

THREAT ASSESSMENT OF DISEASES OF MILITARY
IMPORTANCE IN THE TROPICS

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1. Dengue as an Etiology of Undifferentiated Fevers at Bangkok Children's Hospital, 1983

PROBLEMS: To determine the relative importance of dengue virus as the etiology of undifferentiated fevers in children in Bangkok.

METHOD: The outpatient clinic of Children's Hospital, Bangkok is visited each day. Blood specimens and clinical information are collected from a sample of children with fevers. From 10 to 14 days later, follow-up specimens are collected. Acute specimens are cultured for dengue virus. HAI serology for dengue is done on each serum.

PROGRESS : This study has been in progress since 1979, excluding 1982 (Table 1). During that time, a total of 523 cases were evaluated. 25% of the fully evaluated cases had evidence of flavivirus as the etiology of fever.

Table 1. Dengue isolations and serology from cases of undifferentiated fever at Bangkok Children's Hospital, 1979 - 1983.

Year Dengue	Cases Evaluated	Serotype				Seroconversions		Not Flavi	% Isolates
		1	2	3	4	Primary	Secondary		
1979	166	1	8			1	10	155	9
1980	263	38	25	4	1	42	52	169	36
1981	35	4				5	4	26	26
1982	Not done								
1983	59	Not complete				4	11	44	20
Total	523	43	33	45	5	52	77		25

Overall, dengue 1 has been the predominant isolate, in contrast to the predominance of dengue 2 in DHF patients. Most of the isolates occurred in 1980, a year in which a record number of DHF cases and isolates occurred. In that year, 36% of children cultured yielded a dengue isolate, attesting to the fact that much of the increased load of febrile children was due to the dengue epidemic.

CONCLUSION :

1. 25% of undifferentiated fevers in children are attributable to flavivirus infections, presumably dengue.

2. Dengue 1 and 2 are the most frequent isolates, accounting for 94% of isolates.

3. In contrast, dengue 2 is a relatively more common isolate from DHF cases seen at the same hospital.

FUTURE OBJECTIVES : This study represents a long term surveillance of dengue isolates in patients with a clinical syndrome likely to be seen in US troops affected by epidemic dengue. Moreover it allows longitudinal determination of serotypes of dengue virus causing dengue fever. Comparison of these data with those from the DHF study will allow determination of the relative importance of dengue serotypes in the two syndromes. The longitudinal study should be continued and expanded to include more epidemiological evaluation.

2. An Epidemic of Dengue Fever Due to Dengue 1 in a Rural Thai Village

PROBLEM : To determine the etiology and characteristics of an outbreak of an illness characterized by fever and rash in rural northern Thailand.

BACKGROUND : On 20 July, 1983 the AFRIMS Department of Virology was requested to assist in the laboratory aspects of an investigation of an outbreak of an epidemic of fever and rash in Phayao, a province in the extreme north of Thailand.

PROGRESS : In the village where the outbreak occurred, 25% of the total of 523 persons reported an illness that met the case definition of fever or history of fever plus headache, myalgia or rash. The epidemic curve revealed a sharp increase in cases beginning in the middle of June and peaking in early July. The epidemic was characterized by fever (100%), myalgia (85%), headache (81%) and rash (80%). Hemorrhagic manifestations were seen in very few cases. Age specific attack rates revealed that from 20 to 30% of individuals in each age group over 1, including 20% of those over 60 years of age were involved.

Evidence that the outbreak was due to dengue included the following (in order of their completion) :

1. Significant difference in the HAI dengue 2 GMT's of cases and controls in the bleeding from the initial days of investigation (Controls = 10 (sd = 4.4, n = 8), Cases = 60 (sd = 9.1, n = 30), $p < .02$ by Student's t test).

2. Significantly more cases than well controls experienced HAI rises. (21 of 30 cases vs. 2 of 8 controls had HAI rises, $p = .03$ by Fisher's exact test).
3. Significant rise in HAI titer in cases between acute and convalescent sera (acute GMT = 30 (sd = 8), convalescent GMT = 251 (sd = 10), $p < .001$).
4. Isolation of dengue 1 from acute sera of seven human cases (Identified by ELISA).

In addition to confirming the etiology of the epidemic, several other aspects of the diagnosis of dengue epidemics were investigated :

1. Comparison of HAI and ELISA definition of primary and secondary cases. In 22 infected patients, paired sera were available. By HAI, primary infections were those with low or absent antibody in S1, 4 fold rise in S2, but no titer > 160. Secondary infections were those with high titered antibody against several antigens in S2. By ELISA, primary infections were those with MAC index > GAC index, while in secondary infections, GAC > MAC. In 21 out of 22 sera, there was concordance between these two classifications, suggesting that either method may be used.

