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STUDIES OF TAIWAN LEECHES

II. Field Tests of Effectiveness of Insect Repellents Against Aquatic Leeches at Cha'o Chow, Pingtung, Taiwan[1]

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ABSTRACT

Field tests were conducted at Cha'o Chow, Pingtung, Taiwan, to develop techniques for testing effectiveness of repellents against aquatic leeches; to determine effectiveness of M-1960, benzylbenzoate, and two formulations containing diethyltoluamide against leeches of genus *Hirudo*; and lastly, to compare the degree of protection afforded by untreated Army fatigue trousers with that given by fatigue trousers impregnated with M-1960 or benzylbenzoate. Both a military issue lotion repellent containing diethyltoluamide and a commercial, pressurized spray diethyltoluamide formulation applied to the skin of the legs and feet quickly lost effectiveness when test subjects stood in water in leech infested ditches. Although both formulations gave complete protection against leech attachment for one five minute exposure period, this effect was lost by the end of a second five minute period. Untreated cotton socks plus untreated Army fatigue trousers, and trousers treated with either M-1960 or benzylbenzoate gave complete protection against attachment by aquatic leeches. This was true whether trouser bottoms were tucked into sock tops, or were worn loose but rolled to the level of sock tops. Aquatic leeches at Cha'o Chow attached to hosts throughout daylight hours, and also at least in the early hours of darkness.

In many portions of Southeast Asia blood-sucking terrestrial and aquatic leeches are annoying pests of man and domestic animals. Current interest in the leech control problem is reflected in the numerous recent papers describing leech attacks and effectiveness of insect repellents and clothing in giving personal protection against them (1-5). In spite of this interest, generally accepted techniques for judging effectiveness of repellents against leeches have never been developed. Field and laboratory techniques described to date have varied in detail to a considerable extent, and have involved less than a dozen species of leeches.

This paper was written to describe techniques used in field tests of effectiveness of insect repellents against aquatic leeches in Southern Taiwan; to present specific results obtained in field tests with three repellents, diethyltoluamide, M-1960, and benzylbenzoate; and to record observa-

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tions on habits of blood-sucking leeches of genus *Hirudo* in irrigation ditches in the vicinity of Cha'o Chow, Pingtung, Taiwan.

MATERIALS AND METHODS

1. *Materials:* Repellents used in these tests consisted of three items of military issue, and one commercial preparation. These were:

a. Insect repellent, personal application (75 per cent diethyltoluamide).

b. Insect repellent, clothing application (Formula M-1960).

c. Insect repellent, clothing application (90 per cent benzylbenzoate).

d. A commercially available repellent in pressurized spray form (active ingredients: N,N-diethyl-meta-toluamide—12.75 per cent; other isomers—2.25 per cent).

Clothing used in field tests consisted of U. S. Army fatigue trousers (cotton, sateen) and loosely woven cotton socks purchased on the market in Cha'o Chow.

2. *Personnel:* Test subjects were residents of the Cha'o Chow area. Both adults and children participated in field tests. There was a considerable size variation among test participants.

3. *Leeches:* Blood-sucking leeches available in the Cha'o Chow area were examples of a species of genus *Hirudo* close to *H. nipponia* Whitman. Their biology and taxonomic position is now under investigation in this laboratory.

4. *Testing Procedures:* The basic procedure followed in tests to determine leech population density, and to test effectiveness of repellents applied to the skin, was to have test subjects remove shoes and socks, and either remove trousers or roll trouser legs up above the mid-thigh level. Each subject was then assigned a number, which he retained throughout the test series. Test subjects were directed to specific points along the ditch margin by monitors. Upon a signal, each subject stepped into the water and for one minute either "walked in place" or gently kicked his feet. At the end of one minute a signal was given and all subjects then remained immobile for four minutes. At the end of this time, a signal was given and all subjects stepped up on the bank, where they remained motionless until a leech count had been taken by a test monitor. In early tests, all leeches present were rated as equal and only the number present was recorded. However, since some leeches only "explored" and did not actually attach to the host, this system was changed to separately record those present on the skin but not attached, and those actually attached and feeding.

Tests with untreated clothing and clothing impregnated with repellents were conducted in two ways. First, bottoms of trousers were tucked into the tops of socks. Second, the bottoms of the trousers of test subjects were not tucked into the sock tops, but were rolled to the level of the tops of the socks. *Figures 1* and *2* are photographs taken during these field tests, and show test subjects in position in leech infested ditches.

