitoes in this experiment were fed on the most heavily infected blood that the writer was able to obtain. The carrier was an eleven-months-old baby girl, and a thin smear showed 3 macro- and six micro-gametocytes per 200 leucocytes.

Eleven of the *A. bachmanni* that took blood were dissected. Of these, 3, or 27.3 per cent, were positive. Two of the mos-

quitoes that were infected died before sporozoites developed. The third positive mosquito died on the ninth day after its infective blood meal. No oöcysts were present in the stomach, but along one edge of the stomach was found a group of sporozoites, which had apparently just ruptured from an oöcyst. From the relatively small number of sporozoites it was estimated that only

TABLE 2

EXPERIMENT NUMBER	SPECIES OF MOSQUITO	MOSQUITO NUMBER	NUMBER OF OÖCYSTS
5	*A. bachmanni*	10	3
		12	3
	A. albimanus (control)	2	8
		3	15
		6	3
		7	2
		8	17
		9	3
		11	3
7	*A. bachmanni*	3	7
		4	2
		6	1
	A. albimanus (control)	1	2
		2	5
		3	7
		4	1
		5	3
11	*A. bachmanni*	6	6
		7	1
		9	?*
	A. albimanus (control)	1	94

* Sporozoites present.

one oöcyst had developed in the stomach wall. The salivary glands were negative.

The single *A. albimanus* control that was finally induced to feed, was found to have approximately 94 oöcysts in the stomach.

Table 2 shows the number of oöcysts found in the stomachs of the mosquitoes that became infected. Experiments 5 and 11 indicate that not only was a larger percentage of *A. bachmanni*

refractory to the infection, but that even the susceptible mosquitoes exhibited more immunity than the *A. albimanus* controls.

It is possible, therefore, that although *Plasmodium vivax* and perhaps *P. falciparum* also, can develop in *A. bachmanni*, this mosquito is a much less favorable host for the parasites than is *A. albimanus*.

HABITS OF A. BACHMANNI

As Curry (1931) has pointed out, the larvae of *A. bachmanni* are found, on the Isthmus, within the crowns of water lettuce, *Pistia stratiotes*. Occasionally they also breed in bladderwort, *Utricularia mixta*, and in *Jussiaea natans*. This mosquito exhibits the same selectivity in its choice of breeding places as has been observed with so many other mosquitoes, not only in its choice of *Pistia* as a larval habitat, but also among certain patches of these plants when they are widely prevalent in a certain area. During the month of December, 1934, when the writer first began collecting *A. bachmanni*, most of the larvae were taken from *Pistia* plants that were growing very extensively in a lagoon on the edge of the Chagres River, near the village of Las Guacas. Once a year this area is thoroughly sprayed with a mixture of caustic soda and arsenic; this is done in order to prevent the multiplication of water lettuce and water hyacinth into numbers so large that they might spread down the river and into the Canal. The spraying was done shortly after the first collections were made, and all of the *Pistia* plants were killed, except for a few small patches in one of the far corners of the lagoon. Prior to the spraying, *A. bachmanni* larvae had been found scattered rather uniformly throughout the extensive areas in which *Pistia* was growing, although not enough collections were made to be able to show definitely that some patches might not have been supporting more breeding than others. After the plants had been killed, the gravid females all began to deposit their eggs in one of the remaining patches of the water lettuce. Unfortunately, these plants had been overlooked prior to the spraying, so that it is impossible to say if they had been more attractive to the ovipositing females than the rest of the many plants. The

large number of larvae found here leads the writer to believe that only after the other breeding places had been destroyed did the females begin to concentrate all their eggs in this area. The striking thing was that this particular patch was so much more attractive than were the four or five other patches that were present within a radius of about 200 feet. For over four months, collections were made almost every week, and a large number of larvae and pupae were always found in the first patch, averaging one or more to the dip, while in the others only an occasional one would be encountered. As the favored patch grew in size, it was observed that more larvae were consistently found in one edge of it than in the rest, indicating that the females were even selective in their choice of plants within a small area. In the month of May these patches became very large, and then it was found that many larvae were also beginning to appear in the plants that were situated farther away from the shore.