CONCLUSIONS :

1. An outbreak of dengue fever due to dengue 1 occurred in a sharp epidemic in all age groups of a rural population, suggesting that more than 60 years had passed since this serotype was last introduced into the population.
2. Presumably, any cohort of individuals previously unexposed to the circulating serotype would be susceptible to such an epidemic.
3. Outbreaks of dengue, like epidemics of influenza, may be diagnosed during a single visit by bleeding a number of cases and non-cases and comparing HAI GMT's using a t-test. Confirmation of etiology may await more detailed serology and virus isolation.

FUTURE OBJECTIVES :

1. More information on the transmission of dengue in rural Thailand and the relationship between the strains causing dengue fever and dengue hemorrhagic fever is needed to understand the complex ecological interrelationships.
2. Because of the possibility that this sort of outbreak could have serious adverse effects on military operations in any area of dengue transmission, field studies such as these should be pursued.
3. Flavivirus Transmission in Rural Thailand.

PROBLEM : To determine the risk of flavivirus infection and the environmental risk factors influencing transmission of flaviviruses in rural Thailand.

FUTURE OBJECTIVE : This study may reveal important information about the transmission of flaviviruses in a rural setting. The study should be completed and follow-up studies planned.

4. Outbreaks of Encephalitis in Thailand, 1982 - 1983.

PROBLEM : To determine the proportion of cases of acute encephalitis which is due to Japanese encephalitis virus using the JE MAC ELISA test.

BACKGROUND : Encephalitis, probably due chiefly to Japanese encephalitis virus, continues to occur in Thailand some 17 years after its demonstration in humans. Diagnosis is not possible in most of the regional hospitals, and when it is attempted, the HAI method is used. This method is unreliable because : 1. Paired sera are required, thus negating the possibility of a diagnosis in rapidly fatal cases, and 2. The widespread presence of dengue causes confusing cross reactions in secondary infections that make differentiation between dengue and JE impossible in any second flavivirus infection. In recent years, this laboratory has developed a MAC ELISA for diagnosis of JE (Burke, DS., et al. Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. J. Clin. Microbiol., pp 1034-1042, (1982).) which is positive in 80% of patients at the time of admission, and in 100% within three days. Moreover, since the test measures CSF antibodies, the presence of large amounts of flavivirus antibody in the serum do not interfere. Thus the test has the potential of providing a specific diagnosis in many cases where HAI serology is inadequate. Its usefulness in the field suggests that it is a test which will be very helpful in clarifying the etiology of the ongoing problem of encephalitis in Thailand.

To further evaluate the usefulness of this test, we have sought outbreaks of encephalitis and investigated them ourselves, or encouraged their investigation by Ministry of Public Health epidemiology trainees.

PROGRESS : Outbreaks of encephalitis occurring during the rainy season were sought prospectively based on the reporting of encephalitis cases in the national surveillance system. Four such outbreaks were investigated : one each in the provinces of Kampanghet, Uttaradit, Chiangmai, and Yasothon.

RESULTS :

1. Kampanghet : For the third consecutive year, (1981, 82, and 83) we conducted epidemiological and clinical studies of Japanese encephalitis in Kampanghet Province, Thailand. During the three years of the study, 40, 41, and 66 cases occurred, respectively, marking 1983 as a year of particularly intense JE activity in that province. The 1983 epidemic began abruptly in mid June and rose to a sharp peak during the week of 13-19 July (Table 1). In that week alone, 14 encephalitis cases, of which 11 were confirmed as due to JEV, were admitted to the hospital requiring the dedication of a special hospital ward exclusively for the care of encephalitis patients. By the third week in August, admissions due to encephalitis were decreasing.

Table 1. Weekly number of cases of encephalitis in
Kampanghet province, 1983.

<u>Week</u>	<u>Cases</u>	<u>JE*</u>
6/15 - 6/21	3	2
6/22 - 6/28	8	5
6/29 - 7/ 5	7	6
7/ 6 - 7/12	7	4
7/13 - 7/19	15	11
7/20 - 7/26	10	10
7/27 - 8/ 2	4	4
8/ 3 - 8/ 9	7	4
8/10 - 8/16	5	3
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Total	66	49

* confirmed by anti JE IgM in CSF

Of the 66 cases in 1983, Japanese encephalitis was established as the etiology of 49 by detection of IgM anti-JE antibody in the admission cerebrospinal fluid with a JE MAC ELISA test. Twelve confirmed cases were fatal, for a case fatality ratio of 24%. Post-mortem brain biopsies or autopsies were performed in 10 of the 18 fatal encephalitis cases, and JE virus was isolated from brain tissue in at least 8 of these cases. Cultures of CSF, performed by inoculating AP-61 (*Aedes pseudoscutellaris*) cells at the bedside, yielded JE virus in 5 out of 47 attempts from confirmed cases (Table 2).

