

Antibody to Tick — borne Viruses in Thailand

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OBJECTIVE: This study is an attempt to determine if humans and small animals have naturally acquired serum antibody to tick — borne viruses.

BACKGROUND: Three different viruses were isolated from *Haemaphysalis* ticks in two provinces of Thailand during the spring and summer of 1973. One tick from Loei Province was removed from a domestic dog. The other ticks were collected while resting on vegetation in Khao Yai National Park (KYNP), and so gave no information on the potential hosts for the viruses they carried (SMRL Annual Report 1974).

The *Haemaphysalis* genus of ticks has a wide range of animal hosts including man. There are an estimated 90 species in the genus. The viruses were isolated from *H. conigera* and *H. papuana*. Although these species will bite mammals, it is not known if they will bite humans.

A serologic survey of animals and people living in the vicinity of the viruses was conducted to try to determine their natural hosts.

DESCRIPTION: A program for serologic screening of residents of KYNP was initiated with the cooperation of the Chief, National Park Division, Department of Forestry. Surveys were conducted in September, 1973 and February, 1974.

Residents of KYNP include the families of employees of three different administrative agencies working in the park: Department of Forestry (FD), Highway Department (HD) and the Tourist Organization of Thailand (TOT). Each agency has its own work responsibilities and housing areas. TOT families as a group are the highest paid, best educated and enjoy the best living conditions. Members of each group travel widely throughout the park and have access to the areas where the ticks were collected, even though some tick collection sites are long distances from some of the housing areas. (Figure 1).

In each housing area, the study was explained as a survey for malaria and viral diseases. Residents were encouraged to participate on a voluntary basis. Each participant was questioned regarding his or her age, length of residence in KYNP, area of residence, names and relationship of family members, and history of illnesses and hospitalization. Residents were encouraged to provide this information even if they refused to allow blood to be drawn.

Two to 10 ml of whole blood was drawn from each individual, allowed to clot at ambient temperature and centrifuged. Serum was transferred to storage tubes and frozen at -70°C until tested.

Thick and thin blood smears were made for each participant for malaria screening. Malaria smears were stained and interpreted by members of the Department of Microbiology.

Table 1. Characteristics of Residents of KYNP. Combined Information from Surveys in September 1973 and February 1974

Agency	No. of Residents	Sex		Age in years Mean (Range)	Years of Residence Mean (Range)
		Male	Female		
Forestry Dept	166	1.81		21.0 (9/12-60)	3.6 (1 day-13 yr)
Highway Dept	134	1.38		26.2 (3-59)	6.9 (15 day-43 yr)
TOT	248	1.05		20.1 (6/12-48)	3.8 (7 day-10 yr)
TOTAL	548	1.32		21.4	4.5

At least one serum specimen was obtained from 77.9% of the 548 residents (Table 2). Paired sera were obtained from 29.4%

Table 2. Serum Samples Collected from Residents of KYNP

Agency	Number of Sera			Percent of Residents	
	First Serum	Second Serum	Second Serum Feb	At least one Serum	Paired Sera
	Sep	Feb			
Forestry Dept	100	48	60	89.2	36.1
Highway Dept	34	39	8	54.5	6.0
TOT	156	50	93	83.1	37.5
TOTAL	290	137	161	77.9	29.4

Neutralizing antibody was detected by plaque reduction neutralization test (PRNT) for T-1642 and T-1674. Neutralization tests with T-870 were done in suckling mice. Sera showing at least 50% neutralization at a dilution of 1:10 were considered to be positive and were retested in serial dilution.

Microtiter complement fixation (CF) and hemagglutination inhibition (HI) tests were performed by standard techniques using antigens prepared by sucrose-acetone extraction of suckling mouse brains.

Sera were collected from live-caught small animals trapped between Km 30 and 33 and near Km 40 (Figure 1). Each animal was identified by species and location, examined for ticks, bled and released. Some rodent sera were obtained from other areas of Thailand.

Table 3. Frequency of Antibody in Residents of Khao Yai National Park (Sept, 1973)

Agency	T-870		T-1674			Dengue-2	JEV ¹	Chik ²
	SMB-NT ³	CF	PRNT ⁴	CF	HI	HI	HI	HI
Forestry Dept.	0/76*	0/98	8/98	7/56	33/97	57/98	57/98	45/98
Highway Dept.	0/24	0/34	0/33	1/7	21/32	32/34	33/34	22/34
TOT	0/87	1/151	13/151	34/94	69/149	101/150	103/150	75/150
Combined	0/187	1/283	21/282	43/157	123/278	190/282	193/282	142/282