The patch that supported the most breeding differed from the others as follows: In the first place, it was located near the shore, and was shaded in the morning by trees that overhung the water. Secondly, because this patch was situated near the shore, it was the first to be encountered by a female returning from the jungle to deposit her eggs. The temperature of the water of this patch was 28°C., while that of the water of the other patches was about a degree and a half higher. These three characters: shade, proximity to blood supply, and temperature of the water, seemed to be the only noticeable differences between this patch and the unattractive ones, and of these, the second mentioned appeared to be the most important. The age of the plants, their size, and their density were no different from that of the other patches.

Anopheles bachmanni is a mosquito that apparently, on the Isthmus, does not enter houses in search of blood, as does *A. albimanus*. Clark and Komp (1932), in their malaria studies in five Chagres River villages, reported that the only other Anopheline besides *A. albimanus* that was encountered in house catches was *A. punctimacula*, so far as could be determined by the characters of the females. Three of these villages are situated near the area where the writer has been collecting *A. bachmanni*.

In the laboratory, *A. bachmanni* is a vicious biter, and in the infection experiments, the writer was often able to get a greater number of *A. bachmanni* to take blood than *A. albimanus*. On December 11, 1934, at about nine o'clock in the morning, while the writer was looking for *Anopheles eiseni* in a small shady pool, located near a jungle trail several hundred yards from the village of Santa Rosa, he was considerably surprised to observe, among the many *Mansonia* that were attacking him, a number of white hind-footed Anophelines. Several of these were caught, and later identified as *A. bachmanni*. On April 23, 1934, a second observation on the day-biting habits of this species was made. A native helper and the writer stationed themselves, at 9:00 a.m., on the edge of a clearing, about 400 yards from the village of Las Guacas, and about 150 feet from the patch of *Pistia* in which so many larvae were found. Within twenty minutes, four *A. bachmanni* females came to obtain blood. Three of them filled up with as much blood as they could hold, the fourth was disturbed when the catching tube was placed over it before it had begun to suck in blood, and it refused to bite. It should be emphasized that on both these dates, these mosquitoes were not merely disturbed from the surrounding vegetation, but appeared to be actively searching for blood, as were the hordes of *Mansonia* with which they were associating. It is unfortunate that identification had to be based on female characters. The females that were taken on April 23 were caught alive, brought back to the laboratory, and imprisoned in lantern globes above water. If viable eggs could have been obtained, identification would have been absolutely certain from the larvae. However, only non-viable eggs were deposited. Nevertheless, not only the wing-markings, but the small size of these day-biting Anophelines leave little doubt in the writer's mind that these mosquitoes were *A. bachmanni*.

If the malaria parasites are capable of developing in a mosquito that has the habit of attacking man by daylight, then it may not be impossible for an occasional malaria infection to take place in the jungle during the day.

SUMMARY

1. In two separate experiments, *Anopheles bachmanni* females were infected with *Plasmodium vivax*. In one experiment, 3 out of 7 *A. bachmanni* were infected with oöcysts, and all of the 5 *A. albimanus* controls were found to be positive. In the other experiment, 3 out of 11 *A. bachmanni* females were infected with either oöcysts or sporozoites; while approximately 94 oöcysts were found in the stomach wall of the single *A. albimanus* control.

2. Sporozoites were found in one of the positive *A. bachmanni* nine days after the infective blood meal.

3. Mosquitoes that quite probably were *A. bachmanni* were also infected with *P. falciparum*. Two out of 13 of these mosquitoes had oöcysts in the stomach wall, while 7 of the 14 *A. albimanus* controls were positive.

4. Not only were more individuals of *A. bachmanni* refractory to infection, but it appeared that those mosquitoes which didbecome infected displayed a higher degree of immunity than *A. albimanus*.

5. *A. bachmanni* larvae in Panama are found chiefly in *Pistia stratiotes*. In a limited area that was under observation for over four months, a certain patch of *Pistia* seemed to be more attractive to ovipositing females than other patches, and this appeared to be true even as regards certain areas within a single patch.

6. *A. bachmanni* females have been observed to attack man in the jungle by daylight.

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