Table 2. Isolations of JEV in Kampanghet, 1983.

<u>Source</u>	<u>Number Attempted</u>	<u>Number Positive</u>
Brain	10	8
CSF	47	5
Pig	4	1

There are approximately 560,000 people in Kampanghet Province, thus the attack rate for JE was 7 cases per 100,000 population. Age specific attack rates were highest in 6-10 (22/100,000) and 1-5 year olds (21/100,000). Within the province, the geographic distribution of the 1983 cases extended approximately 50 km further south than in 1981 or 1982, suggesting some southward movement of the disease.

We conclude that :

1. Japanese encephalitis epidemics continue to recur on an annual basis in Thailand.
2. 82% of cases of encephalitis were confirmed as due to JEV.
3. Age specific attack rates are such that any immunization programs for JE must be targeted at pre-school age, as well as school age children.
4. The diagnosis of JEV encephalitis can be confirmed by post-mortem needle brain biopsy in a high percentage of fatal cases.
5. The technique of bedside inoculation of CSF into AP-61 cells yields a virus isolate in 10% of cases.

2. Uttaradit : Uttaradit is a province in the northern part of Thailand which has generally experienced some of the highest rates of encephalitis in Thailand. Encephalitis cases were diagnosed by the pediatrician at the provincial hospital. Serum and CSF specimens were collected and sent to AFRIMS for testing using the JE MAC ELISA test.

The monthly number of cases demonstrated the typical sharp July peak seen in virtually every outbreak which has been studied in recent years (Table 3).

Table 3. Monthly number of encephalitis cases seen in Uttaradit in 1983.

<u>Month</u>	<u>No. cases</u>	<u>No. fatal</u>
January	2	0
February	3	0
March	1	0
April	4	1
May	1	0
Jun	16	2
July	59	10
August	17	0
September	3	0
Total	106	13

Clinical specimens were collected on 31 cases and sent to AFRIMS. Of 29 with paired sera, 25 had a positive test for anti JE IgM. Of the other 5, only two had a firm diagnosis of encephalitis, one was felt to be a reaction to typhoid vaccine, one was accompanied by a rash and felt to be due to another agent, and one was a brain tumor. Thus of 27 encephalitis patients, 25 (93%) had evidence of acute JE infection as the etiology.

3. Chiangmai : On July 20, 1983, Drs. Supramit and Surachai of the Thai Ministry of Public Health came to AFRIMS to discuss their plans for an investigation of an outbreak of encephalitis in Chiangmai, a province in northern Thailand long known to contain many cases of encephalitis each year. Discussion of the optimum specimens for diagnosis ensued. In addition to the requirement for CSF for the MAC ELISA test, the recent success in isolation of JE virus from the CSF of acute cases suggested that field inoculation of AP-61 cells should be attempted.

CSF specimens were received on 17 individuals and from 33 well family members of cases. JE MAC ELISAS were strongly positive in 14 of the 17 cases (82%). Of the 33 well contacts of cases, 5 (15%) had evidence of JE IgM in their serum, suggesting that they had been recently exposed and infected, but had not developed encephalitis. Of the 17 cell cultures received back at the lab, only 4 were not heavily contaminated. These 4 yielded no evidence of JE virus.

CONCLUSIONS :

1. JE virus accounted for 82% of cases of encephalitis tested in this outbreak in Chiangmai.
2. 15% of asymptomatic controls had evidence of recent flavivirus infection, suggesting that transmission to humans is quite intense.
3. Field inoculation of cell cultures for isolation of JE from CSF was unsuccessful in this case, but the technique should be improved and retired under more felicitous circumstances.
4. Yasothon : In a recent review of encephalitis in Thailand, the province of Yasothon had the highest mean rates of any province of reported encephalitis during the last decade (Table 4).

Because Yasothon was far from any other province which had high encephalitis rates, its own high rate was a prominent feature of maps showing encephalitis rates by province. Moreover, pressure had been growing from local groups for the government to institute control programs. Therefore, a request was made of local surveillance officials that they provide more detailed information and specimens from cases of encephalitis.

Table 4. Provinces in which the encephalitis rates were among the 10 highest for six or more of the 12 years of surveillance between 1970 and 1981.

