

Title: Plague Study in Southeast Asia

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Objective: To perform an ecological and epidemiological plague survey in South East Asia.

Progress: A. Thailand. Plague surveys including collection of rodents and ectoparasites were carried out in the following areas of Thailand: Arranyaprathet, Bangkok, Chiangmai, Kanchanaburi, Korat, Mukdaharn, Nongkhai, Pakchong, Rajburi, Tak, Trad, Ubon and Udon. In addition rodent tissues and fleas collected by the Department of Medical Zoology from other areas of Thailand were submitted for examination; rodent tissues and ectoparasites, as well as human and domestic animal sera collected by the rickettsial project, Thai Component were similarly examined for evidence of Pasteurella pestis infection. Wild-caught or colonized fleas were tested for insecticide resistance.

1. Isolation of Pasteurella Pestis. Attempts to isolate P. pestis from rodent tissue or fleas of Thai origin have been carried out at the Institute Pasteur, Saigon, RVN. Of 90 such attempts, none has been successful. Further tissue or fleas collected during the past year remain to be processed.

2. Serologic Evidence of P. pestis Infection. Six hundred twenty-five rodent sera and 1053 human sera have been examined for the presence of hemagglutinating antibody to P. pestis. None were positive. A single positive serum, with a titer of 1:64 was found among 472 canine sera similarly tested. This positive serum came from a dog trapped by the Bangkok municipality in its anti-rabies program. The exact locality in Bangkok from which this dog came could not be determined.

3. Flea Index. Flea indices for the various regions of Thailand (Table 1) show some seasonal variation, with maximum indices being seen in those months during which human plague cases were formerly recorded. Except for Kanchanaburi, the reported flea indices refer to Xenopsylla cheopis on various Rattus spp. In the Kanchananaburi area, the predominant rodent trapped in November 1966 and February and March 1967 was Bandicota bengalensis; the indices for this area and time period refer to X. vexabilis on this rodent.

It is noteworthy that although it is commonly held that X. cheopis indices in Thailand rarely exceed 2, we have found an index of 3 or higher not unusual.

4. Insecticide Resistance in Fleas. Wild-caught fleas which were tested for insecticide resistance generally showed resistance to DDT and sensitivity to other insecticides tested (Table 2). Fleas from Ban Pong, Rajburi appear to be somewhat more sensitive to DDT than those from other areas. As noted, regression lines for mortality are fitted only with difficulty in the case of wild-caught fleas, since there is considerable mortality, probably associated with the trapping procedure and handling of fleas with attendant injury. In addition numbers available for testing from any given area are often limited, preventing replicate determinations. These problems are avoided by the establishment of flea colonies, especially from trapping

areas of low flea density. Fleas collected from rodents in Thailand thus far have appeared to be either X. cheopis or X. vexabilis. These species can be readily identified and separated under a microscope, alive.

Colonization. Fleas separated by species are transferred to large screw capped jars, in which a layer of saw dust has previously been placed. Ground dog biscuit is added, as larva food. A suckling mouse is placed in the jar to provide blood meals for the adult fleas. The mouth of the jar is then closed with muslin. This simple procedure can be carried out in the field and the fleas transported to the laboratory in the colony jars.

About 2 months is required to establish a colony large enough for a series of resistance tests. Colonized fleas appear to be superior for testing purposes to wild-caught fleas, inasmuch as insecticide dose-response is more uniform and reproducible. This is probably due to varying age and vigor of the wild-caught flea population.

Flea colonies have been established using X. cheopis from Bangkok and from Korat. Table 3 summarizes insecticide resistance data obtained from these colonies.

B. Viet Nam. Studies in Nha Trang RVN are being carried out as a joint project with the Institute Pasteur and USA Medical Research Team (WRAIR) Vietnam. Nha Trang is of interest since the current plague outbreak in RVN apparently made its first appearance in that city, in 1962. No organized rodent or flea control program has been attempted there, so the city presents an undisturbed urban rodent-flea ecology.

1. Human Plague. From 1963 through November 1966, of 1084 suspected human plague cases, for which bacteriologic diagnosis was attempted by the Institute Pasteur of MRT, 525 were confirmed. Sporadic cases are reported year-round, but the majority of cases have occurred in February, March and April each year; in 1967 a major outbreak of human cases again occurred in Nha Trang, with at least 70 confirmed cases.

2. Evidence of P. pestis Infection in Rodents and Fleas. Table 4 summarizes the Nha Trang data for 1966-67. Suncus murinus, the house shrew, was the most common rodent encountered; one isolation of P. pestis was made from this source, and 6 of 16 sera tested showed the presence of hemagglutinating antibody. Rattus norvegicus, trapped in slightly lower numbers than S. murinus was the source of at least 3 isolates of P. pestis; 56 of 206 sera thus far tested were positive for P. pestis antibody. R. exulans was trapped less than half as often as the other two species; no isolates were made but 2 of 13 sera tested were positive for HA antibody.

Flea indices varied with rodent species; R. norvegicus, which had the most consistent evidence of P. pestis infection, had consistently high X. cheopis index, from 6.3 to 8.5 Three of 21 flea pools from this rodent were positive for P. pestis. The flea index on R. exulans was consistently low, from 0.9 to 2.0. One P. pestis isolation was made from 21 flea pools from this species. The flea index on S. murinus was as high or higher than that on R. norvegicus (8.7) during the "plague season" and as low (2.5) as R. exulans in October. Four flea pools of 20 tested were positive for P. pestis. Data presented are incomplete, since laboratory results of the March 1967 field trip are not yet available.