5. *Selection of Test Areas:* All tests were conducted in irrigation ditches between rice paddies near the town of Cha'o Chow, Pingtung, Taiwan. This area was selected because of its proximity to the Taiwan Provincial Malaria Research Institute where technical assistance was available, and because there were reported to be many leeches in the area. The ditches in which the tests were held varied considerably in width, depth, and density of aquatic vegetation. Width of the ditches varied from 2½ to 10 feet; depth of the water varied from less than one foot to over two and one half feet. In many areas, test subjects sunk five or more inches in bottom mud. At some points ditches were almost choked with matted aquatic plants, while in other areas they were relatively clean. Flow of water and water level varied from day to day. The rate of flow was not sufficient to constitute a flushing action, and produced no noticeable effect on leech populations.

Figures 1. and 2. Photographs taken during field tests of leeches repellents near Cha'o Chow, Pingtung, Taiwan.

Although previous reports by Mr. Lien and his staff had indicated that blood-sucking leeches were numerous in these ditches, the general test area was selected only after visual inspection, and after a test to determine whether blood-sucking leeches were present in sufficient numbers for testing purposes. It had previously been observed that, in most ditches, leeches would appear soon after the marginal vegetation was disturbed with a stick, or when a person stepped into the water. In a preliminary test to determine whether the leeches seen swimming in the ditch were actually blood-suckers, twelve test subjects were employed. These test subjects removed shoes and socks, and either removed their trousers or rolled them up above the mid-thigh level. Subjects were placed approximately ten feet apart along the ditch margin. At a signal they stepped into the water, "walked in place" or kicked their legs for one minute, then stood motionless for four minutes. At a signal they stepped from the water and stood motionless while test monitors counted the leeches present. A second five minute exposure period, conducted in the same manner, commenced five minutes after the end of the first period. Results of this test are summarized in TABLE I.

Observations made during this field test indicated that leeches were not evenly distributed in the ditch, but occurred in "pockets" along the margin of the ditch, or in "islands" of aquatic vegetation. This uneven distribution was reflected in the variation in numbers of leeches found on test subjects exposed for equal periods simultaneously in the same ditch. While this may have been the result of individual attractiveness for leeches, it was more likely due to the uneven distribution mentioned above. It was noted that leeches would often engorge to a considerable extent during a five minute period. When leeches were removed, the wounds caused by their cutting teeth bled freely for some time. In one instance a wound produced by attachment of two leeches at the same site bled freely for 35 minutes, and oozed blood for over five hours. It was observed

TABLE I

Numbers of leeches on bare legs and feet of test subjects at the end of each of two five minute exposure periods in a leech infested ditch at Cha'o Chow, Pingtung, Taiwan

Subject no.	Leeches present after	
	1st exposure period	2nd exposure period*
1	8	6
2	4	5
3	6	3
4	5	3
5	3	6
6	6	14
7	2	0
8	0	0
9	8	5
10	1	2
11	1	0
12	1	3
Total	45	47
Mean	3.75	3.92

* The second exposure period commenced five minutes after the end of the first period. Between periods, all leeches were removed, and blood was wiped off legs. At the conclusion of the second period, test participants washed their legs, then wiped them with 70 per cent ethyl alcohol.

that fresh wounds would quickly be re-attacked if the subject entered the water while blood was still flowing.

Upon reaching the host, the leeches sometimes attached and commenced feeding immediately, but more frequently they "explored" on the skin surface for 10 to 20 seconds or more before selecting a site for attachment.

RESULTS AND DISCUSSION

1. *Tests with personal application "skin" repellents containing diethyltoluamide.*
On 13 April 1964, field tests were conducted with two repellents containing diethyltoluamide. These were the standard Army issue item "insect repellent personal application" (75 per cent diethyltoluamide), and the commercially available product in a pressurized container described previously. Tests were conducted in an irrigation ditch with an average width, at the test site, of 10 feet. Depth at mid-ditch was about three feet, and at the ditch margins varied from one to two feet. At some points test subjects sunk in the bottom mud to a depth of several inches. At the time of the tests water temperature was 32.75 C, Water pH, as determined with pHydrion papers, was 6.5–7.

Test subjects, 14 in number, were divided into three groups. All subjects removed shoes and socks, and either removed trousers or rolled them up to above the mid-thigh level. The four individuals in *Test Group A* received no repellent, and served as controls. The five persons in *Test Group B* each received an application of the previously described commercial spray repellent on feet and legs to the mid-thigh level. Each of the five subjects in *Test Group C* received an application of the issue item "insect

repellent, personal application" on the feet and legs to the mid-thigh level. Because of the considerable variation in the size of test subjects, no effort was made to insure that exactly equal amounts of repellent were applied to each individual. The repellent was applied to all persons in *Group B* by a monitor (REW), and to persons in *Group C* by another monitor (HLK).