<u>Province</u>	<u>Region</u>	<u>No. yrs in top 10</u>	<u>Mean Annual rate</u>
Yasothon	NE	11	12.8
Kampangphet	N	11	12.4
Chiangmai	N	11	9.4
Chiangrai	N	10	12.1
Nan	N	9	12.1
Lampang	N	7	6.8
Lamphun	N	7	6.8
Uttaradit	N	6	10.9

RESULTS : During the surveillance period, 4 cases of possible encephalitis occurred. These specimens have not been sent to AFRIMS for analysis as of this time. However, even if they are all positive, the rate will be less than 10% of most previously reported rates.

CONCLUSION : One result of improved diagnostic tools is improved surveillance. In this case, the availability of an improved diagnostic test occurred simultaneously with the marked reduction in reported encephalitis cases from the province.

SUMMARY : These 4 investigations (Table 5) reveal that JE continues as a serious health problem in northern provinces, but not in Yasothon. About 80% of cases of encephalitis occurring in the rainy season are due to JEV.

Table 5. Summary of epidemiological investigations of JE, 1983.

<u>Province</u>	<u># cases</u>	<u># tested</u>	<u># with JEV infection</u>	<u>%</u>
Kampangphet	66	66	49	74
Uttaradit	76	27	25	93
Chiangmai		17	14	82
Yasothon	4	-		

FUTURE OBJECTIVES : Control measures for this disease are urgently needed in northern Thailand and should be evaluated.

5. Seroconversion of Pigs in Northeast Thailand to JEV

OBJECTIVE : To determine in swine the monthly seroconversion to JEV in Northeast Thailand.

PROBLEM : Pigs are known to be an amplifying host for JEV. Studies on pig seroconversion rates in Chiangmai, Japan, and Sarawak have shown high rates of seroconversion that are not constant throughout the year. Infection in pigs are a means for monitoring the JE season. Increased seroconversion in pigs is an area with a high human attack rate. However, in Southern Thailand there are many pigs with JE antibody and few reported cases of JE in man. Multiple factors may account for this including climate and vector differences and differences in strains of virus. This study at Sri Kiu pig farm near Korat will allow calculation of seroconversion rate in a province with an intermediate attack rate. This information will help in determining the importance of swine in JE transmission and possible differences in various locations in Thailand. Attempts will be made to isolate virus from these pigs so it can be compared with recent isolates from an area where clinical JE infection in man is common.

PROGRESS : The testing of pigs for HAI antibody to JEV began on 15 June 1983. Twenty pigs, three months of age were bled and 12 had positive HAI titers. To date, 4 months into the study, the average monthly seroconversion has been 23.3% (range 17-28%).

6. Hepatitis A Outbreaks in Thailand, 1982 - 1983.

PROBLEM : In the past two years, two epidemics of Hepatitis were investigated. Both epidemics were investigated because initial data suggested that young adults were most heavily affected, a characteristic that was thought to suggest non-A non-B hepatitis. Unexpectedly, both outbreaks were due primarily to Hepatitis A virus, suggesting that susceptible cohorts of young adults are developing as sanitation improves in Thailand. The first outbreak may have been due to both A and NANB viruses.

1. Acute hepatitis in Nakorn Sawan : In November, 1982, routine surveillance of hepatitis indicated a marked increase in reported cases in the province of Nakorn Sawan, about 200 kilometers north of Bangkok. An investigation by the Ministry of Public Health epidemiology trainees and AFRIMS was initiated. Surveillance of acute hepatitis cases was instituted at the provincial hospital, and convalescing cases which had been previously reported in the national surveillance were sought. Controls for cases seen at the hospital were chosen from patients seen with traumatic illnesses.

Seventy-nine convalescent cases and 21 acute cases were found in the surveillance program. The epidemic lasted from May until November. A peak in hepatitis A cases occurred in August, while a peak in apparent non-A non-B cases occurred in October. Cases were concentrated in a neighborhood where the water supply to several blocks of shop houses was compromised by poor quality, submerged, leaking pipes and considerable poorly-drained, standing water. It was hypothesized that the disease had been transmitted through the drinking water, and recommendations for improving the quality of the water supply were made.

Laboratory studies revealed that 63% of convalescent cases, but only 20% of controls, were positive for IgM anti HAV by radioimmunoassay ($p < .01$). Only 1% of cases and no controls had evidence of hepatitis B infection. Among the patients found in the prospective surveillance, 29% of cases and 22% of controls had evidence of hepatitis A. The incidence of hepatitis A was highest in 10 to 19 year olds. 72% of acute hepatitis cases in this age range had evidence of hepatitis A infection. In older patients, about 40% of those with hepatitis had evidence of neither A nor B, suggesting that a form of non-A non-B hepatitis may have occurred in conjunction with the hepatitis A epidemic. When possible, stool specimens had been collected from early cases with the intention of seeking an infectious agent if the case appeared to be due to a non-A non-B hepatitis. In one case of NANB hepatitis, such a specimen was collected. Immune electron microscopy revealed the presence of a small number of 27 nm particles consistent with hepatitis A virus or non-A non-B hepatitis.

