BODY OF REPORT

SEATO Medic Study No. 13	Ecology of Arboviruses in Thailand. Yearly Dissemination in Vertebrates and Arthropods.
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	Division of Medical Research Laboratories
	Department of Virology
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Principal Investigator:	Dr. Joe T. Marshall, Jr.
Associate Investigators:	Major Scott B. Halstead, MC Dr. Rapin Snitbhan Dr. Pricha Singharaj Dr. Suchinda Udomsakdi 1st Lt James M. Neely, MSC 1st Lt Dick L. Deonier
Assistant Investigator:	Mr. Somsak Pantuwatana, MS
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Objective: To assess quantitatively and temporally the natural dissemination of arboviruses among mosquitoes and vertebrates within a discrete habitat.

Description: The principal study site is an island in the river 11 miles north of Bangkok. This island, Koh Kret, represents typical Central Plains environment with its heavy usage by men. It is about 3 square kilometers in area, has 4,500 persons living in 600 hourses around the periphery, and is given over in the interior to rice paddies with sugar palms and to fruit orchards. (Chicken sera

were gathered in the similar environment of Pitsanuloke, and blood samples from large egret, stork and swallow colonies near Bangkok have been preserved. Recently, vertebrate sera have been collected in additional representative habitats: semi-cultivated limestone hills east of Saraburi, and forest edge near Chiengmai).

Engorged mosquitoes are captured, maintained alive until their blood meal is digested, then frozen and stored for virus isolation. School children are bled serially at three-month intervals and the sera stored. Wild vertebrates are trapped and netted, bled, marked and released. After their heparinized blood samples are centrifuged, the cells are stored for virus isolation attempts and the plasma for serology. Representative study specimens of mammals, reptiles and amphibians and of birds from areas away from Koh Kret are preserved for taxonomic study in the scientific collections of the laboratory. Original plans called for field inoculations of suckling mice in order to isolate arboviruses from vertebrate blood. However, a paucity of mice necessitated recourse to isolation attempts in tissue culture with only the follow-up tests being made with mice. Sera are tested for hemagglutination inhibition.

Progress: Mosquitoes. The 89,466 specimens of mosquitoes identified from Koh Kret belong to 58 species. Of these, 8,448 specimens in 600 pools belonging to 15 species were frozen for virus isolation. So far, 210 pools have been screened for viruses, yielding positive results in 9 pools of Aedes aegypti. All have been identified as chikungunya virus. Aedes aegypti feeds on humans by day and most specimens were caught resting in houses. We may conclude that the chikungunya virus in Aedes aegypti is derived from humans and not from wild birds and mammals. The numerous entomological results regarding comparative methods of capture, taxonomy, behavior, and relative numbers are reported in the medical Entomology Department's study No. 40.

Human Sera. Serial bleeding of 292 school children at Koh Kret was accomplished on 3 June, 7-8 September (39 absent) and 14-15 December under the direction of Miss Prabhasri Umpaivit.

Japanese Encephalitis at Pitsanuloke. To the north, at the opposite end of the Central Plain from Koh Kret, is Pitsanuloke with its paddies, sugar palms, orchards and bird life all very similar to those of Koh Kret. An outbreak of Japanese encephalitis among Thais occurred there during the summer; it is reported in SEATO Medic Study No. 8. There were 67 cases from March to August, concentrated during June and July, and mostly of young persons from pastoral environments. At some of these farms chickens had died of disease. Accordingly, blood samples from 99 chickens were preserved by the ornithology team, from 26 to 28 August. HI antibodies against Japanese encephalitis were found in 34% of these chickens. These sera will be retested by JE neutralization test to evaluate the specificity of these results.

Mammals. The ornithology section obtained 93 blood samples for virus study from 17 species of wild mammals. Microfilariae were found in blood smears of

Rattus rattus at Koh Kret. Additionally, 165 scientific specimens (skin with skull) of 36 species were preserved. There are many very similar species of bats and of rodents in Thailand, and no single standard text for identification. Therefore, in order correctly to identify even common mammals, a good reference collection is needed.

