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TRANSMISSION, CONTROL AND TREATMENT OF INFECTIOUS DISEASES OF MILITARY IMPORTANCE IN EQUATORIAL ASIA

ANNUAL REPORT

Dr. G.F. de Witt
LTC D.L. Huxsoll

1976

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U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D. C. 20314

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U.S. Army Medical Research Unit
Institute for Medical Research
Kuala Lumpur, Malaysia

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75 10 - 76 09 Studies of febrile patients in indigenous populations have shown that scrub typhus is a common but frequently undignosed, cause of febrile illness. The clinical syndrome whether mild or severe, was difficult to distinguish clinically from that due to other infections. The antigenic properties of human isolates and the antibody response to infection have shown that the Karp strain group produces the preponderance of symptomatic infections in the study area. The strain activity in humans, rodents, and chiggers in selected study sites is being compared. In studies with →

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U.S. Army Medical Research Unit
Institute for Medical Research
Kuala Lumpur, Malaysia

U.S. ARMY MEDICAL RESEARCH
AND DEVELOPMENT TECHNICAL REPORT

1 October 1975 - 30 September 1976

SUMMARY

Studies of febrile patients in indigenous populations have shown that scrub typhus is a common, but frequently undiagnosed, cause of febrile illness. The clinical syndrome whether mild or severe, was difficult to distinguish clinically from that due to other infections. The antigenic properties of human isolates and the antibody response to infection have shown that the Karp strain group produces the preponderance of symptomatic infections in the study area. The strain activity in humans, rodents, and chiggers in selected study sites is being compared. In studies with immunosuppressants cyclophosphamide treated mice died following inoculation of a normally nonlethal strain. In a study of mammals and chiggers from a primary forest only a single specimen of a known vector of R. tsutsugamushi was collected. L. L. fletcheri and L. L. arenicola colonies infected with R. tsutsugamushi have been employed to study the effects of temperature on chigger development and transmission of the infection.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals, DHEW Publication No.(NIH) 73-23, as Prepared by the Institute of Laboratory Animal Resources, National Research Council."

TABLE OF CONTENTS

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23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23.(U) To investigate the incidence of scrub typhus infection in selected local and foreign populations, to relate this to the prevalence of vector chiggers and their common rodent hosts, to determine the strains of R. tsutsugamushi causing human infections and relate to clinical disease, to develop improved serological methods, to study the basis for immunity, to define vector-rickettsia relationships, to develop a laboratory animal model for the disease, and to produce laboratory animals in support of the mission. Scrub typhus is an incapacitating disease of military importance.

24.(U) Coordinated studies are being conducted at selected field sites in an effort to relate the incidence of scrub typhus in humans to numbers of vector chiggers, rodent hosts, and the strains of R. tsutsugamushi prevalent in each. Standard techniques developed here and in other laboratories are employed in other aspects.

25.(U) 75 10 - 76 09 Studies of febrile patients in indigenous populations have shown that scrub typhus is a common, but frequently undiagnosed, cause of febrile illness. The clinical syndrome whether mild or severe, was difficult to distinguish clinically from that due to other infections. The antigenic properties of human isolates and the antibody response to infection have shown that the Karp strain group produces the preponderance of symptomatic infections in the study area. The strain activity in humans, rodents, and chiggers in selected study sites is being compared. In studies with immunosuppressants cyclophosphamide treated mice died following inoculation of a normally nonlethal strain. In a study of mammals and chiggers from a primary forest only a single specimen of a known vector of R. tsutsugamushi was collected. L.L. fletcheri and L.L. arenicola colonies infected with R. tsutsugamushi have been employed to study the effects of temperature on chigger development and transmission of the infection.

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Project 3A762759A831 TROPICAL MEDICINE

Task 00 Tropical Medicine

Work Unit 071 Field Studies of Rickettsioses and Other Tropical Diseases

Investigators:
Principal: LTC David L. Huxsoll, VC
Associate: LTC David M. Robinson, VC; MAJ Grahaem W. Brown, RAMC; MAJ Richard D. Montrey, VC; MAJ Alexander L. Dohany, MSC; Miss Elsie Gan, B.S.

CLINICAL AND EPIDEMIOLOGICAL STUDIES OF SCRUB TYPHUS

The studies on "normal" populations, described in the Progress Report for 1975, yielded good evidence for a high incidence of scrub typhus transmission in the areas studied. Due to the success of other studies, the surveys at Pos Iskandar, Bukit Lanjan and Elmina Estate have been discontinued for the present. Likewise, the study of indigenous soldiers has been suspended, useful data having been obtained. The investigation of febrile patients in rural Malaysia, described in the Progress Report for 1975, has been continued this year. Over 120 human isolates of R. tsutsugamushi have been made, and a wealth of clinical and serological data collected.

Normal Populations

Orang Asli (Aborigines): Pos Iskandar is situated in an area of secondary jungle, 35 miles N.E. of Kuala Pilah and 67 miles East of Kuala Lumpur. It is the focal point for several Orang Asli kampongs scattered around a marshy lake, Tasek Bera, and has a school, and a clinic staffed by a medical orderly. About 1000 people live in the area, subsisting largely on shifting cultivation of hill rice, tapioca, etc., in the surrounding jungle. Rubber has recently been planted in a few areas close to the villages.

Sera were collected from 208 volunteers of all ages during the first visit in January 1975. The system of agriculture necessitated families moving out of their village houses to guard the crops in the jungle ladangs for periods of several weeks at a time. Thus it was difficult to find all of the same individuals regularly. Sequential specimens of serum were collected from 127 individuals (71 twice, 44 three times, 11 four times and 1 five times), over a period of 8 months (January-August 1975). A total of 462 man-months of exposure was studied.

The age specific prevalence rates of scrub typhus antibody at Pos Iskandar are shown in Table 1, using data from the largest group bled simultaneously, in January 1975.

During the following 8 months of observation, 46 individuals showed a rise in titer (see Table 2). In 18 individuals, there was a 4-fold or greater rise in antibody to a titer of at least 1/200, and in none of these were malaria parasites or leptospiral antibody found. In 8 of the 18, the maximum titer was 1/200, including one person who showed a rise in OXK titer from 1/40 to 1/320. In 5 the titer rose to 1/400, and in a further 5, to 1/800 or more. Only 5 (28%) of these 18 people admitted having had a febrile illness during the intervals between collection of the sera. Of the remaining 28 whose antibody titer rose from <1/25 to 1/50, or from ≤1/25 to 1/100, 6 (21%) had malaria parasites. None showed a rise in leptospiral antibody. Two (7%) showed a rise in OXK titer from 1/40 to 1/160. Only 3 (11%) admitted having had a relevant illness.

Only the 18 subjects showing a 4-fold rise in FA titer to at least 1/200 were regarded as confirmed infections. This is equivalent to an incidence of 468/thousand/annum. However, the study was carried out during only part of a year, and most of the sera were collected from January to April.

Bukit Lanjan: Bukit Lanjan is a small forested hill 5 miles West of Kuala Lumpur. Approximately 250 Orang Asli live in a kampong on the jungle fringe, and pursue a variety of urban and rural occupations, including much travel through the jungle. The preliminary survey of the population, using filter paper blood spots, was carried out in 1974, and reported in last year's Progress Report. Sequential sera and clinical data were collected from October 1974 to May 1975, by which time the numbers of volunteers had fallen to an unproductive level.

Of 61 individuals from whom sequential sera were obtained over a total of 234 man months, 8 showed a rise in FA titer (see Table 3) and 2 a concurrent rise in OXK. Of the 8, 4 recalled an illness during the relevant period, and one (H48) had been admitted to Gombak hospital for fever and chills; an isolate of R. tsutsugamushi was obtained from her by mouse inoculation. Five of the 8 had significant antibody rises, an incidence of 5/234 man months, or 256/thousand/annum.

Elmina Estate: A pilot survey using filter paper blood spots showed that 54/135 (40%) of oil-palm workers and 14/93 (15%) of rubber workers had demonstrable antibody to R. tsutsugamushi. In a longitudinal study of oil-palm laborers, sera were obtained on one occasion only from 73 workers, on 2 occasions from 26, 3 times from 12, 4 times from 11 and 5 times from 2. Thus multiple observations were made on 51 individuals over varying periods from 1 to 10 months, and a total of 273 man months of exposure were studied.

Table 1

Prevalence of Scrub Typhus Antibody at Pos Iskandar

Age Group	Prevalence of Antibody[1]
<5	1/3 (33)[2]
5-9	2/28 (7)
10-14	11/44 (25)
15-24	16/37 (43)
25-34	20/34 (59)
35-44	29/38 (76)
>44	20/24 (83)

1. A titer of 1/50 or greater.
2. Positives/total (percent)

Table 2

Seroconversions to Scrub Typhus at Pos Iskandar

Age Group	Numbers Showing Antibody Rise	
	Possible[1] Significance	Definite[2] Significance
<5	1	0
5-9	9	0
10-14	7	4
15-24	6	1
25-34	1	5
35-44	3	3
<44	1	5

1. Includes rises in titer from <1/25 to 1/50 and from ≤1/25 to 1/100.
2. Includes 4-fold or greater rises in titer to 1/200 or more.

Table 3

Seroconversions to Scrub Typhus at Bukit Lanjan

Patient	Age	Sex	FA	OXK	Illness	M.P's
H 48	15	F	1/25 1/800	1/80 1/5120	+	-
H 130	8	F	1/50 1/800	1/40 1/40	-	P. vivax
H 1014	14	F	<1/25 1/50	1/40 1/40	+	-
H 1003	6	M	1/50 1/200	1/80 1/80	-	-
H 146	6	F	<1/25 1/50	1/80 1/320	+	P.falciparum
H 144	14	F	<1/25 1/400	1/80 1/40	+	-
H 21	8	F	<1/25 1/50	1/80 1/40	-	-
H 154	12	M	<1/25 1/100	1/80 1/80	-	-

Two workers showed a significant rise in titer of antibody to R. tsutsugamushi during this time (see Table 4), and two more lesser rises, one from 1/25 to 1/100, the other from <1/25 to 1/50. Three of the 4, including the 2 showing definite rises (1254 and 1225), admitted having had a febrile illness between the dates of bleeding. None of the illnesses had been prolonged or serious and none, as far as is known, required antibiotic therapy. The subject showing a rise to 1/50 did not recall any illness during the relevant period. From this data, the incidence of definite infections was calculated as 2 per 273 man months, or 88/thousand/annum.

