REPORT OF BUREAU OF MALARIA CONTROL 1926–27

Paper IV

OBSERVATIONS ON THE BLOOD FEEDING HABITS OF A. ALBIMANUS AND GRABHAMII

In order to obtain as much definite information as possible concerning the habits of the two mosquitoes the precipitin test as reported by Bull and King was used. Because of the inability to find in nature sufficient numbers of engorged females practically all the tests here reported were on samples obtained experimentally. The main object was to determine what protection for man might be expected from animals, especially horses and oxen.

SOURCE OF MATERIAL

A house essentially like that described by Bull and Root in their first paper was constructed adjacent to a small swamp near the beach at Fajardo where both albimanus and grabhamii were generally breeding in abundance. It was a region in which in spite of continued breeding of both species there was very little malaria as shown either by sickness, parasite or spleen rates. Instead of breeding out the mosquitoes in the laboratory, entrances were made for the mosquitoes on each side of the house away from the ocean breeze. The entrances were horizontal openings extending across the entire side, with the opening wide (8 inches) toward the outside and narrow (1 inch) toward the inside. Thus the mosquitoes could enter fairly well, but as the entrances projected into the house and were small toward the inside, it was difficult for mosquitoes to get out. A mosquito that is full of blood does not seem to try to get out as hard as a hungry one to get in. Men were then placed within the house on various nights with various combinations of animals. Since the more individuals there were in the house the larger the catch, it was the rule to use at least two men and on some occasions, as many as six. By the use of several, there is the possible advantage that one eliminates the variation in attractiveness of individual men which is known to exist and one gets results applicable more to human beings as a whole. Several sets of men were used, though in the later experiments it was generally the same group. Due to

the warm weather it was possible for the men to sleep with the greater part of their bodies exposed and while at the beginning possibly the object of the experiment was not clear to them, they soon learned that they were not to disturb the mosquitoes and another man was placed there to see that all went as directed. Due to the large numbers of non-anophelines at times, possibly the men were unusually bitten, but certainly not more than they usually were in their own homes. The mosquitoes were caught early in the morning and the stomachs removed as soon as possible and the blood placed on filter papers.

THE PRECIPITIN TEST

The technic was essentially that recommended by Bull, though it was found that a high mortality among rabbits resulted from the large doses of serum recommended. In most cases as potent a serum as he reported was not obtained nor was it found necessary. The usual titer was between 1 to 4,000 and 1 to 8,000, but at times results were obtained with sera as low as 1 to 2,000. The average blood meal found here, weighed 1 mg. though an unusually large meal often found especially in grabhamii probably weighed 2 mg. Many were encountered, however, which did not average over 0.5 mg. When one is removing the stomach one can estimate fairly well the size of the blood meal, but after the blood has dried on filter paper it is very difficult and, besides, one cannot always know that it will all go into solution later. The attempt was made to try and work with a dilution of antigen of about 1 to 1,000 and with an average blood meal of 1 mg. this was obtained by adding 1 cc. of salt solution to each sample. The routine was first to add 0.5 cc. until all the sample was dissolved and then just before doing the test, 0.5 cc. more was added, except to those which by the color of the solution were shown to contain small quantities of blood, or at least a small quantity in solution. The strongest solution that was used then was a 1 to 500 in those where the blood meal was unusually large.

In spite of many precautions it was difficult at times to avoid cross reactions, especially between horse and ox. These difficulties were greatest in the earlier work and later it was found safer to use only sera that was highly specific and not try to determine the source of the blood by the first one that precipitated. With the weaker sera, observations were usually continued up to an hour before making a final reading. It was found more convenient in the handling of the tests to use porcelain test plates with 12 de-

pressions in each, the capacity of each being about 3 cc. The majority of the mosquitoes caught each day had taken some blood, though the number with engorged stomachs was usually less than one-half.

RESULTS OF THE TESTS

Albimanus.

Experiments were made mainly with man, horse and ox, though some were also made with pig and goat. When the work was first started mosquitoes were abundant, but because of difficulties with the test full advantage could not be taken of the opportunity until the breeding began to diminish considerably. For that reason the numbers of mosquitoes concerned in individual tests at times was rather small and results need further checking.

Man–Horse.

