

## Japanese Encephalitis Among United States Military Personnel in Vietnam

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**OBJECTIVE:** To determine the etiology of "Vietnam summer encephalitis" by conducting coordinated clinical, entomological, epidemiological and laboratory studies of encephalitis occurring in U.S. Forces in Vietnam during the monsoon season, 1970.

**DESCRIPTION:** Long Binh Post in 1967, 1968, and 1969 experienced a rise in incidence of encephalitis that occurred each year between May and September. Clinical studies of the outbreak beginning May, 1969, was carried out at the 93rd Evacuation Hospital, Long Binh. Ninety patients were admitted to the study, and paired serum from 11 of these patients and brain tissue from 2 fatal cases were sent to SMRL. Significant rises in antibody titer for JEV was found in 9 of the 11 paired serums, and JEV was isolated from one brain specimen.

Although the original objective of the 1970 study was to focus on Long Binh Post, an extensive mosquito control program was instituted on that post beginning in May which lasted through the monsoon season. Conventional mosquito control methods and the aerial spraying of malathion was associated with a lower mosquito population on post than found previously. Only one mild case of encephalitis, which could not be serologically associated with JEV infection, was encountered on post from May to 15 Oct 1970 when the study was concluded. Thus the project, minus most of the original entomology protocol, evolved into an epidemiological, clinical, and laboratory study of all suspected cases of Japanese encephalitis occurring among U.S. military personnel stationed in the IIIrd and IVth military regions, R.V.N. Cooperating in the clinical aspects were medical officers from the 12th (Cu Chi), 24th (Long Binh), and 93rd (Long Binh) Evacuation Hospitals and the 3rd Field Hospital (Saigon).

**PROGRESS:** A uniform protocol was followed on each of the 33 patients with encephalitis admitted to the study. Their evaluation included a detailed medical and epidemiological history, a careful physical

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exam (emphasizing frequently repeated neurological examinations), and diagnostic laboratory tests. The presumptive diagnosis of encephalitis was made on the basis of the clinical triad of headache, fever and central nervous system signs and symptoms associated with an abnormal cerebrospinal fluid (CSF) which was sterile for bacteria on culture. A CSF was considered abnormal if greater than 10 white cells/cmm were found or if the protein was elevated above 45 mg%.

Acute and convalescent phase sera were transported to SMRL where hemagglutination-inhibition (HI) and complement-fixation (CF) tests against JEV, dengue serotypes 1-4, and chikungunya antigens were performed. Six of 33 patients initially diagnosed on hospital admission as having encephalitis were later found to have undetectable JEV antibody titers by HAI ( $<20$ ) and CF ( $<4$ ) tests; two more patients had low-fixed HI (40-80) and CF (4) serum titers. These 8 patients were not considered to have Japanese encephalitis. Twenty of 25 patients with serological evidence of JEV infection showed a 4-fold rise in titer to JEV by either the HI or CF test and were considered as confirmed JEV infections of recent origin. Five patients with high fixed JEV titers on HI ( $\geq 640$ ) or CF ( $\geq 32$ ) testing were considered to be "probable" acute cases of Japanese encephalitis. Chikungunya HI titers were negative ( $<20$ ) in all patients. See Table I for a summary of the positive serological results.

The plaque reduction neutralization test (PRNT) was performed on nine pairs of serum in order to confirm the results of the HI and CF tests. In addition we wanted to determine if the PRNT eliminated the antibody cross-reactions to dengue antigens found in 6 of these 9 patients. A total of 22 of the 25 confirmed or probable JEV cases had diagnostic titer rises or high fixed titers to dengue in addition to JEV by the HI and CF techniques. A comparison of the HI, CF and PRNT results are shown in Table 2. The PRNT confirmed the results of HI & CF tests in these 9 patients; however cross-reactions to dengue were also found. Therefore the test proved no more specific than the CF and HI test in diagnosing a recent JE infection. The occurrence of serological cross-reactions to group B arbovirus antigen in these soldiers experiencing an acute JEV infection was not unexpected. They were exposed to dengue in Vietnam and all patients had presumably received yellow fever virus immunizations before they entered Vietnam and were thus presensitized to group B arbovirus antigens.

During the course of this study a laboratory procedure was developed that provided increased serological specificity for JEV infection in encephalitis patients showing cross-reactive antibody titers to dengue. The technique is described in more detail under a separate section of this annual report (see, Search for Humoral Specificity in Group B arbovirus infections: Increased specificity provided by serum IgM). Using this serum fractionation technique we were able to confirm a recent JEV infection in 21 of 22 patients who had group B cross-reactive antibody. We were unable to uncover IgM antibody reactive against JEV in the two patients with low-fixed arbovirus titers: the absence of detectable IgM in these 2 patients further mitigated against a recent JEV infection.

A frozen aliquot of cerebrospinal fluid from each of the initial 33 patients was sent to SMRL for viral studies. Aliquots of CSF from 7 of 8 patients with low and undetectable group B arbovirus titers, and 5 of 5 patients with high-fixed titers were put into primary cynomolgous monkey kidney cell cultures in an attempt to isolate a possible viral pathogen other than JEV. The cultures were blind-passed once and held for 21 days before they were discarded. No virus was isolated.