Rodent trapping from a grid system consisting of 200 traps was accomplished at Elmina throughout the entire year. The trapping was conducted for 1 week per month with marking and releasing of the trapped rodents. A total of 696 rats were trapped from which 623 blood samples were taken for isolation studies. R. tsutsugamushi was isolated from only 2 (0.32%) of the specimens. Chiggers were collected from all trapped rodents. In addition to collection of chiggers from rodents, a black plate grid of 22 sites within the trapping grid was established, with 10 plates per site for a total of 220 black plates.

The primary vector found within this mature oil-palm estate was Leptotrombidium (L.) deliense (61.1%). Other Leptotrombidium collected were L. (L.) fletcheri (0.1%), L. (L.) bodense (0.2%) and L. (L.) near-arenicola (0.03%). Other than L. (L.) deliense, Ascoschoengastia (L.) indica was the only other chigger collected in large numbers (30.0%). The remaining species were Gahrliepia (W.) lewthwaitei (3.7%), G. (W.) enode (0.02%), G. (G.) fletcheri (0.6%), Walchiella impar (0.06%), and A. (L.) lorius (0.4%).

In comparing the chiggers collected from rodents with a black plate chigger index (number of chiggers collected each month divided by the total number of plates) the data tends to follow a parallel line and may prove to be a valuable tool for comparing chigger populations in the field (Fig. 1).

Military Populations (Malaysian Soldiers): Two hundred and seventy five recruits were bled before and after 6 months basic training at Port Dickson. Forty seven (17%) of the patients had FA titers of 1/25 or more. Of these, 6 (2.2%) showed a significant rise in titer between the two specimens, indicating infection in the interval. Three of the 6 admitted having had a febrile illness during training, and one man had been ill for 2 weeks. The remaining 3 men denied any illness during the study period. Four hundred and seventy six soldiers from two infantry battalions (2 Ranger and 11 MTA) were bled before and after a period of operational duty near the Thai border, in June and October 1975. On the second occasion information was obtained, by means of a questionnaire, on any illnesses experienced. Both specimens were

Table 4

Seroconversions at Elmina Estate

Patient	Age	Sex	Date	FA Titer	OXK Titer
1254	22	F	20 January	1/50	1/40
			20 March	1/50	1/40
			19 June	1/400	1/320
			20 August	1/100	1/320
1225	23	M	20 January	<1/25	1/40
			20 March	1/200	1/40
			19 November	1/200	1/80

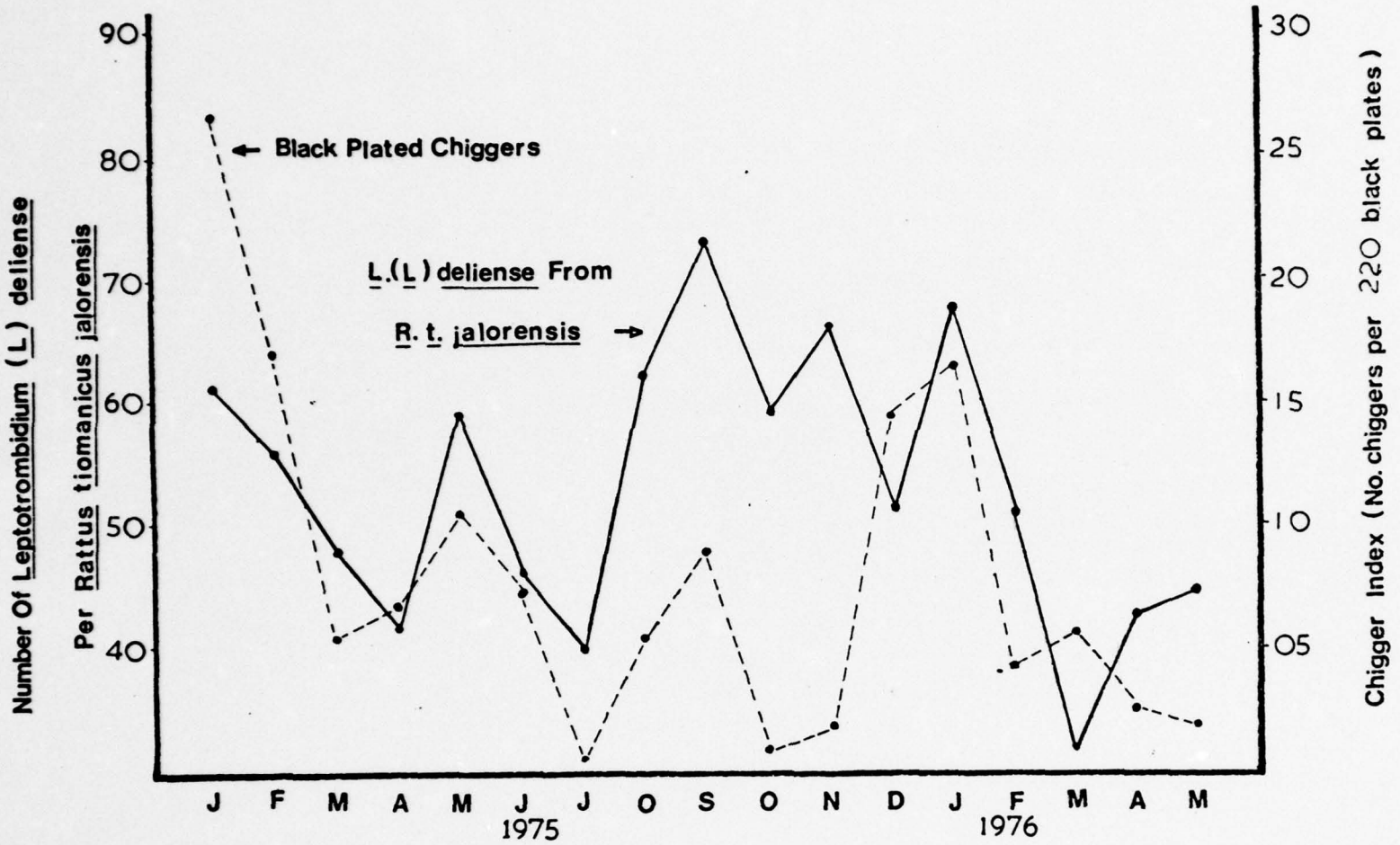


Figure 1. Comparison of black plate collections of chiggers with the number of Leptotrombidium (L.) deliense collected from trapped Rattus tiomanicus jalorensis within an oil-palm estate, Elmina Estate, Selangor.

negative in 235 (49%). In 210 (44%) one or both sera were positive at low levels, though no rise was detected. Eight of 262 (3.1%) of the 11 MTA group had a titer of $\geq$1/400 in the first serum, indicating infection during the few months prior to our study. This evidence was re-inforced by OXK titers of $\geq$1/160 (1/2560 in one) in 6 of the 8. Small rises in FA, from <1/25 to 1/50 or $\leq$1/25 to 1/100 occurred in 11 (2.3%) of the whole group, but in only one was this accompanied by a rise in OXK titer (1/40 to 1/160). Of the 11, 5 (45%) admitted a febrile illness, though no details were available. Significant rises in FA were seen in 2 (0.4%) of the group: 1/100 to 1/400 in one case (no rise in OXK) and 1/50 to 1/400 in the other (OXK 1/80 to 1/160). Neither subject admitted any illness during the period.

Military Populations (British Soldiers): In last year's progress report, the occurrence of low level seroconversions amongst visiting soldiers, often without illness, was reported. This was examined further in 98 additional men, of whom 13 (13%) developed antibody to a titer of 1/50, after a 6-8 week period of jungle training. Sera collected prior to the training exercise had no demonstrable antibody (<1/25). None had clinical malaria nor serological evidence of leptospirosis. The majority denied any illness whatsoever. Blood was collected from each of the 98 men and inoculated into mice. No isolates were made from the mice and the significance of these small rises remains uncertain, as in the indigenous soldiers.

Febrile Patients

Studies were carried out at the district hospitals of Mentakab and Kuala Pilah, the Orang Asli hospital at Gombak, the General Hospital and Kinrara Military Hospital in Kuala Lumpur, and the health sub-center at Bukit Mendi.

Bukit Mendi health center is located on a Federal Land Development Authority (FELDA) oil-palm plantation in southern Pahang. An area of disturbed primary jungle, approximately eight miles by ten has been partially cleared, burned and planted over the past nine years. Most of the plantation is now in production, and approximately 10,000 people have settled, in four villages, on the scheme. Adults of both sexes work in the fields, and children sometimes accompany them. A health sub-center, visited weekly by a doctor, provides medical services for the inhabitants, and was the location for the study.

Mentakab District Hospital serves an area of central Pahang containing many similar FELDA schemes, and also a large population of rural villagers. Kuala Pilah Hospital, in Negri Sembilan, serves a rather different, semi-urban population, and rubber estate and rice field workers predominate over relatively few oil-palm laborers. Gombak Hospital admits Orang Asli patients from all over

peninsular Malaysia though many patients, including those with fever, find the long journey too difficult to make. At the General Hospital, Kuala Lumpur, the study was attempted on one of the 3rd class male medical wards and at Kinrara on febrile soldiers. However, sera were obtained from only a small number of febrile patients, and these two studies had to be abandoned after several months.

At each location, clinical and epidemiological data, and paired sera were collected from unselected febrile patients. In many instances, blood was also inoculated into mice for isolation of R. tsutsugamushi. Patients complaining of headaches, cough or general malaise were also included, even if not febrile at presentation. Young children, from whom venous blood could not easily be obtained, were largely excluded from the study. Convalescent sera were collected two weeks after the acute specimens wherever possible, though early discharge from hospital necessitated a shorter interval in many instances. All clinical data was gathered by the clinic and hospital staffs. With the exception of Kuala Pilah, most of the specimens were collected by our technicians.

The paired sera from all study sites were examined for R. tsutsugamushi antibody by indirect immunofluorescence, and for Proteus OXK agglutinins by the Weil-Felix test. OX2 and OX19 agglutinins were not sought. All sera were stored at -20°C from the time of separation. Results were notified to the responsible doctor as soon as possible after collection of specimens.

Isolation and identification of the organism was performed using the standard technique, in which fresh whole blood is inoculated intraperitoneally into mice. Isolates were confirmed by direct immunofluorescent examination of peritoneal monoclear cells from the infected mice.