2. Hepatitis A in Chiangrai, Thailand : In August of 1983, investigators from the Thai Ministry of Public Health epidemiology program, requested the assistance of the AFRIMS Department of Virology in investigating an epidemic of hepatitis in Chiangmai, a province in the far northern part of Thailand.

The largest number of cases occurred in August, when 61 cases occurred. Again, most cases were in young adults between 15 and 24 years of age. Blood specimens from 24 acute cases were obtained. Of these, 20 contained IgM anti HAV, suggesting that hepatitis A virus was the etiologic agent. Epidemiological studies are in progress.

CONCLUSIONS :

1. These two outbreaks suggest that hepatitis A can no longer be considered exclusively a disease of children in Thailand. Presumably, as sanitation improves, the numbers of children exposed to hepatitis A virus in young childhood is lessening. As a result, cohorts of adults are developing.

2. The first outbreak suggests that NANB hepatitis may occur in conjunction with hepatitis A in young adult populations in Thailand.

FUTURE OBJECTIVES :

1. Further studies to isolate the agent(s) and define the epidemiology of NANB hepatitis in Thailand are needed. Either investigations of acute outbreaks or prospective surveillance among high risk groups, such as those along the Cambodian border, would be appropriate.

2. With respect to efficacy trials of hepatitis A vaccine in Thailand, attack rates in younger children may be lower than previously thought, while those in older people may be higher. Vaccine protocols should take these findings into account.

7. Rotavirus as a Cause of Severe Gastroenteritis in Adults

OBJECTIVE : To determine the role of rotavirus as a cause of gastroenteritis in adults in Thailand.

PROGRESS : Rotavirus was identified as the only etiologic agent in five percent (28/526) of adults with diarrhea who were admitted to Bamrasnaradura Hospital in Nonthaburi, Thailand during a one year period. Infection was determined by detection of rotavirus in diarrheal stools by ELISA accompanied by a greater than fourfold rise in serum CF and RIA antibody titers to rotavirus. Adults with clinical rotavirus infections were as severely ill as patients with most bacterial enteric infections; only patients with cholera passed more watery stools and were more dehydrated than those with rotavirus infections. Only two of the 28 adults with rotavirus infections had known recent contact with young children with diarrhea. Rotavirus infections in these adults occurred most frequently in the cooler, drier months in Thailand than during the rest of the year. In some settings, rotavirus should be considered in the differential diagnosis of severe diarrhea in adults as well as in young children.

FUTURE OBJECTIVES: To collect rotavirus from infants and adults and compare the serology and immunological response for the different serotypes of rotavirus.

8. Prevalence of Heat-stable II Enterotoxigenic *Escherichia coli* in Pigs, Water, and People at Farms in Thailand as Determined by DNA Hybridization

OBJECTIVE : To determine the prevalence of heat-stable II enterotoxigenic *Escherichia coli* in pigs, water, and people at farms in Thailand as determined by DNA hybridization.

PROGRESS : The DNA hybridization assay employing a 460 base pair fragment of DNA encoding for ST-II enterotoxin was used to determine the prevalence of heat-stable II enterotoxigenic *Escherichia coli* (ETEC) in pigs, people, and water at 57 farms in Sri Racha, Thailand. ST-II ETEC infections were found in 248 (12%) of 2145 suckling pigs, none of 560 weanling, or 455 adult pigs examined. Evidence of ST-II ETEC was found in pigs at 13 of 32 (41%) farms with suckling pigs with diarrhea and at three of 25 (12%) farms with pigs of the same age without diarrhea ($p < 0.025$). ST-II ETEC was detected in water collected from three of 57 clay jars used to store bathing water at three pig farms, in one jar outside of the barn, and from one asymptomatic farmer at a pig farm. Evidence of this organism was not found in 245 other individuals living at the pig farms, or in 220 inhabitants and 114 water specimens at tapioca farms nearby. ST-II ETEC was associated with young pigs with diarrhea in Thailand, but was infrequent in man.

FUTURE OBJECTIVE : Study completed.

9. Mosquito Survey and Taxonomic Studies

PROBLEM: To elucidate the mosquito fauna of Thailand and Southeast Asia. Primary emphasis is put on the determination of diagnostic characters that separate the vector species and groups containing vector species that transmit parasites detrimental to humans.