In most cases the animals were placed on the side farthest from the entrances so that the mosquitoes needed to fly over the men to reach the animal. Even with this slight advantage on the side of the men, only six of forty-seven albimanus fed on men in one night, making a percentage of thirteen. The house was then divided into two parts by a screen of mosquito netting into which two openings were made, one at each end, large enough to permit a man to pass through. All of the mosquito entrances were on the side upon which the men were usually placed. It was thus still more difficult for the mosquitoes to reach the animals. While there was some variation with different horses only on one occasion, when only nine mosquitoes were caught, did more than half of the mosquitoes bite the men. On this occasion a new man was used to catch mosquitoes and it is not certain that he did the job well. Repetition on night following showed the usual low per cent of bites of man though on the second night one of the horses and one of the three men were changed. The lowest per cent of man bites was twenty-one and the highest forty-three. Three men were always used and on two occasions two horses, the lower per cent of bites on man being when two horses were in the house. The summary of all the eight observations in which men and horses were concerned, is shown in Table XII, where it is seen that a little less than one-third of the bites were on man. More men than horses were always used and the mosquitoes were obliged to pass over the men to get to the horses in six of the eight trials.

TABLE XII

RESULTS OF TESTS WITH MEN AND HORSES IN THE HOUSE

		Man bites	Horse bites	Man Horse	Total	No. reaction	Percent Man
Horse 3.....	ALB...	6	40	1	47	1	13
Man 7, 8.....	GRAB.	.....	24	.....	24	.....	0
Horse 1.....	ALB...	10	18	2	30	6	33
Man 1, 2, 3....	GRAB.	9	19	1	29	1	31
Horse 2.....	ALB..	16	16	5	37	2	43
Man 1, 2, 3....	GRAB.	15	56	4	75	.....	20
Horse 3.....	ALB...	4	7	.....	11	.....	36
Man 2, 3. 4....	GRAB	3	34	5	42	1	7
Horse 4, 5.....	ALB...	8	1	.....	9	.....	88
Man 3, 5, 6....	GRAB.	6	10	1	17	.....	35
Horse 4, 7.....	ALB...	7	25	2	34	.....	21
Man 5, 6, 9....	GRAB.	4	77	1	82	.....	5
Horse 4, 6.....	ALB...	10	20	.....	30	.....	33
Man 1, 2, 3....	GRAB.	1	60	.....	61	.....	2
Horse 4, 6.....	ALB...	11	36	2	49	2	22
Man 1, 2, 3	GRAB.	4	37	.....	41	.....	10
Total.....	ALB...	72	163	12	247	11	29
	GRAB.	42	317	12	371	2	11

Man–Ox.

With no screen, but with six men and one ox only five out of twelve bites were on man. It was necessary for the mosquitoes to pass over the men to reach the oxen. With the screen and with two men, an ox and calf thirty-five per cent of sixty-five bites were on man (average of two tests), the mosquitoes being obliged to fly over the men first. With two oxen on the side of the entrance, so that the mosquitoes reached oxen first, and five men on the other side of the screen only six per cent of 93 bites were on the man, though there was some trouble with cross reactions at this time. Even considering all the crossed ones as man bites, the per cent is raised only to seventeen, which is still only one-half the number when the position of the ox and men was reversed. Summing up the four observations with man and ox it is found that twenty per cent of the bites were on men though if all of the crossed reactions are considered as man bites the per cent is raised to twenty-nine. Oxen gave a great deal of protection to men and were preferred even when men were more accessible.

Man–Horse–Ox.

Of five observations with no screen, using a bull and horse and two men, only seven per cent of 332 bites were on the men. With a screen between the men and animals the percentage was raised to nineteen. Some difficulty was encountered with double reactions with the horse and ox, but a higher per cent of bites were on the ox than on the horse. In one case the screen was made as tightly as possible between the animals and men, but still four out of thirty-four mosquitoes managed to get through and bite the animals along with a few that bit the men first. Again, the animal blood was distinctly preferred. (Table XIV.)

TABLE XIII

RESULTS OF TESTS WITH MEN AND OXEN IN THE HOUSE

		Man bites	Ox bites	Man bites	Total	Doubtful	Percent Man	Observations
Ox 1.	ALB...	5	7	1	12	....	41	No screen-men
Man 1, 2, 3, 4, 10, 11	GRAB.	6	6	....	12	...	50	nearest entrances
Ox 1.....	ALB...	0	4	....	4	....	0	No screen-bull
Man 1, 2, 3	GRAB.	6	23	....	29	....	21	nearest entrances
Ox 2, 4	ALB ..	8	21	1	30	...	27	.....
Man 1, 2, 3	GRAB.	9	12	2	23	....	40	....
Man 1, 2, 3, 4, 10, 12	ALB...	7	74	12	93	....	6	Screen-ox on side
Ox 2, 3	GRAB.	5	89	9	83	1	6	of entrances
Total	ALB...	35	125	14	174	2	20	
	GRAB.	26	150	11	187	....	14	