A compatible clinical history, plus any of the following criteria was regarded as confirming the diagnosis:

1. isolation of the organism
2. a four-fold rise in FA titer to 1/200 or more
3. a four-fold rise in OXK titer to 1/160 or more

Either of the following findings was regarded as indicating concurrent or recent infection:

1. an FA titer of at least 1/400, with no demonstrable rise
2. an OXK titer of at least 1/160,with no demonstrable rise

A summary of the results from the six studies is given in Table 5.

Table 5

Incidence of Scrub Typhus in Febrile Patients

	Definite	Probable	Total
Mentakab	47 (19.2)[1]	11 (4.5)	245
Kuala Pilah	33 (5.3)	20 (3.2)	621
Bt. Mendi	49 (13.1)	17 (4.5)	374
Gombak	8 (7.4)	12 (11.1)	108
G.H.K.L.	0	0	15
Kinrara	0	0	15

1. Number of patients as a percentage of the total studied at each location.

The low number of cases documented at Gombak was probably the result of self-selection by febrile Orang Asli, who appeared to stay at home rather than go to hospital. The large number of Orang Asli with high titers, but no rise, may well represent recent but not current infections. Some cases may have been treated with tetracycline at the jungle medical posts. The much higher incidence at Mentakab hospital as compared with Kuala Pilah probably reflects the difference in habitats - a lot of the area surrounding Mentakab is now planted with oil-palm, or is 'scrub' vegetation, whereas the padi fields and rubber estates so common around Kuala Pilah offer less favorable ecological conditions for the vectors.

From the results obtained, it is evident that scrub typhus is a much more common cause of illness than was previously suspected, and that the clinical syndrome, whether mild or severe, is difficult to distinguish from that due to other infections. Eschars, rashes and adenopathy were not usually observed. The infection was particularly prevalent in oil-palm workers, causing an estimated 400 cases annually in the population of 10,000 people living on the Bukit Mendi scheme alone.

Rodent and chigger collections were begun in Bukit Mendi in July 1975, with initial collections being made as a general survey of the various habitats of the entire scheme. When it was determined that a specific area (Phase III) was producing a high incidence of scrub typhus cases, study sites within this Phase were established. Grid trapping systems were established allowing for trapping, marking and releasing, and retrapping of the rodents, with collection of chiggers and blood specimens from each rodent trapped. Two such grids consisting of 100 traps each were set up in Phase III oil-palm. More recently, similar grids have been established in a Phase I oil-palm site which has yielded only a few positive cases of scrub typhus.

From the general survey prior to the establishment of the grids, a total of 10 species of small mammals were trapped, with R. tiomanicus being the dominant species within the entire area, and R. argentiventer and R. exulans being sub-predominant. The rest of the trapped animals included: Tupaia glis, R. muelleri, R. cremoriventer, Callosciurus nigrovittatus, C. notatus, C. caniceps, and Sundasciurus tenuis.

During the general survey, R. tiomanicus had an infection rate of 41.6% (32/77) from Phase I oil-palm, 37.5% (3/8) from Phase III, 37.5% (20/56) from lalang and 27.0% (10/37) from fringe habitat. In R. argentiventer only 16.7% (1/6) from lalang was positive, and none from Phase I oil palm (2) and fringe (4) was positive, thus giving an overall rate of 8.3% (1/12). R. exulans from lalang had 23.8% (5/21) positive. Two R. exulans from Phase I oil-palm and 1 from the fringe were negative. Tupaia glis 22.2% (4/18) from Phase I oil-palm and fringe habitat were found to be infected with R. tsutsugamushi. T. glis within lalang (1) and Phase III (10) were

all negative. None of the other species was found to be positive for R. tsutsugamushi.

L. (L.) deliense was the predominant vector chigger species collected from the four types of habitat during the general survey of Bukit Mendi, with 76.8% from lalang, 49.8% from forest fringe, 70.5% from Phase I and 72.6% from Phase III. L. (L.) fletcheri was also collected from the lalang habitat (14.0%). The collection of large numbers of L. (L.) deliense from the lalang demonstrates the active movement of the chigger hosts between the lalang and adjacent scrub, as L. (L.) fletcheri is the predominant species collected from black plates within West Malaysia.[1]

Within the grid systems of Phase III the first area (FF) is a mixed habitat of oil-palm and adjacent swamp forest. Results of the prevalence rate of R. tsutsugamushi isolation from small mammals trapped in this area are shown in Table 6. The trapping and recapture rates were so low that it is not possible at this stage to assess any population fluctuation of any species of the small mammals obtained. Altogether eight species of small mammals were trapped, of which only two species, R. tiomanicus and R. argentiventer were found in the oil-palm habitat. The remaining six species were all from the swamp forest. R. tsutsugamushi was isolated from R. tiomanicus and R. argentiventer. The rest of the species from the swamp forests were negative. Of 60 blood samples from R. tiomanicus 8 (13.3%) were found infected and from R. argentiventer 33.3% (3/9) were infected.

The second area (YY) is exclusively planted with 3-4 year old oil-palm trees. Only two species of rats, R. tiomanicus and R. argentiventer were trapped in this area. (Table 6) Like the first area the trapping and recapture rates were very low. However, 26.3% (10/38) blood specimens collected from R. tiomanicus were positive for R. tsutsugamushi as compared to 43.48% (10/23) from R. argentiventer.

In both these study areas, R. argentiventer was shown to have a higher rate of isolation than that of R. tiomanicus, although the numbers caught were lower than R. tiomanicus.

The trees of the FELDA scheme are planted in rows approximately 6 meters apart. The litter from pruning the trees is placed in a straight line between every other row of trees. The alternate rows are left clear. This is the case in both Phase I and Phase III of Bukit Mendi. These piles of litter provide good harborage for the rodent hosts of the vectors.

The most noticeable difference between the study areas of Phase I and those of Phase III, is the lack of grass covering within Phase I sites. The covering within Phase I is primarily ferns, while that of Phase III is grass and vines.

Table 6

Prevalence of *Rickettsia tsutsugamushi* Isolation (Blood) from Small Animals in Phase III, Bukit Mendi.

Site	Species of Mammals	Habitat	Total Mammals Tested	Number Positive	Percent Positive
FF	*R. tiomanicus*	Oil-palm	60	8	13.3
	R. argentiventer	Oil-palm	9	3	33.3
	R. whiteheadi	Forest	4	0	0
	Tupaia glis	Forest	5	0	0
	R. muelleri	Forest	1	0	0
	S. tenuis	Forest	7	0	0
	C. notatus	Forest	2	0	0
	E. gymnurus	Forest	1	0	0
YY	*R. tiomanicus*	Oil-palm	38	10	26.3
	R. argentiventer	Oil-palm	23	10	43.5

Table 7 presents a comparison of the vector L. (L.) deliense collected from R. jalorensis from both the Phase I and Phase III study sites. During the three months from which data is available, the number of L. (L.) deliense per rodent collected from Phase I has remained fairly constant, ranging from 7.4 to 10.7, whereas the L. (L.) deliense per rodent from Phase III has fluctuated considerably, ranging from 3.6 to 27.7. During much of this time the area was experiencing extreme drought. However, just prior to the high number (27.7), showers were occurring daily for approximately 10 days. As the rodents within Phase I can only acquire the chiggers from the litter piles, and as these would probably retain a more suitable environment for the chiggers even during a dry period, one might expect the number of chiggers per rodent to remain more constant. Whereas within the Phase III area, where the rodents would not only acquire the chiggers from the litter, but also from the open grassy areas, which would be highly affected by changes in moisture, a greater fluctuation of numbers of chiggers per rodent might be expected.

Weil-Felix Test

A comparison was made of three methods for the Weil-Felix test, using paired sera from 50 scrub typhus patients. The methods were:

1. the tube agglutination technique, with a 4 hour incubation period, recommended by the manufacturer of the antigen (Wellcome Reagents, Beckenham, England).

2. the standard tube agglutination technique of Shaffer and Goldin[2] with overnight incubation.

3. the microtiter adaptation of (1), using v-type microtiter plates (Cooke Engineering Co., Alexandria, Va) as described by Gaultney, Wende and Williams.[3]

The OXK results obtained by each method were essentially the same, but the microtiter method was easier to read, and quicker to perform, and much more economical of antigen and serum.

Using the microtiter technique, OXK agglutination titers were measured in 225 control subjects (see Table 8). There was confirmation of earlier observations, that rises in titer occur also in leptospirosis, and malaria. OXK titers measured in 209 sera obtained from 112 isolate positive scrub typhus patients are shown in Table 9.

LEPTOSPIROSIS

The sera collected in the Mentakab, Kuala Pilah and Bukit Mendi studies have also been examined by the HL technique for leptospiral antibody. The results are shown in Table 10. These

Table 7

Comparison of Total Chiggers and Vector Chiggers L. (L.) deliense Collected from Rattus tiomanicus jalorensis from Phase I and Phase III Oil-Palm, Bukit Mendi, Pahang.

Month	Number of Chiggers per R. t. jalorensis		Number of L. (L.) deliense per R. t. jalorensis	
	Phase I	Phase III	Phase I	Phase III
February	26.5	36.5	10.7	13.8
March	33.7	64.3	7.8	3.6
April	28.5	52.5	7.4	27.7

Table 8

OXK Titers in 225 Control Subjects

Source		Reciprocal Titers						Total Sera
		≤40	80	160	320	640	1280	
50	British soldiers	39	11	-	-	-	-	50
50	Malaysian soldiers	41	9	-	-	-	-	50
25	Malaria[1,2] patients	34	12	3	-	1	-	50
100	Leptospirosis[1,3] patients	66	68	51	8	6	1	200

1. Acute and convalescent sera from each patient.
2. A 4-fold rise in titer occurred in 3 patients.
3. A 4-fold rise in titer occurred in 12 patients.

Table 9

OXK Titers in 112 Scrub Typhus Patients[1]

Day of Illness[2]	Reciprocal Titers						Positive[3]/Total (Percentage)
	≤40	80	160	320	640	≥1280	
1-7	42	17	16	9	6	3	34/93 (37)
8-14	4	11	13	9	10	18	50/65 (77)
15-30	3	4	6	8	8	22	44/51 (86)

1. 209 sera from 112 isolate positive patients.
2. As reported by the patients.
3. Titer ≥1/160.