PROGRESS : During the past year, morphological studies were continued on the sibling species of the *balabacensis* and the *maculatus* complexes. These studies are being done in collaboration with the WRAIR Biosystematics Unit at the Smithsonian Institution. Manuscripts are in preparation to describe new species in the *balabacensis* complex, with the members currently being referred to as *Anopheles dirus* A, B, C, or D. Discriminating morphological characters have been found that will differentiate some of the new species in the *maculatus* complex. A key has been prepared and is included in a manuscript still under preparation (1). Work on the *Aedes (Finlaya)* manuscript continues with over 22 plates having been completed. Progeny rearings of vector species from many locations in Thailand and several locations in West Malaysia have been completed and correlated with genetic identifications.

FUTURE OBJECTIVES : Taxonomic studies on members of the *balabacensis* and *maculatus* complexes will continue. Morphological characters to discriminate field-collected *An. dirus* A, B, C, and D will be tested with populations in Thailand and Malaysia. The key to separate the *maculatus* complex also will be tested in the field with large population samples. The final ten plates for the *Aedes (Finlaya)* manuscript will be prepared.

REFERENCE :

1. Rattanarithikul, R., Green, C., Baimai, V., Andre, R., and Tremongkol, A. Morphological differentiation within the *Anopheles maculatus* complex of species. (Manuscript in preparation).

10. Mosquito Cytogenetic, Electrophoretic and Cross Mating Studies

PROBLEM : To define and delimit the taxa in the vector *Anopheles* species complexes by cytogenetic, electrophoretic and cross mating techniques for the following reasons : a. to check against current morphological species concepts; b. as an accurate determination of chromosomal polymorphisms and genetic variations in natural populations of malaria vector species and/or suspected vector species; and c. to correlate genetic variations in natural populations of malaria vector species with habitat differences, innate susceptibility to the human malarial, and behavioral patterns that may facilitate more effective control measures in the future.

PROGRESS : Analyses of metaphase chromosomes of the four taxa of the *Anopheles balabacensis* complex (*An. dirus* A, B, and C, *An. takasagoensis*,) using the Hoechst 33258 fluorescent staining technique have revealed remarkable differences in fluorescent banding patterns of the sex chromosomes, particularly the Y chromosome of these taxa. These differences are mainly the amount and distribution of constitutive heterochromatin. Two largely independent studies of chromosomes from natural populations of *Anopheles maculatus* provide evidence for several genetic species within the taxon. Polytene chromosome variation shows four different rearrangements of arm 2 and three rearrangements of the X chromosome. There is unequivocal evidence for three species within this complex. The progeny of five isofemale lines of *Anopheles indefinitus* exhibited a supernumerary (B) chromosome. Some preparations of the B-chromosome manifested 2 sister chromatids indicating some normal duplication and segregation.

Electromorphic variation for some esterases and 6-phosphogluconate dehydrogenase enzymes in *An. maculatus* is controlled by four loci which are unlinked to sex. Esterase loci are linked to each other; Est 1-36%-Est 4-16.5%-Est 3; but unlinked to Pgd 2. Linkage data were obtained by selfing F-1 from selected parents and analysing genotypes in the F-2. Cross mating experiments have provided additional evidence for the species status of members in the *balabacensis* and *maculatus* complexes.

FUTURE OBJECTIVES : Large samples from natural populations of members of the *balabacensis* and *maculatus* complexes will be collected and analyzed by cytogenetic and electrophoretic techniques. Correlations with morphology will be attempted. Recombinant DNA techniques will be tried to differentiate members of the *balabacensis* complex.

11. Comparative Susceptibility of Known and Suspected Species/Strains of *Anopheles* to *Plasmodium* Parasites

PROBLEMS : The objectives of this investigation are as follows : a. to determine and compare the susceptibility of primary and potential secondary vectors of malaria to *Plasmodium* parasites; b. to delineate the development of malaria parasites in *Anopheles* species with varying degrees of susceptibility; and c. to observe the feeding behavior of colonized vectors of human malaria under laboratory conditions.

PROGRESS : Studies on the susceptibility of various anophelines to human malaria continued this year at the Malaria Control Center, Tha Muang, Kanchanaburi Province. *Anopheles dirus* A was compared to other colonized Thai *Anopheles* species/forms in paired-feeding experiments. Thirty-two patients infected with falciparum malaria gave rise to infection in *dirus* A. Over three-quarters of the mosquitoes dissected had developed oocysts, but only forty percent had positive salivary glands. Half of the other mosquitoes (*dirus* B and C, *maculatus* A and B) became infected and of those, forty-one percent had sporozoites. Results from forty-five paired feeds on *Plasmodium vivax* patients showed that the mosquitoes were more susceptible to this parasite. Overall, *dirus* A was more susceptible to human malaria parasites than the other species/forms tested.