This section,accordingly, sought to supplement the collections made previously by the Medical Zoology and Medical Entomology Departments. During this year Dr. Marshall trained Mr. Somsak, MS, and Mr. Somchai in mammalogy in order that identifications and taxonomically useful material will be obtained by: skinning with cornmeal or sawdust to absorb blood, removing the brain with a syringe through the intact foramen magnum, using small insects to clear the skull, and finally by providing a label with unequivocal sex symbols, standard measurements, size of gonads, locality and date. There are a half-dozen departments and sections concerned with medical studies of wild mammals here. The welcome addition to our SMRL staff of a full-time mammalogist, Dr. Norman Negus, provides an excellent opportunity to unite our various collections and to straighten out some of the taxonomic snarls, under his direction. Such cooperation, utilizing the extraordinary skills of our Thai expert, Mr. Kitti Thonglongya of Medical Entomology, should secure a remarkable advance in this field - - so important to zoology and epidemiology. The following tentatively identified mammals were collected this year (an asterisk denotes those occurring at Koh Kret):

Tupaia glis
Hylomys suillus
Euroscaptor klossi
Suncus murinus
Rousettus amplexicaudatus*
Cynopterus sphinx
Cynopterus brachyotis*
Eonycteris spelaea*
Macroglossus minimus
Myotis adversus
Pipistrellus imbricatus
Tylonycteris robustula
Scotophilus temminkii*
Scotophilus heathii*
Megaderma spasma
Rhinolophus lepidus
Rhinolophus ferrum-equinum
Rhinolophus affinis

Manis pentadactyla *
Herpestes javanicus
Menetes berdmorei
Callosciurus erythraeus *
Callosciurus caniceps
Callosciurus finlaysoni
Tamiops macclellandi
Belomys pearsoni
Hylopetes sagitta
Hylopetes phayrei
Rattus mülleri
Rattus berdmorei
Rattus rajah
Rattus exulans *
Rattus rattus *
Rattus norvegicus
Bandicota indica
Bandicota bengalensis

Reptiles and Amphibians. A remarkable observation of mosquitoes feeding upon toads is recorded from Koh Kret of 4 February. At dusk two toads were seen in a trail beside a small marsh. Mosquitoes of at least two species were so thickly settled along each side of the head that it appeared to be covered with whiskers!

Table 1

PERCENTAGES OF WILD BIRDS WITH HI ANTIBODY TO JEVE, BANGKOK, 1962-1963
(RECENT ANALYSIS AND REEVALUATION OF RAW DATA OBTAINED DURING SEATO MEDIC STUDY NO. 9)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
Number of blood specimens	23	4	180	229	22	293	191	306	140	80	163	157	172	31
Number with antibody	11	2	15	11	2	117	92	43	35	25	27	26	5	0
% with antibody	48	50	8	5	9	40	48	14	25	31	17	18	3	0

After dark and at dawn and dusk of subsequent nights, no such feeding was seen again. Blood samples were taken from 23 of these toads, Bufo, which is a common nocturnal species at this island. Two sera were positive for Japanese encephalitis antibody. A few other reptiles and amphibia were encountered there, but none was found commonly except for the two prevalent species of gecko (one of which feeds onmosquitoes). Snakes were looked for by day and by night, because snakes have recently been shown in the United States to harbor arboviruses gained from mosquitoes. A short-tailed nocturnal burrowing snake was the only seen regularly. Identification of our scanty collection still awaits receipt of the necessary books by Professor Edward H. Taylor, recently published by the University of Kansas: "The Amphibian Fauna of Thailand","Lizards of Thailand" and "Snakes of Thailand".

Birds. The success of an earlier SEATO Medic Study No. 9 (Annual Progress Report, SMRL, 1 April 1964) in demonstrating large scale transmission of Japanese encephalitis virus to wild birds in the Bangkok area is largely responsible for the present study and its emphasis on birds in the ecology of arboviruses. During this year, the raw ornithological data of Study No. 9 was put on punch cards for analysis and possible future publication. Analysis was handicapped by the loss during 1963 of all skins of unknown birds which had been prepared for identification, and by the use of equivocally-numbered bands which prevent analysis of what would have been rather extensive and valuable paired serological and recapture data of these birds which were marked, bled, and released. Blood samples from 1,733 birds of 61 species (and additional 187 chickens and 71 bats, Cynopterus

brachyotis) (yielded 411, 21% of 34 species, seven of them migratory) with HI antibodies for Japanese encephalitis virus. Frequency of positive antibodies varied with the seasons but did not depend importantly on species, migratory vs. resident status, or site of collection in Bangkok -- whether near buildings, in orchards, field, garden, marsh, brush patch, etc. Table 1 shows the fluctuation in proportionate antibody response by months, with high points in June, July, September, and October of 1962.

Accordingly it seemed desirable and logical to bleed birds throughout the year at Koh Kret. Of the 98 species of wild birds we found there, 64 species were netted to yield 1,073 blood samples, 225 of which are of recaptured individuals. Since herons and egrets are important hosts of Japanese encephalitis virus in Japan, we also bled their nesting colonies which were closest to Koh Kret (168 samples) and the other birds about Bangkok, including barn swallows from the Jawarat Street roost (cooperating with the MAPS Project), 54 species were bled in the hills east of Saraburi (287 samples), 42 species at Doi Pui, Chiengmai Province (258 samples, on an expedition cooperating with MAPS Project), and 27 species at Chiengmai University (119 blood samples). These blood specimens total about 2,391 from 118 species of birds for the year.