Table 10

Leptospirosis[1] in Unselected Febrile Patients

	Mentakab Hospital	Kuala Pilah Hospital	Bukit Mendi clinic
Leptospirosis	26 (8)[2]	41 (6)	19 (4)
Total Patients[3]	318	688	431

1. Criterion for diagnosis: a 4-fold or greater rise in HL titer.
2. Number of patients as a percentage of the total at each location.
3. Includes all patients with paired sera collected 3 or more days apart.

results differ from those in previously reported series from Malaysia in that these patients were not selected for symptoms, signs, age or sex. Thus a balanced indication of the overall importance of the infection as a cause of fever is obtained. Noteworthy features in this series are (1) the number of cases in the Bukit Mendi group who were treated as outpatients, often with no diagnosis or specific treatment, (2) the fact that jaundice was present in only 2 (2%) of the 86 cases, (3) no mortality was documented and (4) the majority of the cases were not suspected clinically.

IDENTIFICATION OF STRAIN ACTIVITY IN NATURALLY OCCURRING SCRUB TYPHUS INFECTIONS

Isolates recovered from human scrub typhus infections have been classified on the basis of antigenic composition and antibody response to infection with the isolates. Although the studies are still in progress, findings to date indicate that the Karp strain group produces the preponderance of symptomatic infections in the study area.

NEAR-L. (L.) arenicola

A species of chigger taxonomically intermediate to L. (L.) deliense and L. (L.) arenicola has been found to occur in several locations in the central part of West Malaysia. This species is currently being referred to as near-L. (L.) arenicola. The scutal measurements matches that of L. (L.) arenicola but the scutal setae measurements are closer to L. (L.) deliense. Traub[4] in his original description of L. (L.) arenicola described it as having a palpal formula of NNNNN but indicated that "on an occasional specimen, the dorsal seta of the palpal tibia is frayed or, rarely, even slightly branched", thus giving a palpal formula of NNbNN. Subsequent collections of L. (L.) arenicola have shown that the palpal formula is more often NNbNN. The palpal setae of this intermediate species is also NNbNN.

The name arenicola (or "sand loving") was originally coined because the chigger was only collected from sandy beaches, not from rocky beaches in which the scrub or forest came down to the water's edge. L. (L.) arenicola has been collected throughout West Malaysia from only sandy beaches.

The chiggers which are currently being referred to as near-arenicola have not been collected from a pure sandy habitat. In fact, some of the black plate collections have been from a highly organic soil adjacent to a stream bed. This species, collected from rodents at Bukit Mendi, tends to be somewhat host specific. Of 64 collections, 58 have been from Tupaia glis, with the remaining being collected from R. tiomanicus jalorensis (4) and R. argentiventer (2). If this species is determined to be a true L. (L.) arenicola,

the significance of the finding in regards to the distribution of scrub typhus vectors is apparent.

We are currently attempting to establish a colony of this species of chigger from the central part of West Malaysia. If a colony can be established, then cross-mating of this species with L. (L.) arenicola and other taxonomically similar species can be attempted. In addition taxonomic studies of post-larval stages (nymphs and adults) can be undertaken.

AN ECOLOGICAL STUDY OF RICKETTSIA TSUTSUGAMUSHI IN THE PRIMARY FOREST OF TAMAN NEGARA, WEST MALAYSIA

There has been considerable reference to the possible existence of a jungle cycle of scrub typhus within the literature, but little actual evidence of such occurrence has been presented. To answer this question, rodents and their ectoparasites were collected from the Taman Negara, West Malaysia. Much of the area is primary forest and reachable only by foot or boat. A site at Lata Berhad located approximately 6½ miles from the park headquarters at Kuala Tahan was selected. This area was fairly accessible by boat and ranged from approximately 250 feet to 2000 feet in altitude.

The forest canopy was contiguous and the streams were mostly dry, having water in them only during the rainy season. A similar area across the Sungai Tahan was described by Soepadmo[5] as being undulating hill or ridge dipterocarp forest.

Mammals were trapped in small animal basket traps (Harrison)[6]. The chiggers were removed and returned to the laboratory for identification. All the mammals were held in traps and returned to the laboratory for identification and serological examination. The presence of rickettsial antibody was determined by the microfluorescent antibody test and R. tsutsugamushi isolation attempts were made using the mouse passage technique (Jackson et al.)[7].

Altogether 35 animals comprising 10 species of rats, squirrels, and tree shrews were collected (Table 11). These included: Rattus whiteheadi (8), R. surifer (6), R. rajah (4), R. cremoriventer (1), R. sabanus (10), R. muelleri (1), Tupaia glis (2), Callosciurus nigrovittatus (1), Iomys horsfieldi (1) and Lariscus insignis (1). The trapping success rate was 0.6 percent (35/5479 trapping nights) which is low compared to success rates in secondary forests in Malaysia (Lim & Heyneman)[8], but comparable to other primary forest studies (Lim)[9].

Two individual rodents, a R. surifer and a R. sabanus were trapped at 250 feet elevation in the fringe habitat by the side of the Kuala Tahan river. The rest were caught in primary forest. All the animals caught were lowland forms (Medway)[10]. R. surifer

Table 11

Mammals Collected at Varying Altitudes from Taman Negara, West Malaysia

	Altitude (ft.)						Total
	250	500-600	700-800	1000-1100	1500-1600	>1800	
Rattus whiteheadi	-	3	3	2	-	-	8
R. surifer	1	-	-	2	-	3	6
R. rajah	-	-	-	4	-	-	4
R. cremoriventer	-	-	-	1	-	-	1
R. sabanus	1	2	3	4	-	-	10
R. muelleri	-	-	1	-	-	-	1
Tupaia glis	-	-	1	-	1	-	2
Callosciurus nigrovittatus	-	-	1	-	-	-	1
Iomys horsfieldi	1	-	-	-	-	-	1
Lariscus insignis	-	1	-	-	-	-	1
	3	6	9	13	1	3	35

and Tupaia glis are known to occur from sea-level up to 6000 feet (Harrison)[11]. R. sabanus and R. whiteheadi have been found to live as high as 2000 feet in forested areas around Kuala Lumpur (Lim)[12] but none of these two species was trapped above 1100 feet.

Of the 2,107 chiggers collected during this study (Table 12), only a single specimen of a vector, L. (L.) deliense, was taken from a R. rajah. This one chigger was the only chigger present on the animal. This animal was trapped at approximately 1000 feet in elevation. Additionally, the only other species of Leptotrombidium collected were L. (L.) bodense (243: 11.5% of the total chiggers collected) and 2 specimens of a Leptotrombidium closely related taxonomically to L. (L.) arenicola.

By far the largest number of species and specimens collected were of the genus Gahrliepia: 8 species in two different subgenera (Walchia and Gahrliepia) totalling 1205 specimens (57.2%). G. (W.) cuspidata had the largest number of specimens collected; 587 or 27.8%. Walchiella oudemansi (419: 20.0%) and Ascoschoengastia (L.) audyi (214: 10.2%) were the only other species in which large numbers of chiggers were collected.

Sera collected from the rodents were screened at 1/25 dilution by FA methods for antibody to the Karp, TA 678 and Gilliam strains of R. tsutsugamushi, epidemic typhus, R. canada, siberian tick typhus, and Q fever. Of this spectrum of antigens only two R. surifer rats were positive for Q fever antibody. No antibody was detected to any of the other strains or species of rickettsia.

Both whole blood and spleen/kidney pools were examined for R. tsutsugamushi by the standard technique. The tissue pool from a R. sabanus and both the blood and tissue pool from a Tupaia glis yielded isolates. All three isolates were antigenically similar and related to the Karp and TA 763 strains. These antigens have been the most common detected in surveys conducted in inhabited areas of the east and west coast of Peninsular Malaysia.

EFFECT OF CYCLOPHOSPHAMIDE, AZATHIOPRINE, AND 5' FLUOROURACIL ON RICKETTSIA TSUTSUGAMUSHI INFECTIONS IN MICE

The class of compounds most widely used to block the immune response to infectious and non-infectious antigens are alkylating agents. Of these the most widely studied is cyclophosphamide (CY). Kazar et al.[13] studied the effects of CY on Rickettsia akari, R. prowazeki and Coxiella burneti infections in mice. Their studies showed that CY increased the virulence and the number of rickettsia present in tissues and was effective in suppressing antibody formation when given at the specified intervals. Tachibana and Kobayashi[14] found that CY increased the virulence and enhanced the growth of R. sennetsu in mice. Maximum titers in spleen tissue were approximately two $\log_{10}$ higher in chemoimmunosuppressed mice than in control animals.

Table 12

Species of Chiggers Collected from Mammals Trapped at Taman Negara, West Malaysia

MAMMAL SPECIES	*Leptotrombidium (L.) deliense*	*L. (L.) bodense*	*L. (L.) near-arenicola*	*Gahrliepia (W.) caspidata*	*G. (W.) leuthwaiti*	*G. (W.) rustica*	*G. (W.) turmalis*	*G. (G.) fletcheri*	*G. (G.) neterella*	*G. (G.) rutilla*	*G. (G.) insigne*	*Ascoschoengastia (L.) audyi*	*A. (L.) ctenacarus*	*Walchiella nadchatrami*	*W. oudemansi*	*Siseca rara*	Total
Rattus rajah	1										18						19
R. surifer											3			2			5
R. muelleri		26		3		9		21	67						250		356
R. sabanus				579		14	5	97	296	1				13	15		1020
R. whiteheadi				4	66									1			71
Tupia glis		216	2	1		14		1	6						173		418
Callosciurus nigrovittatus		1										214	2		1	5	218
TOTAL	1	243	2	587	66	37	5	119	369	1	21	214	2	16	419	5	2107

Antimetabslites such as purine and pyrimidine analogs have also been shown to have significant effects on immune response. For this study the pyrimidine analog 5 fluorouracil (5-FU) and the purine analog azathioprine were selected as representative of this group of compounds.

Many strains of R. tsutsugamushi are not lethal for mice, and death occurs in mice infected with virulent strains coincident with the appearance of antibody. The response of chemoimmunosuppressed mice to inoculation with a mouse virulent and a mouse avirulent strain was studied in anticipation that such studies would further elucidate the mechanisms responsible for pathogenicity.

The Rickettsia tsutsugamushi strains used in the study were propagated in specific pathogen free hens' eggs purchased from SPAFAS, Inc., Norwich, Connecticut. The Karp strain was uniformly lethal following inoculation, but the TA 686 strain was not lethal to our particular strain of mice. A 20% yolk sac suspension of the Karp strain contained $10^{8.5}$ LD_{50} and a 20% suspension of the TA 686 contained $10^{7.6}$ median immunizing doses for mice (ID_{50}).