1 Japanese Encephalitis Virus

2 Chikungunya

3 Suckling Mouse Brain Neutralization Test

4 Plaque Reduction Neutralization Test

* Number of antibody positive sera over number tested

Table 4. Residents of KYNP with Plaque Reduction Neutralizing Antibody to T-1674

Serum No	Occupation	Sex	Age (years)	Years in KYNP	Housing Area	HI Titer		
						T-1674	D-2	JEV
54034	FD-Dependent	M	4	4	1	<10	<10	<10
54039	FD-Dependent	M	7	7	1	<10	<10	<10
54040	FD-Dependent	M	4	4	1	<10	<10	<10
54060	Housewife	F	24	2	1	<10	<10	<10
54228	TOT-Dependent	F	4	4	2	<10	<10	<10
54220	TOT-Dependent	M	12	10	2	<10	10	<10
54092	Receptionist	M	22	1/12	2	<10	<10	10
54090	Restaurant worker	F	28	4	2	<10	10	20
54116	TOT-Dependent	F	12	6	2	<10	20	20
54094	Janitor	M	28	1	2	10	20	20
54019	Guest House worker	F	54	9	6	<10	10	40
54077	Janitor	M	22	3/12	2	<10	20	40
54038	Housewife	F	42	11	1	10	40	20
54111	Housewife	F	38	6	2	20	20	40
54107	Guest House worker	M	22	2	2	40	20	40
54029	Guest House worker	M	26	6	1	20	40	40
54091	Carpenter	M	24	4	2	20	80	40
54036	Housewife	F	23	1	1	20	40	80
54076	Receptionist	M	33	5	3	20	80	80
54082	Accountant	M	23	3/12	2	40	80	80
54056	Forest Guard	M	23	2	Waterfall	20	160	160
54078	Restaurant worker	M	22	7/12	2	160	160	160

Table 5. Frequency of Antibody to Tick-borne Viruses

Virus Strain: Test Used: Serum Dilution:	T-870		T-1642		T-1674		
	SMB-NT at 1:10	CF ≥ 1:4	PRNT at 1:10	CF ≥ 1:4	PRNT at 1:10	CF ≥ 1:2	HI ≥ 1:10
Human	0/187	1/283			21/282	43/157	123/278
Gibbon							1/8
<i>Tupaia glis</i> (Tree shrew)		3/11					0/19
<i>Hylomys suillus</i> (Pig shrew)							0/2
Dog			1/54	38/100*			
Goat		0/2					
Sheep		0/1					
<i>Melogale personata</i> (Ferret badger)							0/1
Rat		6/103*					0/168

* All 38 reactive dog sera and 4 reactive rat sera reacted with normal mouse brain or were anticomplementary to within one dilution. CF reactive rats were *Rattus fulvescens*-1 and *R. surifer*-1.

No serum samples were obtained from Loei Province, the source of virus strain T-1642. Since this strain was isolated from a tick on a dog, sera were collected from 100 dogs at the Bangkok dog pound. Sera from eight laboratory gibbons were obtained for testing from the Division of Veterinary Medicine.

PROGRESS: Information was gathered by questionnaire on 548 different residents during surveys in September and February. Members of families of Highway Department employees tended to be older and to have lived in the park longer than the other two groups (Table 1). All agencies had a constant turnover in personnel and many people had lived in the park for less than a year.

Antibody to Tick-borne Viruses: CF antibody to T-870 was found in only one of 283 sera collected in September (Table 3). The single reactor titered 1:32 to T-870, 1:8 to normal mouse brain and was anticomplementary to 1:4. A second serum from the same individual collected in February titered <1:2. None of 187 sera, including that from the CF reactor, had neutralizing antibody in suckling mice. Thus, there is no conclusive evidence that T-870 can infect people.

A great many sera (44.2%) reacted by HI to T-1674 (Table 3). Even more reacted to other group B arboviruses. Generally, people reactive by HI and CF were over 10 years of age, and represented a fairly constant proportion of each age group. Because of the high degree of cross-reactivity, analysis of T-1674 reactors was confined to the 21 sera with neutralizing antibody (Table 4).