TABLE XIV

MAN–HORSE–OX

		Man bites	Horse bites	Ox bites	Man-Horse	Man-Ox	Man-Horse Ox	Horse-Ox	Total	Doubtful	Percent Man	Percent Horse	Percent Ox	Observations
Man 13														
Horse 3	ALB.	12	37	68	2			6	125	1	10	29	54	No screen
Ox 1	GRAB.	1	17	18	1	1		6	44		2	39	41	
Man 13, 14														
Horse 3	ALB.	4	10	19		3		1	37	1	11	37	51	No screen
Ox 1	GRAB.	2	14	27		2	1	3	49		4	29	55	
Man 13, 14														
Horse 3	ALB.	3	39	84		1	1	10	138	8	2	28	61	No screen
Ox 1	GRAB.	0	23	40				13	76	1	0	30	53	
Man 13, 14, 15														
Horse 3	ALB.	2	9	9				4	24		8	38	38	No screen
Ox 1	GRAB.	1	42	27		4		17	91		1	46	30	
Man 4, 7, 8	ALB.	2	1	3		1		1	8	1	25	12	37	No screen
Horse 3	GRAB	1	15	11				9	36		3	42	30	
Total	ALB.	23	96	183	2	5	1	22	332	11	7	29	55	No screen
	GRAB.	5	111	123	1	7	1	48	296	1	2	38	42	
Man 1, 2, 3	ALB.	2	8					4	14		14	57	0	Screen-men
Horse 3	GRAB.	4	28	6				12	50		8	56	12	nearest entrance
Ox 1														
Same as	ALB.	7	10	9				12	34		21	29	15	Screen
Above	GRAB.	4	11	5				26	50		8	22	18	
Total	ALB.	9	18	5				16	48	1	19	38	10	
	GRAB.	8	39	15				38	100		9	39	15	

Man–Goat.

Fifteen or 48 per cent of thirty one bites were on man. Three reactions were doubtful. There was no screen in the case of the pigs or goats. Three men were used in one case with two pigs and then three men with four pigs. (Table XV.)

TABLE XV

MAN–PIG

		Man bites	Pig bites	Man-pig	Total	Doubtful	Percent Man
3 men.....	ALB...	2	4	.....	6	.....	33
2 pigs	GRAB.	.....	20	.....	20	.....	0
3 men.....	ALB...	13	9	3	25	1	50
4 pigs	GRAB.	3	16	3	22	2	14
Total.....	ALB...	15	13	3	31	1	48
	GRAB.	3	36	3	42	2	7

TABLE XVI

MAN–GOAT

		Man bites	Pig bites	Man-pig	Total	Doubtful	Percent Man
3 men.....	ALB...	6	2	3	11	1	51
2 goats....	GRAB.	2	3	.....	5	.....	40

Results with Grabhamii.

In most of the catches there were more grabhamii than albimanus so that the results should be more conclusive. In only two instances, and both of these where man and ox were in the house, did the percentage of bites on man exceed that for albimanus. In most cases it was much lower. Almost one-third as many grabhamii bites were on man as for albimanus in the man–horse tests, a little less than one-half where man–ox were concerned and almost one-third as many where man–horse–ox were concerned.

The evidences all indicate that considerable protection was given by the horse and ox and if it was really of importance it ought to be possible to keep the mosquitoes from entering the house by placing animals in front of the entrances outside. Six men in most cases, never less than four, were placed in the house at night with or without animals outside in front of the entrances, generally a night with animals, being followed by a night without them. Thus, conditions would be somewhat comparable to those of the average home of five individuals. Table XIX shows the catches with men alone and with

men inside and various animals outside in front of the entrances. The blood of the mosquitoes caught inside was tested in most cases and it was found that the mosquitoes had bitten only man. Observations during the night on two occasions showed that large members of anopheles were biting the animals outside. It is seen that the results are somewhat variable, but that the number of mosquitoes entering the house when the horses were outside averaged one-third less than when there were no animals there at all, and that with oxen in place of the horses the catches were lower still. Dogs gave practically no protection. The results of these tests are in rather close agreement with the precipitin tests performed when the animals were in the house with the men.

Grabhamii did not go into the house in search of human blood whether animals were outside or not. To determine if this was due to absence of grabhamii from the region, two oxen were put in the house with five men on May 21. Eighty-three grabhamii with ninety-three albimanus were caught, indicating that they were present. Protection was also greater when the animals were placed directly in front of the entrances which were on the side away from the wind. When the animals were put on the other side, more mosquitoes entered.