FUTURE OBJECTIVES : It is planned to continue this study next year in order to test the susceptibility of the new species in the *balabacensis* complex and the *maculatus* complex. Known genetic lines will be used to determine the importance of these sibling species in the natural history of malaria in Thailand.

12. Detrimental Effects of *Plasmodium* Infections on the Survival Rate of *Anopheles dirus*

PROBLEM : The objectives of this study are as follows : a. to determine if the longevity of mosquitoes infected with *Plasmodium* is different to a significant degree from that of uninfected mosquitoes; b. to determine if the longevity among mosquitoes with heavy or light infection rates is significantly

different; and c. to determine if the longevity of mosquitoes infected with different species of *Plasmodium* is different significantly among groups.

PROGRESS : An investigation of the effects of human malaria parasites on the longevity of *Anopheles dirus* continued this year. Over one hundred and fifty lots of mosquitoes have been fed on *Plasmodium vivax* patients. One hundred and ten lots of mosquitoes were allowed to feed on *P. falciparum* patients. About 40% of the feeds were positive. Control lots of mosquitoes were fed on uninfected volunteers on the day of the patient feed. Survival of control mosquitoes and lightly infected mosquitoes was excellent, with some mosquitoes living more than seventy days. Heavily infected mosquitoes usually died within one month. A manuscript on the correlation of survival rates of *An. dirus* with different infection densities of *P. cynomolgi* was revised and is in press (1).

FUTURE OBJECTIVES : Data from these experiments will be entered onto the computer for statistical analysis. Feeds will continue to be made during the next year to increase the number of infected comparisons.

REFERENCE :

1. Klein, T., Harrison, B., Grove, J., Vongprodit, S., and Andre, R. 1983. Correlation of survival rates of *Anopheles dirus* (Diptera: Culicidae) with different infection densities of *Plasmodium cynomolgi*. Bull. W.H.O. (In press).

13. Identification of Field-Collected Sporozoites

PROBLEM : Four different tests are available to potentially identify *Plasmodium* sporozoites in mosquito salivary glands : a. circum-sporozoite precipitin test; b. immunofluorescent antibody test; c. radioimmuno assay test; and d. enzyme-linked immunosorbent assay test. The objectives of this study are to provide *Plasmodium falciparum* and *P. vivax* sporozoites to WRAIR for the development and improvement of these tests, to evaluate these four tests in the laboratory with mosquitoes infected with Thai strains of *Plasmodium*, and to adapt the tests for use in the field to identify natural infections in vector anophelines.

PROGRESS : *Anopheles dirus* females were allowed to feed on patients infected with *Plasmodium falciparum* and *P. vivax* parasites. After 14 days the mosquito salivary glands were removed and the sporozoites harvested, or mosquitoes were frozen, or mosquitoes were killed and dried. *Plasmodium vivax* sporozoites were inoculated into BALB/C mice. Each mouse received an initial inoculum and four boosters over a period of three months. The mice were sent then to WRAIR for the development of monoclonal antibodies to be used in an ELISA test. Mosquitoes infected with *P. falciparum* sporozoites were sent to WRAIR for evaluation of new ELISA test and were sent also to NIH for evaluation of the RIA test (1). Mosquitoes infected with *P. vivax* also were sent for evaluation of the tests. Harvested sporozoites were spotted onto IFA slides for monoclonal antibody analysis and rabbit antiserum specificity evaluation. One *An. dirus* female, captured during a human biting collection, was found to have sporozoite-infected salivary glands. These sporozoites were tested using the IFA and gave

a positive reaction when exposed to a *P. falciparum* monoclonal antibody. Manuscripts describing the ELISA test for both species of *Plasmodium* are under preparation at WRAIR (2).

FUTURE OBJECTIVES : During the next year, evaluation of the different tests will continue. Emphasis will be placed on field-testing the ELISA technique and its applicability to use at a longitudinal malaria study site. Tests will be run to quantify the specificity and sensitivity of the ELISA for both falciparum and vivax malaria sporozoites against local Thai strains of parasites and vectors. The ELISA test will be used to determine the density of anophelines infected with human malaria sporozoites in a village situation.

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14. Correlation of Sporozoite to Gametocyte Ratios in a Village Situation

PROBLEM : The objectives of this study are as follows : a. determine the monthly gametocyte prevalence for *Plasmodium vivax* and *P. falciparum*; b. determine the species of sporozoites found in man-biting *Anopheles* in the same community using the IFA or ELISA tests; and c. compare species ratios for gametocytes with species ratios for sporozoites.