Cyclophosphamide was purchased as Endoxan-Asta^{R} 200 mg vials for injection from Asta-Werke Ag, Chemische Fabrik, Brackwede, Federal Republic of Germany. Azathioprine B.P. was purchased as Imuran, 50 mg tablets from Burroughs-Wellcome and Co., London. 5-fluorouracil was purchased in the injectable form from F. Hoffman-La Roche and Co. Ltd., Basle, Switzerland.

Azathioprine was employed at a dosage of 3 mg/kg/day; and 5-FU at 10 mg/kg/day. CY was given either at a continuous dosage of 45 mg/kg/day, at a single dose of 400 mg/kg, or at a dose of 400 mg/kg followed by a second dose of 200 mg/kg 3 days later depending on the experimental design.

Initial experiments were conducted to determine the effect of chemoimmunosuppression on survival following challenge with the mouse lethal Karp strain of R. tsutsugamushi. Administration of CY was either initiated one day post challenge and given until 7 days post challenge, or given as a single dose concurrent with challenge.

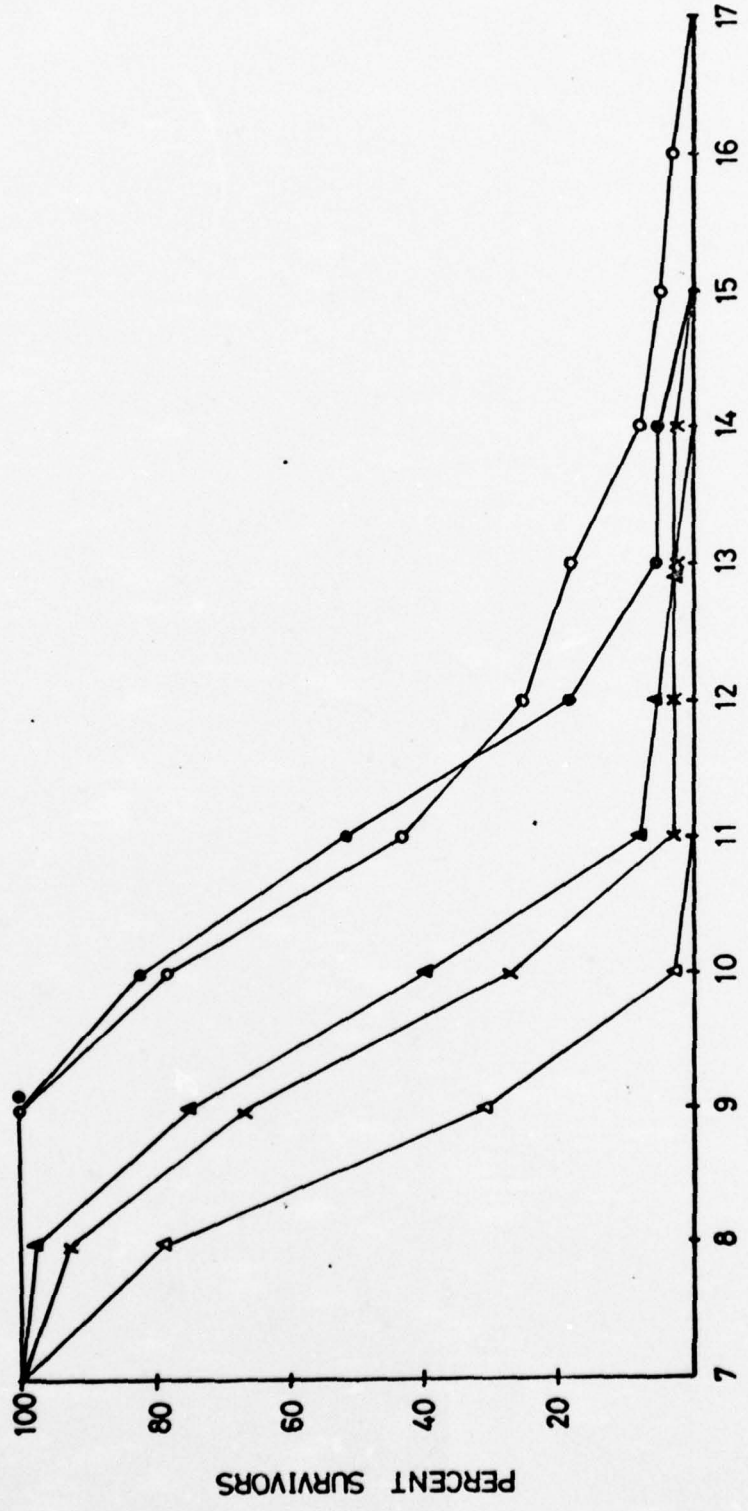
The results are shown in Figure 2. All the experimental mice died following inoculation of the rickettsia and administration of the drugs. The survival time appeared to be prolonged with azathioprine and 5-FU and shortened with daily doses of CY. These changes were consistent when the experiment was repeated. No deaths occurred in control groups administered the drugs alone.

The same parameter was studied under the same conditions following an equivalent infectious dose of the TA 686 strain. This strain is not lethal for mice, but previously exposed mice are immune to challenge with virulent strains. Since no mice died when

Caption

Figure 2. Effect of chemoimmunosuppression on infection with the mouse virulent Karp strain of R. tsutsugamushi.

△ CY daily, days 1-8 post inoculation; ▲ CY single dose on day 1 post inoculation; O azathioprine on day 1; ● FUDR on day 1; X control - Karp strain only.


PERCENT SURVIVORS
100
80
60
40
20
7
8
9
10
11
12
13
14
15
16
17
DAY POST CHALLENGE

inoculated with TA 686 alone or with TA 686 and azathioprine or 5-FU, only the response of mice to CY inoculation is shown in Figure 3. The mice given a single 8 mg dose of CY first evidenced signs of illness on day 10 post challenge which was approximately 2 days longer than the incubation period of the virulent Karp strain. A few mice died each day through day 15 post inoculation when 17/39 (44%) were alive. The daily administration of a small dose of CY with TA 686 produced signs of infection 1 day later, but the death patterns more nearly approximated the Karp strain in that the majority of the mice died over a three day period. At 28 days, the termination of the experiment, 10% (4/40) of the mice were alive.

The effect of varying the day of administration of the drugs on survival and antibody titers is shown in Table 13. No deaths occurred in the experimental groups inoculated with TA 686 strain and given azathioprine or 5-FU. However, CY given by either dosage schedule increased the sensitivity of the mice to this normally avirulent strain. When the single dose was given near to the time of the rickettsial challenge some mice survived, but when the CY was given between days 4 and 7 post challenge there were no survivors.

The titers of rickettsia in liver/spleen pools and in peritoneal exudate were determined when all of the CY mice were showing signs of the disease and approximately 20% had died (Table 14). At 8 days post inoculation little difference could be detected between the titers in peritoneal fluid between mice inoculated with CY and those inoculated with PBS. Also, little difference could be detected between the titers in tissues from mice treated with a single 8 mg dose on day 3 and control titers. When the 8 mg dose was followed on day 5 with a 4 mg dose the maximum mean difference was 1.0 $\log_{10}$.

When peritoneal exudate containing cells was stained by the direct fluorescent antibody method a distinct difference was noted between CY treated mice and the other experimental groups as well as controls. The individual organisms stained much more distinctly and larger numbers were visible. We reasoned that significant levels of humoral antibody would coat the organisms and interfere with the direct staining technique. A series of exudates were stained with the direct fluorescent antibody method using conjugated rabbit origin antimouse globulin, and the rickettsia from the azathioprine, 5-FU, and control animals fluoresced indicating that they were coated with mouse globulin. However, the organisms from CY treated mice could not be observed by the use of antimouse globulin alone.

Smears were prepared from the peritoneal fluid of drug treated, infected mice and stained with monospecific conjugate. No antigens were detected in the isolates that had not been detected in the original seed material.

Caption

Figure 3. Effect of chemoimmunosuppression on infection with the mouse avirulent TA 686 strain of R. tsutsugamushi.

△ CY daily, days 1-8 post inoculation; ▲ CY single dose on day 1; X control - TA 686 strain only.