CONCLUSIONS

It is very evident that there is a marked difference in the attractiveness of man for grabhamii and for albimanus. Previous observations were confirmed that grabhamii does not enter houses much in search of human blood and that animals are much more preferred, especially the horse and ox. Considerable protection from albimanus bites is given by the horse and ox and it is very probable that, after people have gone to bed and shut up their houses as they do in Porto Rico, that very few albimanus reach them if horses and oxen are between the houses and the source of the mosquitoes. Protection is not nearly so great if the mosquitoes can reach the house first even though the animals are near on the other side. When people are sitting outside the houses at night, whether they will be bitten or not will depend upon the proximity and position of the animals with relation to the breeding place and the direction of the wind. Since there are few stables in Porto Rico and the animals are most of the time free in the pastures, at least at night, protection may be considerable at some times and very slight at others. It is very probable that in many regions in Porto Rico, with its large numbers of horses and oxen, this protection is sufficient to avoid con-

siderably in preventing the development of an unusual amount of malaria. Observations are being made to determine if it is possible to make use of the large number of oxen in protecting certain regions that are difficult to sanitate.

TABLE XVII

MAN–HORSE–OX–PIG

		Man bites	Horse bites	Ox bites	Pig bites	Man-ox	Horse-ox	Horse-pig	Ox-pig	Total	Doubtful	Percent man
3 men	ALB...	7	12	19	1	4	1	...	6	53	3	13
1 horse	GRAB.	5	7	15	4	...	1	4	18	54	....	9
1 ox	..	..	....	....	..	...	...	....	....	....	....	....
2 pigs	.....	....	....	....	....	...	....	....	....	...	...	...

TABLE XVIII

MAN–HORSE–OX–PIG–GOAT

		Man bites	Horse bites	Ox bites	Pig bites	Goat bites	Man-ox	Horse-ox	Horse-pig	Horse-goat	Ox-pig	Ox-man horse	Total	Doubtful	Percent man
2 men horse ox-pig goat	ALB..	13	10	43	9	...	6	...	...	...	1	1	83	1	16
	GRAB.	...	6	7	..	1	4	2	1	1	2	...	23	...	...

THE BUDGET

Attached is a copy of the annual financial report as well as a more detailed statement of expenditures for various forms of field work. In addition to the total spent by the Insular Government, $2,700 was spent by municipalities and property owners as follows:

Fajardo Sugar Company	$1,800
Municipality of Fajardo	400
Municipality of Salinas	500
Total	$2,700

Most of the water in which Paris green was used was found in ditches or their equivalent so that cost of work is given on the basis of meters of ditches dusted: Dusting the margins of a river is equivalent to dusting two narrow ditches of equal length. It was found that one man averaged 2.5 kilometers in a day though toward the end of the year the average was nearer 3 kilometers per day. A

half pound of Paris green was used for each kilometer. The total cost was about $0.54 per kilometer dusted.

As the men became more experienced and the campaign well under way the personnel was reduced at Fajardo. Since Fajardo serves as a training base for new inspectors it is difficult to determine actually what personnel is really necessary there, but it is very probable that the campaign can be continued there and the 8,000 people protected for an annual budget of not more than $5,000, of which local funds would amount to one-half. In certain years with low rainfall the cost would be much less.

TABLE XIX

CATCHES OF MOSQUITOES WITH MEN INSIDE THE HOUSE AND ANIMALS OUTSIDE

Date	Inside	Outside	Catch inside house			
			Albimanus		Grabhamii	
			Total	Average	Total	Average
Mayo 15, 1927	4 men	Nothing	26		4	
Mayo 16, 1927	6 men	Nothing	15		1	
Mayo 19, 1927	6 men	Nothing	36		1	
Mayo 22, 1927	6 men	Nothing	33		10	
Mayo 26, 1927	6 men	Nothing	14		4	
Junio 1, 1927	5 men	Nothing	29		4	
Junio 7, 1927	6 men	Nothing	36		2	
Total			189	27	26	4
Mayo 17, 1927	6 men	3 horses	0		2	
Mayo 18, 1927	6 men	1 horses	4			
Mayo 20, 1927	6 men	3 horses	14		2	
Junio 3, 1927	5 men	2 horses	20		3	
Junio 5, 1927	5 men	3 horses	4		1	
Total			42	8	10	2
Mayo 23, 1923	6 men	2 oxen	8		1	
Junio 6, 1927	5 men	3 oxen	1		1	
Total			9	4.5	2	1
Mayo 24, 1927	6 men	5 pigs	11		9	
Mayo 25, 1927	6 men	5 goats	17		7	
Junio 2, 1927	5 men	4 dogs	41		6	