The intent was to apply repellent as it actually would be applied in the field by military personnel. An application was regarded as complete when all exposed skin on feet, ankles, and legs to the mid-thigh level was distinctly "wet" with repellent. As soon as repellents had been applied and had "air dried" for approximately five minutes after application to the last subject, the test participants were stationed at about five foot intervals along the stream margin. Upon signal, all subjects stepped into the water, "walked in place" or kicked their feet for one minute, and then, upon signal, stood motionless for four minutes. At the end of this period, they stepped from the water and stood in place on the bank while leech counts were made by the monitors and attached leeches were removed. Five minutes after termination of the first exposure, subjects again stepped into the water for an identical exposure period of five minutes. Subjects were cautioned not to go into the deeper portion of the ditch where water level would reach unprotected skin above mid-thigh level. Results of this test are summarized in TABLE II.

As indicated in TABLE II, neither of the repellents was so effective as to keep subjects entirely free of leeches. Although the mean number of leeches present on both protected groups after the first exposure was only half that of the control group, this difference was much less following the second exposure.

TABLE II

*Effectiveness of two insect repellents containing diethyltoluamide against aquatic leeches**

	Leeches present on feet and legs after	
	1st five minute exposure period	2nd five minute exposure period
Group A Control group (no repellent).		
Subject no. 1	2	7
2	5	5
3	5	4
4	4	4
Total	16	20
Mean	4	5
Group B Protected by a commercial pressurized spray repellent.		
Subject no. 5	1	3
6	6	7
7	2	3
8	0	3
14	1	4
Total	10	20
Mean	2	4

Group C	Protected by military issue lotion type of repellent.		
Subject no.	9	1	2
	10	1	1
	11	3	5
	12	5	4
	13	0	5
	Total	10	17
	Mean	2	3.4

* Repellent was applied to the feet and legs up to the mid-thigh level.

The results presented in TABLE II were not unexpected in view of the known solubility of diethyltoluamide in water. The increase in leeches present on both control and protected groups, as a result of the second exposure, reflected increased activity of leeches in the ditch area where the test was conducted, probably due to the arrival of specimens from outside the immediate test area. Any disturbances in the water attracted leeches from some distance away. While enroute to the source of a disturbance leeches did not always go directly to the host, but frequently stopped to "explore" objects in the water, and at times bypassed the host, only to double back after a short time.

During these tests, it was noted that not all of the leeches found on test subjects were actually attached and feeding. Some dropped off within a few seconds after the host left the water. This raised the question as to whether leeches might be repelled to the extent that they would not feed, even though they might "explore" on the skin surface, or even adhere briefly when the host left the water. Because of this, is was decided that a more practical criterion for judgement of repellency would be to count leeches actually attached and feeding, in addition to those merely present. With this in mind, tests to determine repellency under this new criterion were carried out in the same test area by the writers. These tests were conducted under the same general circumstances previously described, except that in each case repellent was applied to the left leg only. The right leg was not treated and served as a control. One test, with two exposure periods, was conducted in a shaded portion of the ditch. Two additional tests, also with two exposure periods each, were conducted in direct sunlight in another portion of the ditch. In the latter area, the bottom vegetation was quite heavy, and in several instances the leeches were seen to swim and crawl over the top of the vegetation mat to reach the test subject. Tests commenced at 1015 hours. At this time water temperature was 24.75 C, and pH remained at 6.5–7. During these tests, the distance between subjects was approximately eight feet. Results of these tests are summarized in TABLE III.

Results obtained in this test series seemed to confirm the previously expressed opinion that a more accurate judgement of repellent effect could be obtained if specimens actually attached were counted separately from those only present. Although test results indicated a marked loss of effectiveness of both repellents following the first five minute exposure period, neither subject suffered a leech attachment during the first period, although leeches were present on treated legs in two of the three tests.

2. *Field test of protection against leeches afforded by untreated trousers and socks, and by trousers treated with M–1960 and benzylbenzoate.*

These tests were conducted in the Cha'o Chow area on 15 April 1964. Fatigue trousers were impregnated with repellents

TABLE III

*Individual tests of effectiveness of two insect repellents containing diethyltoluamide against aquatic leeches**

Test 1

(in shaded portion of ditch)

	Leeches present on bare feet and legs after			
	1st five minute exposure period		2nd five minute exposure period	
	Untreated leg	Treated leg	Untreated leg	Treated leg
Subject A**	1*A* 3*P*	2*P*	2*A* 1*P*	2*A*
Subject B***	4*P*	2*P*	1*A* 2*P*	2*A* 2*P*

Test 2

(in direct sunlight)

	Leeches present on bare feet and legs after			
	1st five minute exposure period		2nd five minute exposure period	
	Untreated leg	Treated leg	Untreated leg	Treated leg
Subject A	3*A* 2*P*	0	1*A* 4*P*	4*A* 2*P*
Subject B	1*P*	0	1*P*	1*A* 1*P*

Test 3

(in direct sunlight)

	Leeches present on bare feet and legs after			
	1st five minute exposure period		2nd five minute exposure period	
	Untreated leg	Treated leg	Untreated leg	Treated leg
Subject A	4*A* 4*P*	6*P*	8*A* 2*P*	4*A* 2*P*
Subject B	3*A* 2*P*	2*P*	3*A* 1*P*	5*A*

* Repellent applied to bare feet and legs up to level of mid-thigh.