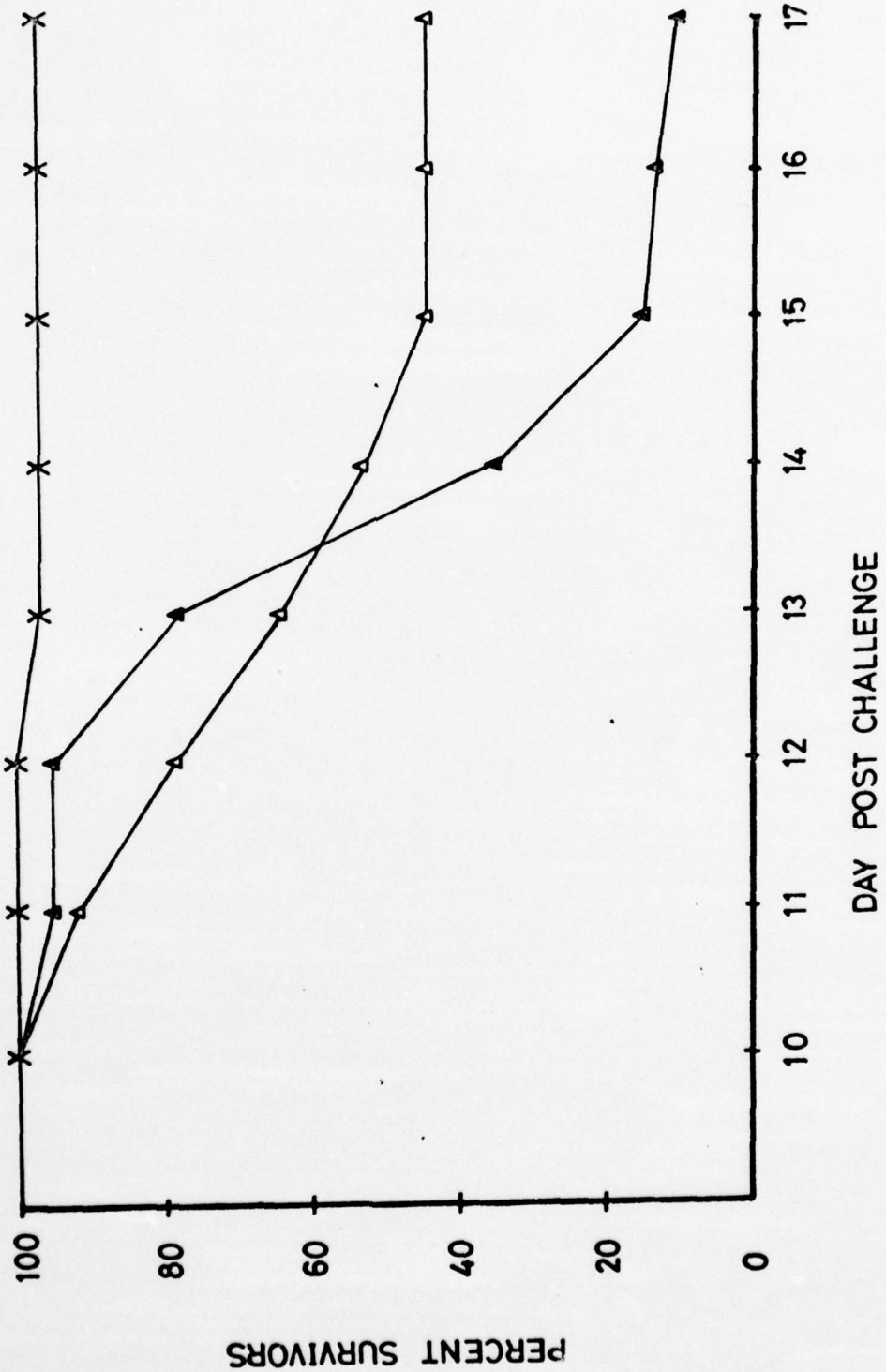

PERCENT SURVIVORS
100
80
60
40
20
0
10
11
12
13
14
15
16
17
DAY POST CHALLENGE

Table 13

The Effect of Varying the Day of Administration of Cyclophosphamide on Survival and IFA Titer in Mice Experimentally Infected with TA 686 Strain of R. tsutsugamushi

Day of Administration of drug[a]	CY				Aza	FUDR
	Single dose		Continuous			
	Survivors[b]	Titer[c]	Survivors	Titer	Titer	Titer
-1	26	20	0	-	320	80
0	33	80	15	40	640	160
1	44	80	10	40	640	160
2	36	80	20	160	640	320
3	5	80	28	160	320	160
4	0	-	33	80	320	160
5	0	-	31	80	640	160
6	0	-	5	160	640	160
7	0	-	80	80	320	160
Control	100	80	100	80	80	160

a. 0 was day of inoculation of TA 686 strain.

b. percent.

c. reciprocal homologous IFA titer of sera pool from survivors at 28 days.

Table 14

R. tsutsugamushi Titers in Normal and CY Treated Mice

Strain	PBS	CY	
		8 mg	12 mg
TA 686 (avirulent)			
Liver/spleen pools	5.6[a], 4.7	5.8, 5.5	6.6, 5.8
Peritoneal fluid	4.6, 4.2	5.2, 4.5	5.3, 4.8
Karp (virulent)			
Liver/spleen pools	6.3, 6.5	ND[b]	7.3, 6.0
Blood	4.5, 3.3	ND	5.4, 3.8

a. Log_{10} values of separate experiments.
b. Not done.

The antibody titers presented in Table 13 are from sera collected at 28 days post inoculation. The titers of the 4 groups of control mice varied from 1/80 to 1/160 which was not significantly different from the titers of the groups given CY subsequent to the inoculation of organisms. Groups given CY prior to or concurrent with challenge usually had lower titers, but the small numbers of survivors in these groups may have distorted the results. Mice given azathioprine had significantly higher titers than controls at 28 days, but no difference was detected between control and experimental values following the administration of 5-FU.

The antibody response of mice following inoculation of the TA 686 strain with drug treatment was studied to determine the production of antibody to each of the 9 putative prototype strains. The results are presented in Table 15. The titers were closely associated in every case with the same 5 strains. This indicated that immunosuppression early in the course of the infection did not allow additional antigens to be expressed in comparison to control mice.

The inoculation of homologous convalescent sera had no effect on the survival of mice inoculated with CY. When CY was given 90% of the mice died following inoculation with the TA 686 regardless of the administration of 0.5 ml of homologous antibody which titered 1/320 in IFA.

The administration of CY to mice infected with an avirulent strain of R. tsutsugamushi increased the virulence of the strain in the treated mice. These results are similar to those found with other rickettsia (Kazar et al)13. The cause of the increased virulence in R. tsutsugamushi is not as clear as it is in the other rickettsial diseases. Increased virulence in immunosuppressed mice has been correlated with increased growth of the organism in R. sennetsu, R. prowazeki, R. akari and C. burneti; but we did not find such a correlation in R. tsutsugamushi. Titers in spleen cells, blood, and peritoneal fluids of immunosuppressed mice were ≤ 1 $\log_{10}$ different than in control animals.

In this study CY treated mice died following inoculation of a normally non-lethal strain. Deaths were not mitigated by passive immunity, and mice which had recovered from the infection several months before became rickettsemic and a small percentage died following CY treatment. These facts indicate that the increased virulence can not be solely attributed to the inhibition of antibody production. Other investigators have postulated a nonspecific toxic effect of CY which may be important in decreasing the resistance of animals following inoculation of normally avirulent strains or species.

There is general agreement that CY is effective in aborting antibody responses when given concurrently with or soon after

Table 15

Homologous and Heterologous Antibody Responses Following Infection with the TA 686 Strain and Drug Treatment

		Karp	Kato	Gill	TA 686	TA 716	TC 586	TA 678	TA 763	TH 1817
CY	(8)[a]	56[b]	<10	<10	24	87	<10	<10	61	56
Azathioprine	(8)	13	<10	<10	470	79	<10	<10	14	54
5-FU	(8)	63	<10	<10	159	100	<10	<10	20	79
Control	(3)	25	<10	<10	32	50	<10	<10	10	32

a. Number of pools of mouse sera tested.

b. Reciprocal geometric mean titer.

antigen (Aisenberg)[15]. In our system, the titers of the survivors did not appear to be reduced when compared to controls which had not received CY. This was a result of the unavoidable bias of necessarily studying the titers in survivors and our choice of 28 days as a time to measure antibody production. *Stockman et al.*[16] showed that mice given an equivalent dose of CY regained B cell responsiveness at 10-14 days. The organism persists for long periods of time in mice; and therefore, was able to act as an immunogen once the mouse had recovered B cell reactivity.

The increase in antibody titer following treatment with azathioprine was not expected since the drug is widely used to reduce the immune response to homografts. However, this property has been used to advantage in the laboratory to produce hyperimmune sera to several strains which are poorly antigenic.

A NEW CAPSULE FOR FEEDING CHIGGERS (ACARINA: TROMBICULIDAE)

In the life cycle of trombiculid mites, the larval stage or chigger must feed on a vertebrate host. Thus, to maintain colonies for Rickettsia tsutsugamushi transmission and general bionomic studies, chiggers are usually fed on laboratory white mice. Two basic feeding methods have been employed at the Institute for Medical Research in Kuala Lumpur. The ear feeding technique as described by Nadchatram[17] has been used for both mass feeding and single rearing, but intensive observation of the feeding of the chiggers is difficult as the mouse must often be anesthetized. The use of a capsule (Baker et al.)[18], glued to the back of a white mouse, has been useful for feeding and provides a means of close observation.

The first capsule for the feeding of chiggers was constructed from the screw top end of a toothpaste tube and was followed by the use of a small piece of laboratory glass tubing that had been flanged to provide a greater surface area for application of glue (Baker et al)[18]. This type of capsule has proved effective for several years, however, a major disadvantage to this type of capsule is the small area in which the chiggers are applied (5 mm), making observation of the feeding chigger difficult.

The development of a new capsule was initiated in conjunction with systemic acaricidal studies (Dohany et al.)[19]. Several types of capsules were tried, including flanged tubing, the neck portion of screw cap glass shell vials and the upper portion of disposable hypodermic syringes with the rubber from the plunger acting as a cap. None of these proved satisfactory for the intended purpose of these studies. The most effective capsule proved to be a portion of a container for a disposable hypodermic syringe (Figure 4).

To prepare the capsule, the expanded portion of a 2.5 cc hypodermic syringe container was cut off and the resulting hole was smoothed of its rough edges. A nontoxic glue (Bostick Universal Cement) was applied to the turned-under edge of the

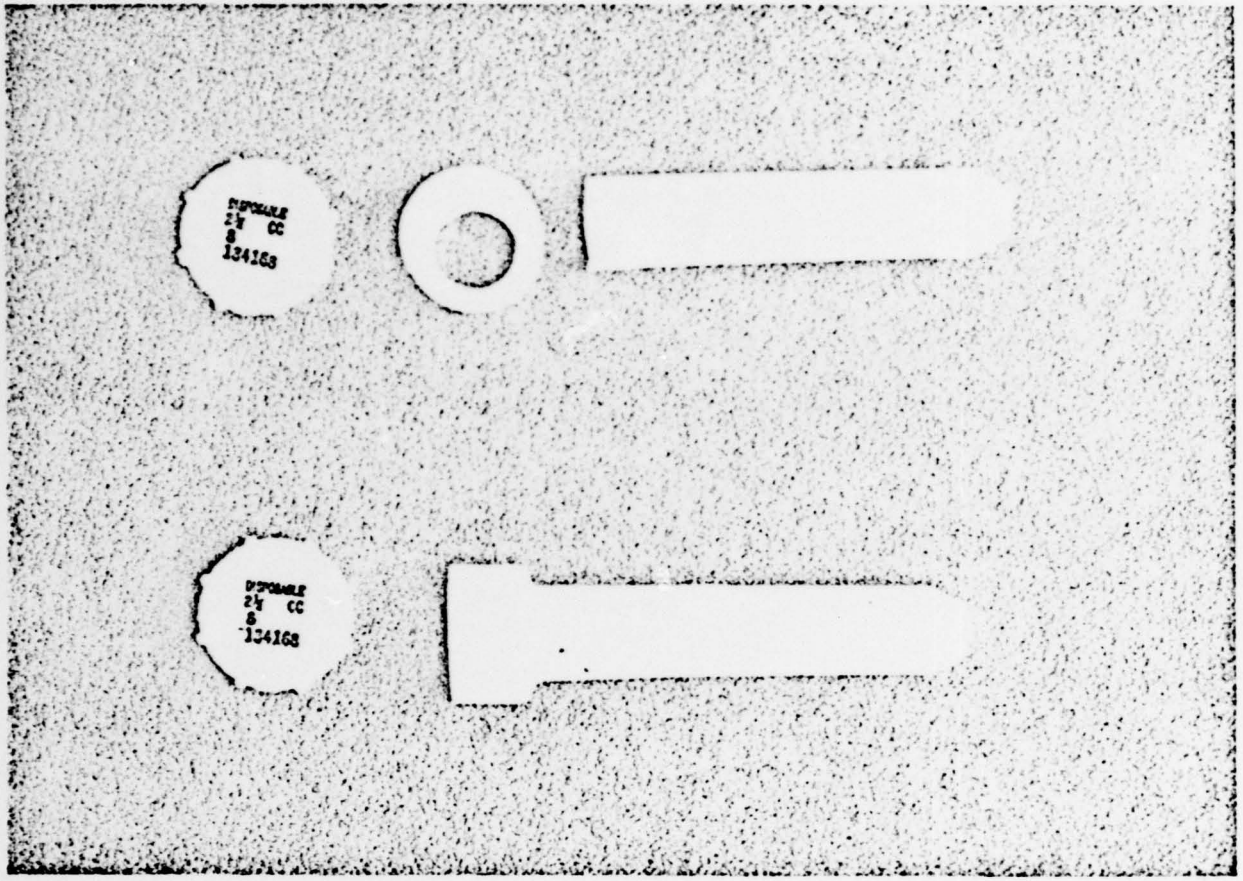


Figure 4. Construction of chigger holding capsule made from the container of a disposable hypodermic syringe.