PROGRESS : Much of the year was spent in a search for a suitable study site which had hyperendemic malaria transmission. Villages in Chumporn, Ranong, Pak Chong, Tak, Petchabun, Trad, and Chantaburi were examined. In May, a village in Chantaburi was found that possessed all the attributes needed to support this investigation. The study site selected was Village 7, Baan Phluang, Amphoe Makaam, Chantaburi Province. Starting in June monthly blood smears were taken from a population of 200 people. The monthly prevalence of malaria ranged from fifteen to forty-five percent depending on the transmission season, which in this village appears to be different from the predicted season. Mosquito collections showed the three major vectors, *Anopheles dirus*, *maculatus*, and *minimus*, to be present in the village. Several mosquito species have been found to carry oocysts, but only *An. dirus* has been found with infected salivary glands. The sporozoites were identified as *P. falciparum* by the IFA test.

FUTURE OBJECTIVES : This study will continue for at least a year. The ELISA test for sporozoite identification will be evaluated in this village. Larval breeding areas will be determined, mapped, and correlated with habitat characteristics.

15. Ectoparasite and *Rickettsia tsutsugamushi* Studies in Thailand

PROBLEM : The objectives are to establish and describe ectoparasites that are or are potential vectors of human parasites or pathogens of human disease in Thailand, and to delineate the distribution of natural populations of larval mites infected with *Rickettsia tsutsugamushi* in Thailand.

PROGRESS : A checklist of the ticks occurring in Thailand has been revised and published (1). The Genus *Miyatrombicula* was redefined and a new species was described. This paper is currently in press (2). Illustrations of new species of the Genus *Leptotrombidium* have been prepared and a manuscript is in preparation. Collections of potentially infected chiggers were conducted at Khao Yai, Sakaerat, and Pak Thong Chai. The chiggers were sent to USAMRU in Malaysia for possible colonization and determination of infectivity status.

FUTURE OBJECTIVES : Because several new species of *Leptotrombidium* have been discovered and were found to carry *R. tsutsugamushi*, a collaborative study with the USAMRU Lab in Kuala Lumpur is being planned. This study would determine the role these new species play in the transmission of scrub typhus. Chromosome studies of *L. deliensis* also are proposed to determine if this species occurs in Thailand or if it is a sibling species and possibly not important in disease transmission.

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16. Serosurvey and Virus Isolation from Rodents to Determine the Hantaan Virus Presence in Thailand

PROBLEM : Recent studies have found Hantaan virus to be the causative agent of Korean hemorrhagic fever (KHF), a disease syndrome of significance in Korea and Manchuria and of potential military significance in the USSR, the Balkans, parts of Western Europe and Scandinavia. Evidence has recently been obtained in Seoul, Korea that urban *Rattus* also are chronically infected with Hantaan virus. Cases of KHF in man have now been linked to infected wild rats in urban Seoul and Osaka, Japan. In addition, antibodies to Hantaan virus have been found in *Rattus* captured near the docks in Japan, Korea and the United States. Chronic infection, rats and international shipping thus provide a likely chain which may have disseminated this virus worldwide. Thus the potential for this agent to cause human disease may be far greater and more widespread than is presently appreciated. Last year we reported that rodents trapped at Klong Toey port in Bangkok had antibody to Hantaan virus. In this preliminary study, approximately 20% of the bandicoots (*B. indicus*) tested had antibody titers to Hantaan. Natural infection with Hantaan virus has not been previously reported in this rodent species.

OBJECTIVES :

1. To identify areas in Thailand where rodents have antibody for Hantaan virus.
2. To test human sera from areas with rodent infection to determine if there is serological evidence of human infection.
3. To isolate Hantaan virus from tissues of rodents in endemic areas.

PROGRESS : Rodent trapping has been completed at Klong Toey (Bangkok Port, Sripacha, and Bangpakong ports). Of 235 rats (*R. rattus* and *R. norvegicus*) trapped in these locations, 10(4.3%) has positive antibody titers \geq 1:32. However, 8/45(17.8%) bandicoots, (*Bandicota indicus*) that were trapped in Bangkok had titers \geq 1:32. A trapping of bandicoots near Kanchanaburi resulted in 6/21(28.6%) with titers \geq 1:32. In addition, 30 residents living in the immediate area of the trapping were tested and 10/30(33.3%) had titers \geq 1:32.

FUTURE OBJECTIVES :

1. Tissue samples (lung, spleen, kidney, urine) from bandicoots will be tested for virus antigens by FA and virus isolation attempts will be made.
2. A group of 35 serum samples from residents at the Klong Toey trapping site who frequently trap and eat bandicoots will be tested for Hantaan antibody.
3. A study of the residents in Kanchanaburi is planned to determine if there is evidence of a KHF like illness in those people with serologic evidence of Hantaan or Hantaan-like virus infection.

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