3. Insecticide Resistance. Table 5 presents resistance data for fleas collected in Nha Trang in 1966-67. A high level of DDT resistance is indicated; sensitivity to other insecticides is present.

Summary and Comment.

Plague has not been detected in Thailand since 1952. Present studies will be continued and will be expanded to include possible sylvatic foci. The finding of a significant HA titer in one dog serum indicates that an enzootic focus may exist in or around Bangkok, or the presence of a rare undetected human case.

Fleas in the areas of South East Asia for which data are available show a high level of resistance to DDT, though remaining susceptible to other insecticides. Flea collection for insecticide tests must be continued to determine the most effective insecticide for use in plague control, to avoid rapid spread of newly introduced epizootic plague. In areas and at times of low flea density, colonization of fleas will insure adequate numbers of fleas are available for testing.

The epidemiologic data necessary for the control of plague in the coastal city of Nha Trang are available. Plague is present throughout the year, with a pronounced seasonal peak in human cases in February-April of each year. Plague appears to be present in the rodents and insectivores throughout the year; with some seasonal variation in *R. exulans* and *S. murinus*, but relatively constant in *R. norvegicus*. Fleas, almost entirely *X. cheopis*, from these animals, are highly resistant to DDT. Flea populations are at their highest during the "plague season" but the index on *R. norvegicus* is high year round. Socio-economic conditions in the area are poor; rat control is probably not feasible on a large scale. Flea reduction, using an insecticide of demonstrated effectiveness, is probably the only practical method for plague control in Nha Trang.

Table 1

Rodent Flea Indices* by Trapping Area and Month of Collection, Thailand 1966-1967.

Area Month	Bangkok	Korat	Pakchong	Kanchanaburi	Rajbiri	Arranyaprathet	Chiangmai	Tak	Udon	Nongkhai	Ubol
May 66	1.6	—	—	—	—	—	—	—	—	—	—
Jun	—	—	—	—	—	—	—	—	—	—	—
Jul	1.5	1.1	—	—	—	—	—	—	—	—	—
Aug	1.0	1.7	2.8	—	—	0.2	—	—	—	—	—
Sep	0.6	2.1	1.1	—	—	—	—	—	—	—	1.0
Oct	—	1.4	0.8	—	—	—	—	—	1.2	1.4	—
Nov	0.9	1.3	1.9	0.6**	1.8	—	—	—	—	—	—
Dec	—	1.0	1.5	—	—	—	3.2	2.9	—	—	—
Jan 67	—	1.6	2.7	—	—	—	—	—	—	—	—
Feb	—	—	—	1.3**	2.7	—	—	—	—	—	—
Mar	3.1	2.8	2.8	9.5**	—	—	—	—	—	—	3.7

* Except as noted, *X. cheopis* on various *Rattus* Spp.

** *X. vexabilis* on *Bandicota bengalensis*.

Table 2

Insecticide Sensitivity of Wild-Caught Fleas from Former Plague Foci in Thailand.

Area of Collection	LC ₅₀ in DDT	% Dieldrin	Benzene Hexachloride
Ban Pong (Rajbusi)	1.2	NT	NT
Pakchong	ND	0.07	0.09
Korat	ND	0.07	0.08

Note:

ND=Not determinable; regression line not fitted, 20-40% mortality in each standard test concentration.

NT=Not tested.

Table 3

Insecticide Sensitivity of Colonized X. cheopis from Bangkok and Korat Thailand.

Source of Colony	LC ₅₀ in %		Malathion
	DDT	Benzene Hexachloride	
Bangkok	> 4.0	0.3	0.02
Korat	> 4.0	0.4	0.02

Note: For DDT 23% mortality at 4.0% conc., Bangkok fleas.
40% mortality of 4.0% conc., Korat fleas.

Each figure result of 3 replicates.

Table 4

Summary of Field and Laboratory Studies by Mammal Species and Month, Nha Trang 1966-1967.

Mammal Species	Month of Study	Number Trapped	Plague HA Antibody*	P. pestis Isolates*		Flea** Index
				Spleen pool	Flea pool	
<u>Rattus Novegicus</u>	Apr 66	68	8/34	0/6	0/4	8.5
	May	159	30/106	0/21	2/13	7.1
	Oct	102	18/66	1/10	1/4	6.4
	Mar 67	149	N.C.	N.C.***	N.C.	6.3
<u>Rattus exulans</u>	Apr 66	22	0/2	0/4	0/4	2.0
	May	110	2/9	0/16	0/13	1.2
	Oct	31	0/2	0/4	1/4	1.7
	Mar 67	25	N.C.	N.C.	N.C.	0.9
<u>Suncus Murinus</u>	Apr 66	41	0/3	0/5	1/4	8.7
	May	169	5/9	1/20	2/12	7.1
	Oct	112	1/4	0/12	1/4	2.5
	Mar 67	208	N.C.	N.C.	N.C.	5.4

Notes: * No. positive/no. tested
 ** > 99% X. cheopis
 *** At least 2 rats positive, remainder not complete
 N.C. Not completed.

Table 5

Insecticide Sensitivity of Wild-Caught Fleas (99% X. cheopis) from Nha Trang RVN 1966-1967.

Insecticide	No. of Replicate Tests	LC ₅₀ in %
DDT	22	2.3
Dieldrin	15	0.1
Benzene hexachloride	5	<0.125
Diazinon	2	0.003
Malathion	6	0.023