** Protected with military issue lotion type of repellent.

*** Protected with commercial pressurized spray repellent formulation.

Note: In leech counts the letter *A* stands for leeches attached and feeding. The letter *P* stands for leeches present on the skin but not attached.

in the manner prescribed on repellent containers. On the day before group tests were conducted, the writers carried on preliminary tests with treated trousers and cotton socks. It was found that both treated and untreated trousers and cotton socks gave complete protection against leeches. This was true whether trouser bottoms were tucked into sock tops, or whether they were rolled up to the level of the sock tops. With this experience as a background, field tests, utilizing 20 subjects, were conducted on 15 April. Subjects were divided into four groups of five men each. Men in *Grovp A* wore untreated fatigue trousers with trouser bottoms tucked into the tops of cotton socks. Men in *Group B* wore untreated trousers, but with the bottoms rolled to the level of the sock tops. Subjects in *Group C* wore fatigue trousers impregnated with benzylbenzoate. Subjects in *Group D* wore fatigue trousers impregnated with M-1960. Subjects of *Groups C* and *D*

tucked bottoms of trousers into sock tops. Actual conduct of the tests was as previously described. Exposure periods were of five minutes duration. At the end of the test, trousers and socks were removed and each subject was carefully examined to determine whether leeches had penetrated trousers or socks, or had gained access bewteen the sock tops and loose, rolled trouser bottoms. Results of these tests are summarized in TABLE IV.

Results of these tests were similar to

TABLE IV

Effectiveness of untreated cotton socks plus untreated Army fatigue trousers, and fatigue trousers impregnated with M-1960 or benzylbenzoate in protecting wearers against attacks by blood-sucking aquatic leeehes.

	Leeches present on trousers, socks, or skin after	
	1st five minute exposure period	2nd five minute exposure period
Group A Untreated trousers tucked in sock tops.		
Subject no. 1	0	0
2	1 clinging to cloth.	0
3	0	0
4	0	0
5	0	0
Group B Untreated trousers rolled to level of sock tops.		
Subject no. 6	0	0
7	0	2 inside trouser leg. 2 outside on cloth.
8	0	0
9	0	0
10	0	0
Group C Trousers impregnated with M-1960.		
Subject no. 11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
Group D Trousers impregnated with benzylbenzoate.		
Subject no. 16	0	0
17	0	0
18	0	0
19	0	0
20	0	0

those obtained by Audy and Harrison (2) who found that clothing alone provided quite good protection against the aquatic leech, *Poecilobdella manillensis*, even when the trouser legs were not tucked into sock tops. However, Harrison, Audy, and Traub (4) reported that treated, knitted woolen socks proved of little value against aquatic leeches. Audy and Harrison (2) found that protective value of M-1960 diminished considerably after two washings, particularly when trousers were not tucked in sock tops.

3. *Nocturnal activity of leeches.*

For convenience of observation, as well as to avoid venomous snakes such as the krait and cobra which were active at night in this area, all tests previously described were conducted during the daytime. Moreover, one of the employees of the Taiwan Provincial Malaria Research Institute who had collected large numbers of leeches for this laboratory during January 1964 had stated that leeches in the area did not attack at night, but could easily be collected as they adhered, partially out of the water, on vegetation. He further stated that if leeches were disturbed at night they would swim away and would not attack. In order to test the validity of this information, the writers, and the informant mentioned above, conducted a test consisting of three five minute exposure periods commencing after dark at 1855 on 15 August. At this time the water temperature was 26 C. Test procedures were as previously described. The participants removed shoes and socks, stepped into the water, "walked in place" or kicked for one minute, then stood motionless for four minutes during each exposure. Lights were turned off during exposure periods, and were used only to count leeches present at the end of each period. Results are summarized in TABLE V.

TABLE V

*A field test of feeding activity of aquatic leeches at night at Cha'o Chow, Pingtung, Taiwan**

	Leeches on bare skin of legs and feet after		
	1st five minute exposure period	2nd five minute exposure period	3rd five minute exposure period
Subject A	1*A*	5*A* 2*P*	5*A*
Subject B	2*A*	4*A*	3*A*
Subject C	2*A* 2*P*	3*A* 1*P*	7*A*

* As determined by attachment to skin of feet and legs of test subjects during three consecutive five minutes exposure periods.

Note: The unanimous conclusion reached, after *very little* debate by all investigators, may be briefly stated: In the Cha'o Chow area, at least, aquatic leeches *will* attack at night.

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