The indirect immunofluorescent test for scrub typhus was performed on 8 JEV-negative serum pairs by Dr. Premthavi Bodhidatta, Royal Thai Component, SMRL. The results were negative.

Table 3 summarizes the mosquito species that were collected by light traps on Long Binh Post, pooled, frozen, and shipped to SMRL for viral studies. A total of 1085 mosquitoes, gathered into 38 pools and representing 7 culicine species, were inoculated into suckling mice and MK2 cell cultures. No viral agents were recovered. Few mosquitoes could be collected for viral studies, probably because of the mosquito control program conducted on Long Binh Post during the course of this study.

Table 1.  
 Serological documentation of cases of Japanese encephalitis  
 in U.S. military personnel, Vietnam, 1970.

<u>Diagnostic Status</u>	<u>Number of Cases</u>
<b>1. Confirmed</b>	
a) 4-fold rise in HI titers between acute and convalescent phase sera	17
b) high-fixed HI titres ( $\geq 640$ ) with 4-fold rise in CF titers between acute and convalescent phase sera	<u>3</u>
<b>Total confirmed</b>	<b>20</b>
<b>2. Probable</b>	
a) high fixed HI ( $\geq 640$ ) and/or CF titers ( $\geq 32$ ) between acute and convalescent phase sera	<u>5</u>
<b>Total cases</b>	<b>25</b>

Table 2.  
A comparison of the HI, CF, and PRNT antibody titers  
in 9 American soldiers with Japanese encephalitis

Patient No.	Date Onset illness	Date Serum	Antigens				
			D1	D2	D3	D4	JEV
LB-JE-1	8 May 1970	12 May	80	40	320	320	640
		24 May	2560	640	10240	5120	10240
					<u>CF</u>		
	"	"	<4	<4	8	16	8
	"	"	32	256	512	1024	256
					<u>PRNT</u>		
"	"	55	80	160	160	40	
"	"	800	2560	2560	1280	1300	
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LB-JE-2	14 May	17 May	<20	<20	20	20	40
		28 May	160	160	640	5120	10240
					<u>CF</u>		
	"	"	<4	<4	<4	<4	<4
	"	"	<4	<4	<4	<4	64
					<u>PRNT</u>		
"	"	<10	<10	<10	<10	160	
"	"	100	160	160	160	4000	
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LB-JE-3	4 May	9 May	20	20	80	160	160
		23 May	640	320	2560	2560	10240
					<u>CF</u>		
	"	"	<4	<4	16	16	16
	"	"	32	8	512	1024	1024
					<u>PRNT</u>		
"	"	40	40	<10	100	60	
"	"	640	1000	160	250	1000	

Table 2. (Continued)

Patient No.	Date Onset Illness	Date Serum	Antigens					
			D1	D2	D3	D4	EV	
LB-JE-4	10 May	14 May	<20	<20	40	40	80	
		30 May	640	320	2560	1280	> 20480	
						<u>HI</u>		
LB-JE-5	17 May	19 May	20	20	80	40	160	
		2 June	1280	1280	> 20480	10240	> 20480	
						<u>CF</u>		
LB-JE-6	17 May	23 May	<20	<20	<20	<20	40	
		4 June	20	20	20	40	320	
						<u>CF</u>		
LB-JE-4	10 May	14 May	<20	<20	<20	<20	<20 contaminated	
		30 May	180	45	450	<160	250	
						<u>PRNT</u>		
LB-JE-5	17 May	19 May	20	20	80	40	160	
		2 June	1280	1280	> 20480	10240	> 20480	
						<u>CF</u>		
LB-JE-6	17 May	23 May	<20	<20	<20	<20	40	
		4 June	20	20	20	40	320	
						<u>CF</u>		
LB-JE-4	10 May	14 May	<20	<20	<20	<20	<20	
		30 May	180	45	450	<160	250	
						<u>PRNT</u>		

Table 2. (Continued)

<u>Patient No.</u>	<u>Date Onset Illness</u>	<u>Date Serum</u>	<u>Antigens</u>				
			<u>D1</u>	<u>D2</u>	<u>D3</u>	<u>D4</u>	<u>JEV</u>
LB-JE-7	23 May	27 May	<20	<20	20	20	40
		9 June	1280	320	10240	> 20480	10240
					<u>HI</u>		
					<u>CF</u>		
					<u>PRNT</u>		
LB-JE-8	20 May	27 May	40	20	80	160	640
		9 June	80	40	320	640	2560
					<u>HI</u>		
					<u>CF</u>		
					<u>PRNT</u>		
LB-JE-9	22 May	28 May	<20	<20	20	80	160
		7 June	20	20	20	160	640
					<u>HI</u>		
					<u>CF</u>		
					<u>PRNT</u>		

Table 3. Mosquitoes processed for virus isolation, Vietnam encephalitis project, 1970

<u>Species</u>	<u>Total No. of Mosquitoes</u>	<u>No. of Pools</u>
C. tritaeniorhynchus	55	3
C. gelidus	46	5
C. fuscocephala	30	3
C. annulus	38	2
C. pseudovishnui	94	6
C. Sitiens group	300	9
Culex species	<u>522</u>	<u>10</u>
<b>Total</b>	<b>1085</b>	<b>38</b>