capsule. The capsule was then applied to the previously shaved back of a guinea pig, laboratory mouse (Figure 5), or silvered leaf monkey (Figure 6). Masking tape was wrapped around the capsule and animal to hold the capsule in place until the glue had set, usually for one to two hours.

The plastic top of the container was used as the cover for the capsule. Filter paper was cut to fit into the top and was kept moist throughout its use. This moisture prevented desiccation of the chigger upon detaching.

While applying the chiggers, a piece of lens paper is used as a gasket between the cap and the capsule proper, thus preventing escape of the unengorged chiggers. For laboratory mice the cap is removed after all the chiggers attach to the host, and the engorged chiggers are collected from a pan of water as described by Baker et al.[18]. This method has been used for approximately one year with good success for the rearing of colonies of scrub typhus vector chiggers (Leptotrombidium spp.). Alternatively, the cap can be left on and the chiggers can be collected at specific intervals. Usually the chiggers move to the top of the capsule upon disengaging and become trapped in the moisture droplets of the cap, allowing for easy collection.

Advantages of this capsule over the glass tubing capsule include: (1) ease of production (used hypodermic syringe containers can be obtained from most hospitals), (2) an increased feeding area allows for an increased number of chiggers to feed and easier observation of feeding chiggers, and (3) a tight fitting cap is included.

DEVELOPMENT OF AN ANIMAL MODEL FOR R. TSUTSUGAMUSHI

The laboratory mouse has been used for many years for the isolation of R. tsutsugamushi and as an animal model for the study of R. tsutsugamushi infections. However, the requirement for a larger, intermediate animal model is well recognized. Work in this laboratory has shown that silvered leaf-monkeys, Presbytis cristatus, are susceptible to infection with R. tsutsugamushi. Following intradermal inoculation with virulent prototype strains the monkeys developed fever, eschars, lymphadenopathy, rickettsemia, and specific antibodies to the infecting strain. However, there were several drawbacks to the use of silvered leaf-monkeys: (1) the supply is not unlimited; (2) shipping of the animals would be impossible, difficult or impractical, thus geographically limiting their use; (3) some captured animals have shown evidence of prior natural infections; (4) many animals are lost (up to 40 50%) during the conditioning period; and (5) the apparent inability to restrain leaf monkeys for a sufficient period of time to allow chiggers in a capsule to feed to repletion seriously jeapordized the potential use of this animal as a satisfactory model for scrub typhus.

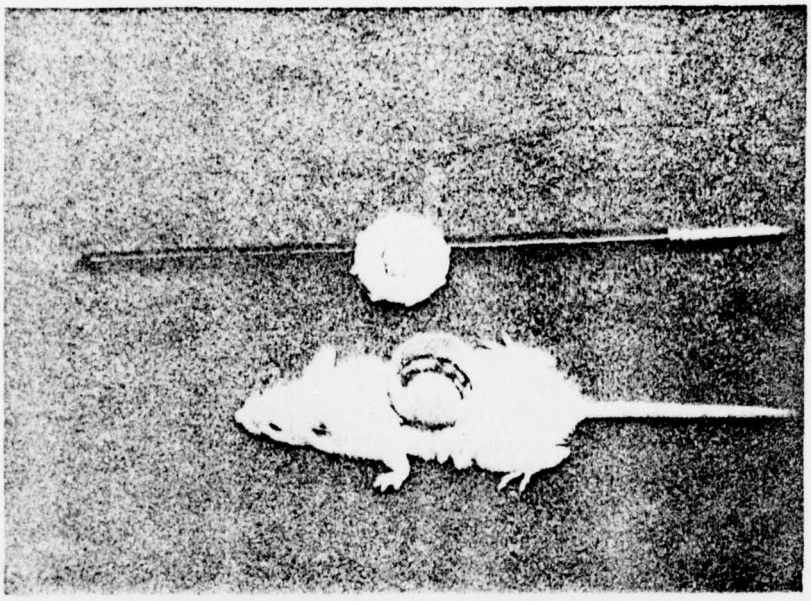


Figure 5. Chigger holding capsule attached to back of laboratory mouse.

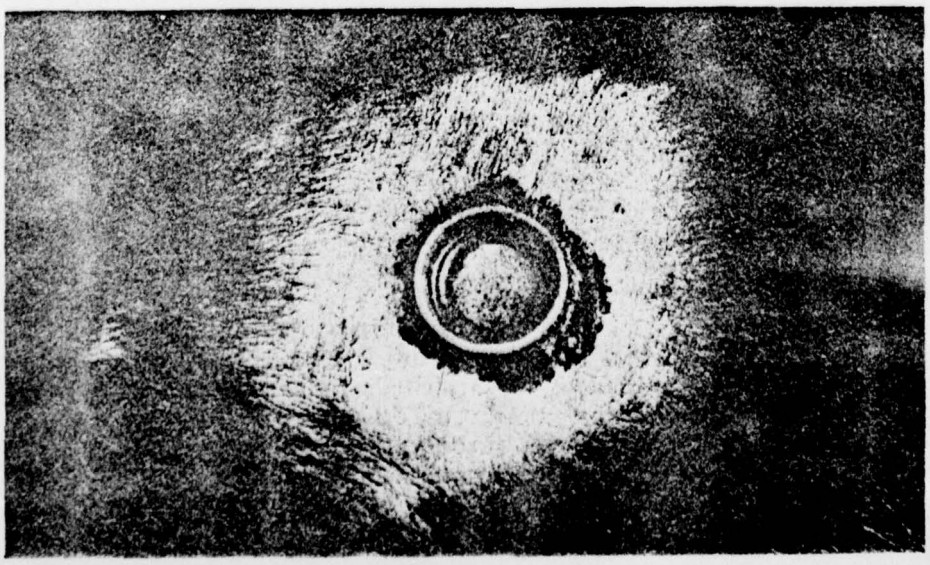


Figure 6. Chigger holding capsule attached to back of silvered leaf-monkey.

These findings prompted us to pursue the development of an intermediate animal model on a broad front. During the past year other animals have been considered and studied, and the above mentioned problems associated with the use of silvered leaf-monkeys have been reexamined with the objective of overcoming or reducing the limitations.

Serological studies[20] have indicated that the dog is often naturally infected in endemic areas. This observation plus the results of recent studies with R. rickettsii which clearly demonstrated that the dog is a good model for the human disease prompted investigation of the dog as a possible animal model for R. tsutsugamushi infection. Additionally, dogs are easily acquired, can be handled readily, have been previously used in well documented laboratory studies in which ectoparasites in capsules were allowed to successfully feed, and are one of the animals on which an enormous amount of basic biological data has accumulated.

In addition to the dog we have recently studied experimental infections in born- and raised-in-captivity cynomolgus monkeys. Early studies with this species indicated that it was relatively resistant to disease following experimental inoculation with R. tsutsugamushi, and previous reports from this laboratory indicated that the resistance to disease in wild caught cynomolgus monkeys may be due to immunity resulting from prior infection, since antibody was detectable in a large number of sera collected from wild caught animals.[21]

Studies on dogs and cynomolgus monkeys are still in progress and final conclusions on their potential as a model for R. tsutsugamushi must await completion of these studies.

The other approach in the development of an intermediate animal model consisted of further examination of the silvered leaf-monkey with the objective of overcoming factors which appeared to limit its use.

It is highly desirable in some studies to infect animals by the natural route (i.e. feeding of infected chiggers). Thus, a means of producing chigger-transmitted disease in controlled studies is essential to the development of a reliable intermediate animal model. As was mentioned above early attempts in this unit to develop a reliable and predictable means of producing chigger-transmitted infections in silvered leaf monkeys failed due to the inability to restrain the animals sufficiently long to allow chiggers to feed to repletion.

Studies have been initiated to develop a more suitable means of restraining silvered leaf-monkeys. In preliminary trials, using a newly designed restraint chair, silvered leaf-monkeys have been restrained for a period of 5 days, sufficient to allow chiggers to feed to repletion.

SYSTEMIC ACARICIDE TESTS USING DIMETHOATE FOR THE CONTROL OF CHIGGERS

Acaricidal spraying for the control of chiggers frequently is not feasible. The habitat for the vectors, particularly scrub habitat of L. (L.) deliense, is often too dense to permit ease of spraying or effective coverage. Use of a systemic acaricide, particularly in conjunction with other control measures, could prove valuable in certain situations.

Systemic insecticides are effective in the control of numerous insects affecting animals, particularly cattle grubs, fleas, lice and ticks. Dimethoate used systemically has proven successful in the control of chiggers in laboratory tests in the United States (Dohany et al, to be published) and in Malaysia (Dohany, unpublished data). Dimethoate is a general usage organophosphate insecticide which is rapidly eliminated from the animal.