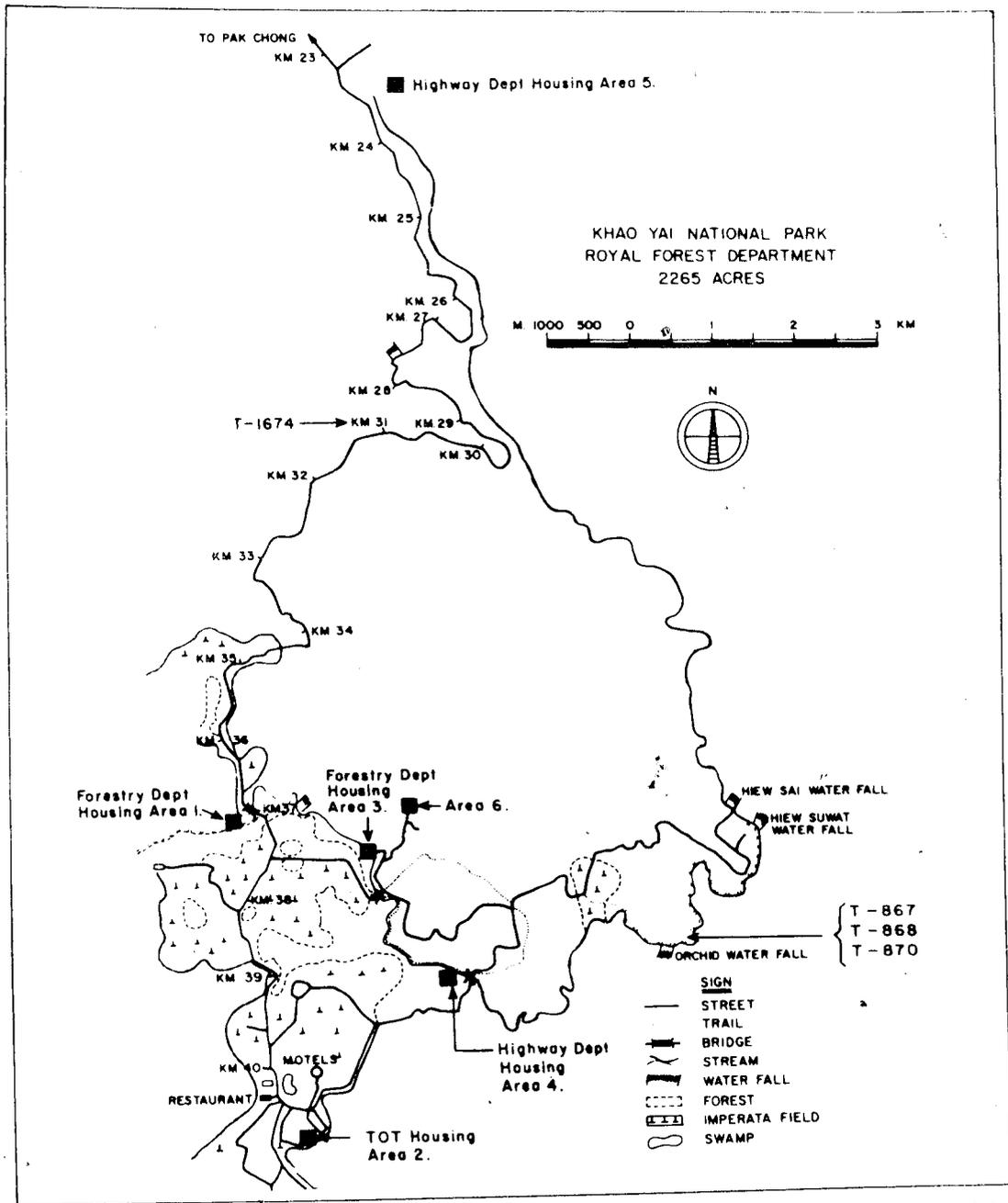


Figure 1. Study area in Khao Yai National Park. Virus isolates were made from ticks collected at Km 31 and Orchid Waterfall. Black squares indicate housing areas of residents.

All of the residents with neutralizing antibody to T-1674 came from families of Forestry and TOT employees. The lack of Highway Department personnel may simply be due to underrepresentation of this group in the September survey.

There was no clear correlation of neutralizing antibody with age, sex, duration of residence in the park, area of residence, occupation or HI titer. On the other hand, HI reactivity to T-1674 closely paralleled HI reactivity to other group B arboviruses.

Eleven of 17 (65%) of the PRNT positive residents reported previous tick bites; however, 55.8% of the PRNT negative residents did too. Some residents reported that tick bites caused fever, local swelling and local pain. None of them had ever been hospitalized. From interviews and examination of skin lesions, it seemed that tick bites were familiar to the residents of KYNP and occurred more frequently in February than September.

One case of *Plasmodium falciparum* malaria was identified in a Forestry Department employee during these surveys.

Screening tests for antibody to tick-borne viruses in other species is incomplete (Table 5). Nevertheless two rats had CF titers to T-870 of 1:16 and one dog had a titer of PRNT neutralizing antibody to T-1642 of 1:60. CF tests of dog sera were unsatisfactory because 38 percent were anticomplementary.

On the basis of this information it can be concluded (1) ticks commonly bite humans at KYNP (2) tick bites may lead to mild fever and local symptoms in some people (3) T-1674 has infected humans at KYNP in the past (4) T-870 may infect rats naturally and T-1642 may infect dogs. The HI and CF tests are of little value for screening people for antibody to T-1674 because of the high level of cross-reactivity with other group B arbovirus antigens.