The 1,576 wild birds sera from Koh Kret and vicinity tested so far, have yielded a scattering of 25 with antibodies for Japanese encephalitis. This virus has now been isolated in two of the first 1,017 blood cell samples (centrifuged from each heparinized blood specimen, isolated in culture of hamster kidney cells and suckling mice). Such results are rather unusual. Indeed, finding a bird with virus is like catching a criminal redhanded at the scene of his crime, for viremia lasts only 3 to 6 days in a bird. The two birds are a myna (Sturnus nigricollis) and a sunbird (Nectarinia jugularis), both permanently resident at Koh Kret, with blood samples taken in July.

Impossibility of isolating virus through direct field inoculation into suckling mice (because of a decline in the mouse colony) deprived the ornithology section of capability to trace virus dissemination at the time of its occurrence. The results obtained in "hind'sight" even negative ones, are of interest however. The field team's proficiency in obtaining sera from vertebrate reservoirs, even in remote areas reached on foot, makes it ready for the outbreaks that are likely to occur. It is hoped that these can be heralded by appropriate mouse isolations from the mosquito vectors.

Meanwhile our scientific knowledge of the birds has advanced. Their importance to medicine is their abundance, susceptibility to the bites of disease carrying insects, and massive migrations. For instance, we have found a huge incidence of bird malaria (Plasmodium) with many heavy infections among perching birds at Koh Kret and some mountain areas (several hundred blood smears already examined from over 1,500 prepared). The night roost of barn swallows (Hirundo rustica) on Jawarat Road in downtown Bangkok numbers about 130,000 individuals throughout the winter, most of them nest in Siberia, Manchuria, and

China. In cooperation with the MAPS Project, 14,024 swallows were banded for migration study, so that recoveries in the north will document their route and summer destinations. The ornithology section independently banded and released 1,789 individuals of 118 species captured for virology study.

Another way we are studying migration is by identifying here in Thailand the race of a particular migrant species which shows consistent geographic variation in color, size, and proportions over its northern breeding areas. Of the 790 species of birds attributed to Thailand by Herbert Deignan (Checklist of the birds of Thailand, U.S. National Museum Bull. 226, 1963), 221 are migrants, mostly from northern or northeastern Asia. In 65 of these, more than one race is known from the Thailand wintering grounds. Correct identification of the race then reveals the general breeding area as surelyas if the birds had been banded. Such racial identification requires standard specimens for comparison; accordingly both MAPS and ornithology sections have enlarged the SMRL bird collection. (294 prepared by Marshall) with careful attention to authenticity of data on the labels. Some travel to museums will be necessary to round out study of these collections.

Considerable data on molt and other physical attributes has been recorded from birds being handled for virology. Information on the two least known aspects of Thailand birds -- ecology and behavior -- has accumulated. For instance, it was found that the stork colony (Anastomus oscitans) north of Koh Kret synchronizes the nesting cycle among the thousands of individuals by vocal and visual displays and that stereotyped rituals coordinate the efforts of the pair.

Ornithologists are in a unique position to make pronouncements -- some of them admittedly speculative - about human ecology. They look for unspoiled natural environments, where the most kinds and numbers of birds can be studied. They are particularly cognizant of trends in human activity which destroy natural habitats together with their faunas. Indeed, it is thought that many human diseases, for which native animals and birds are reservoirs, crop us as a result of such changes and replacement by species associated with man, such as commensal rodents, sparrows and starlings, together with their ectoparasites. In Thailand the native vegatation with its rich fauna is disappearing at an alarming pace. It is a symptom of these interrelated trends: advances in medicine, marked increase in human population, cutting and burning the forest for marginal or shifting agriculture, loss of soil, silting of the streams and rivers, and flooding. It would seem that a need to study the overall human ecology of Thailand exists, especially since medical research is involved in several of its aspects.

Summary: Year-round collecting of mosquitoes and of blood samples from vertebrates has revealed chikungunya virus in the former and sparse Japanese encephalitis virus and antibody in the latter. The large number of mosquitoes and vertebrates processed has made possible a considerable advance in our knowledge of their taxonomy, distribution, migration, behavior, and ecology.

Conclusions: Preliminary results indicates that chikungunya virus occurred at the

the study area and that it is disseminated between Aedes aegypti and man. Japanese encephalitis virus, rare in 1964 may fluctuate from year to year in addition to seasonally (as in the temperate zone). It apparently does not maintain itself at a steady level. Subtle shifts in the ecologic balance among the vectors, reservoirs and hosts might account for the puzzling differences of occurrence in time and place in Thailand.