An area located on the Kuala Selangor-Kuala Lumpur road was selected for a preliminary study. The site consisted of a small island of forest surrounded by lalang. A grid system of 120 traps and 50 dimethoate bait stations was established. Bait, consisting of 0.01 per cent dimethoate mixed with a 1:1 ratio of ground corn and milo, is maintained in the bait stations. The bait is removed only during trapping periods. Rodents are trapped for 4 nights of each month. The chiggers are removed from the rodents and the rodents are marked and released for retrapping. A control site separated from the study site, was established in the same general area with 70 trapping locations. Acceptance of the bait has proved to be good and initial results indicated that there is considerable reduction in the chigger population within the dimethoate area (Figure 7). Although reduction has occurred, complete control has not been obtained due to immigration of rodents from adjacent lalang and nearby forests. Future studies will be concentrated in a location in which such immigration of nontreated rodents does not occur.

DISEASES OF MILITARY WORKING DOGS

Tropical Canine Pancytopenia

Since the early 1970's USAMRU has provided minimal technical support to the Canine Unit of the Malaysian Armed Forces. The unit is located at the Jungle Warfare School, Pulada, Johor Bahru. Pulada is utilized as a home base for dogs used in operations as well as training and breeding. The initial support consisted primarily of surveillance for tropical canine pancytopenia (TCP), a disease which was responsible for the death of approximately 300 U.S. military dogs in Vietnam. In the 1960's numerous military, police, and privately owned dogs died of the disease in Singapore and Malaysia. This included a large number of dogs at the Jungle Warfare School at Pulada.

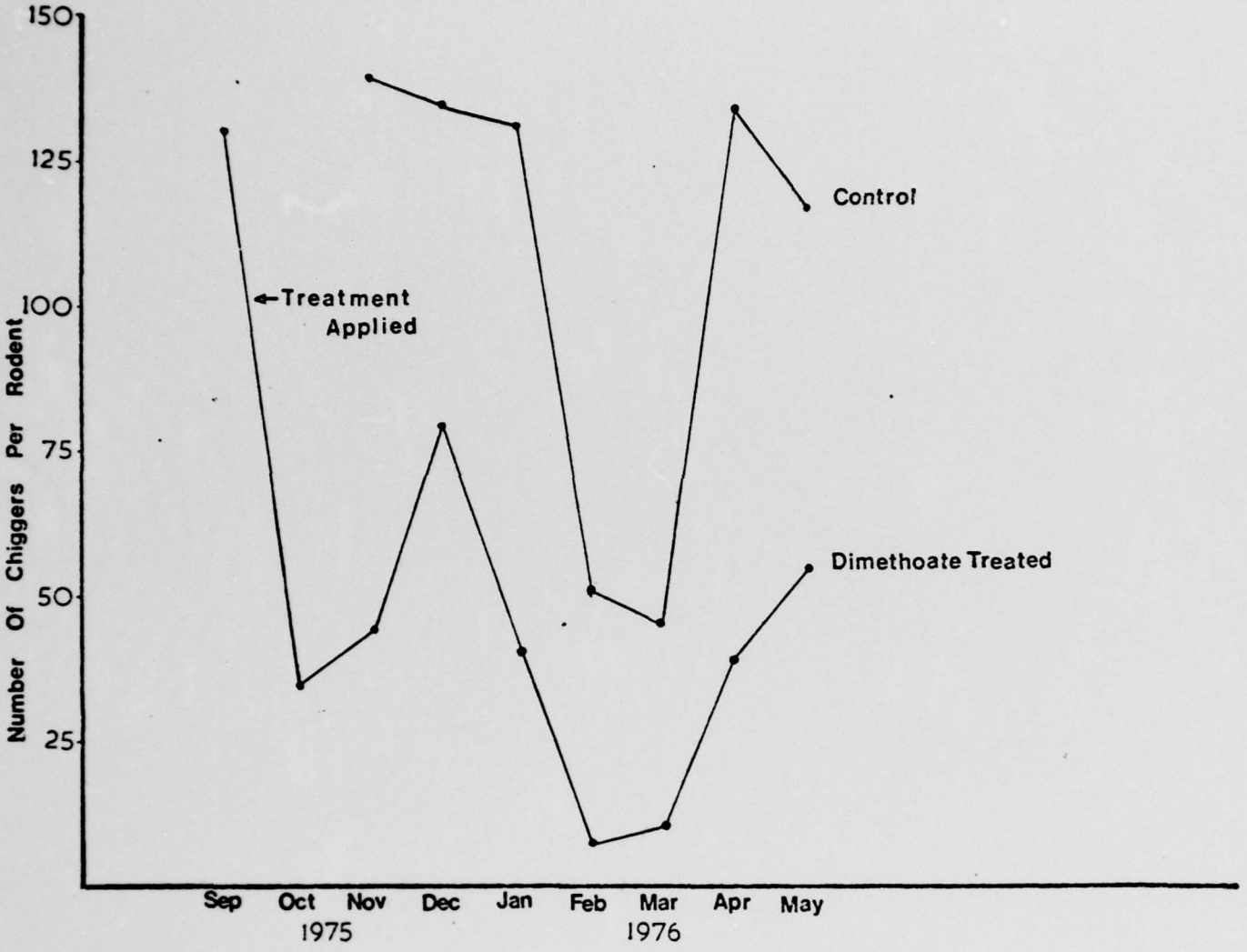


Figure 7. Control of trombiculid mites with the use of a systemic acaricide-dimethoate.

Before a serological test became available periodic hematological examinations provided the only practical means of surveillance. With the development of a serological test for Ehrlichia canis, the causative agent of TCP, a more definitive means of identifying infected animals became available. With the collaboration of investigators at WRAIR and the University of Illinois, periodic serological examinations have been made on the dogs at Pulada.

The Canine Unit at Pulada has afforded an excellent opportunity to observe and study selected clinical and epidemiological aspects of diseases of military working dogs and the efficacy of various therapeutic and prophylactic measures in treating and controlling these diseases.

Following the report of Amyx et al.[22], which showed that dogs given tetracycline hydrochloride orally at a daily rate of 3 mg per pound body weight were refractory to infection with E. canis, a decision was made in 1972 to give each dog in the unit one 250 mg. capsule of tetracycline hydrochloride in its daily feed. The policy has continued for four years.

Sera collected in 1972 from 22 dogs were tested for antibodies to E. canis in the immunofluorescence test. Seven of these sera contained demonstrable antibody. Since E. canis has been shown to produce infections which persist in untreated dogs for many years, one can assume that many, if not all, of the dogs serologically positive in 1972 were indeed infected despite a lack of overt clinical signs. These findings also provide evidence that the organism is endemic in the areas in which the dogs are trained and utilized.

Since 1972 there has been no clinical evidence of infection in the unit. Recent serological studies on 50 dogs showed that only 2 dogs had titers to E. canis, and in both instances the titers were low (1:20). In addition no untoward side effects from continuous administration of tetracycline have been observed, and no impairment of training or working capabilities of the dogs has occurred.

These studies plus those recently reported from Thailand[23] have demonstrated that TCP can be controlled in military dogs by daily administration of tetracycline without untoward effects.

Chronic Anemia in Military Dogs at Pulada

During the past two years a large number of the 60 military dogs of the Malaysian Army at Pulada in Johor Bahru have developed an anemia which rendered them incapable of duty. Sone dogs have had repeated episodes. Serological evidence indicated that the cause was babesiosis. Blood inoculation studies confirmed this diagnosis. Five normal dogs were inoculated with the blood of five military dogs with low packed cell volume. Of the five dogs inoculated two

developed clinical signs typical of babesiosis with Babesia gibsoni organisms visible in blood smears. Four of the five inoculated dogs converted from negative to positive for babesia on serological examination.

Babesiosis probably goes unrecognized in many pet dogs which are seldom required to do vigorous work or exercise. On the other hand, the military dog is exercised over a prescribed course on a regular basis and any loss of stamina becomes immediately evident to the dog's handler. The anemia, which is often severe renders the dog incapable of completing a course, or worse, incapable of properly performing field duty. These dogs are being utilized as tracker dogs in jungle terrain and are sent out routinely for duty. The dogs then return to Pulada to wait for reassignment. In order to combat the problem of babesiosis in this group of military dogs more information is required concerning the clinical disease, the epidemiology, and effective means of treatment or control.

MANAGEMENT OF TROPICAL LABORATORY ANIMAL RESOURCES

Responsibilities are shared with IMR for production of research animals. The major effort is related to mouse production although rabbits, guinea pigs, hamsters, rats and gerbils are also bred. Approximately 100,000 mice are produced each year and USAMRU uses about 60% of that number.

A group of silvered leaf-monkeys were acquired and conditioned for use in scrub typhus work. This species is extremely fragile, and of 51 animals acquired only 26 survived the conditioning period. Necropsies were performed on all dead animals, however, few lesions were seen. Tissues were collected from some animals for histological examination, and the cause of death was generally felt to be of enteric origin.

Cynomolgus Monkey Breeding Colony

Reports in the literature based on serological studies indicate that a large number of wild caught cynomolgus monkeys have been naturally exposed to scrub typhus. In order to supply monkeys which had not previously been exposed to scrub typhus a breeding colony was initiated. The facility used to house the colony has a concrete floor and consists of a wooden supporting frame with 2" x 2" wire mesh walls and a zinc sheet roof. Piped water is available for cleaning and general sanitation purposes. The colony consists of two groups comprised of a male and ten females. Each group is kept in an area approximately 8 feet wide, 12 feet long and 7 feet high. All breeders were tuberculin tested and found to be negative by intradermal test. In addition, each animal was cultured and found to be free of enteric bacterial pathogens and underwent a minimum of two treatments with levamisole* until no intestinal parasite ova

*Nilverm - Imperial Chemical Industries Ltd., Cheshire, England.

were present in stool samples examined by flotation.

The first group was placed together in September 1975 and the second group was placed together between December 1975 and March 1976. Some difficulty was encountered with the second group and several animals were mauled and/or killed before a compatible grouping could be arranged. Thus, in the second group there was an adjustment period over several months with culling and replacement of incompatible animals.

There have been eight births in the first group although only five are surviving. One infant was stillborn and two were killed shortly after birth. Since the last death occurred a new procedure has been instituted whereby pregnant females are placed into smaller cages within the gang cage room. They are left in the gang cage room so their association with other members of their group is not severed completely. It was felt that complete severance of this association might cause fighting when the females were reintroduced after weaning of their infants at 6 months of age.

At present there is a female in the advanced stages of pregnancy in the first group and three pregnant females in the second group.

Infants are weighed once each month and recordings are made of tooth eruptions and weights.

Project 3A762759A831 TROPICAL MEDICINE

Task 00 Tropical Medicine

Work Unit 071 Field Studies of Rickettsioses and Other Tropical Diseases

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