

THE 1981 KAMPANGPHET STUDY OF JAPANESE ENCEPHALITIS
ANTIBODIES IN SERUM AND CSF

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PURPOSE :

1. To determine the kinetics of JE IgM and IgG antibodies in serum and CSF in cases of acute encephalitis due to JE virus.
2. To evaluate the "antibody capture" solid phase immunoassay approach as a rapid diagnostic test in acute JE infections.
3. To determine if cases of acute encephalitis in Northern Thailand which cannot be proven to be due to JE by conventional HAI serology are in fact due to JE virus or are due to another as yet unidentified virus.
4. To identify persons with asymptomatic JE virus infections for participation in future studies of risk factors of encephalitis.
5. To develop baseline data on acute encephalitis patients to be used in the design of future studies on anti-viral chemotherapy of acute JE.

MATERIALS AND METHODS :

Study Location : Kamphangphet Provincial Hospital was selected as the study site for the following reasons : (1) Kamphangphet Province in recent years has been severely affected by epidemics of encephalitis, with an average of over 50 cases per year over the past five years; (2) The timing of epidemics is highly predictable with peak activity regularly occurring between 1 July and 1 September; (3) Kamphangphet is located closer (400 km) to the main AFRIMS laboratory in Bangkok than other provinces with high JE virus activity.

Patient Populations : All patients regardless of age or sex, who presented at Kamphangphet Provincial Hospital with signs and symptoms of acute encephalitis during the peak of encephalitis incidence in 1981 (1 July - 1 September) formed the candidate battery (N = 39). From this candidate battery 32 cases were determined to fulfill the following criteria for a definite clinical diagnosis of acute viral encephalitis :

1. Fever
2. Admission physical examination findings of impaired mental status.
3. Admission lumbar puncture CSF examination with 5 or more WBC per cubic millimeter.

4. No alternative diagnosis clear from history or physical examination (eg., post-measles exanthema, post-rabies immunization, bacterial meningitis).

All study patients were hospitalized and treated by the staff of the Kampongphet Provincial Hospital. Dr. Suchard was the primary clinical physician for all of the cases. Dexamethasone, 0.5 mg per kilogram, was given intravenously to all patients at the time of admission and to most patients on fixed schedules until recovery. Although serum and CSF samples from other groups of patients were also tested, these patients were not studied in detail, other than to exclude viral encephalitis as a working clinical diagnosis.

Study Protocol : In addition to the battery of 39 candidate encephalitis ("E" cases), four other groups were studied :

Household ("H") contacts of index E cases (N = 267).

Asymptomatic ("A") cases were "H" cases found to have high serum levels of IgM anti-JE antibodies on initial screening and who consented to venipuncture and lumbar puncture (N = 5).

Random ("R") cases were all patients with a CSF sample submitted to the routine diagnostic laboratory during the study period and who were not assigned a working diagnosis of acute viral encephalitis and therefore were not studied as "E" cases (N = 18).

Fatal ("F") cases were all hospitalized patients dying during the study period and not studied as an "E" case (N = 10).

Procedures for obtaining specimens :

Venous sera : Five milliliter venous blood samples were obtained from the antecubital vein, held at room temperature for 1-2 hours before centrifugation, and the serum stored frozen at -20°C .

CSF : Two to three milliliters of CSF were obtained from the lumbar subarachnoid space with a 22 gauge needle following local xylocaine anesthesia. Pressures were not routinely measured. CSF protein was estimated by the Pandy method, glucose by Dextrostick, and total RSC, total WBC, and percent mononuclear WBC by light microscopy. Bacterial cultures were performed when clinically indicated. Remaining CSF was frozen at -20°C .

Finger-tip blood serum : Finger-tip blood was obtained by lancing and collected in heparinized capillary tubes. Twenty to thirty microliters of plasma was obtained from each tube after centrifugation.

HAI assays : Hemagglutination inhibition (HAI) assays for antibodies to JE and dengue-2 (DEN-2) mouse brain antigens were performed on all serum and CSF samples after acetone extraction by a microliter adaptation of the method of Clarke and Casals. The lowest dilution of serum and CSF tested was 1:10.

Antibody capture ELISA : Assays for IgM and IgG antibodies to JE and DEN-2 mouse brain antigens (JE MAC ELISA, JE GAC ELISA: DEN-2 MAC ELISA, DEN-2 GAC ELISA) were performed on unextracted serum and CSF samples according to previously published methods, modified for ELISA instead of RIA. All positive samples were also tested using a 1:50 dilution of normal mouse brain antigen. Procedures for MAC and GAC ELISA assays were identical except that anti-human gamma chain was substituted for anti-human mu chain in the solid phase sensitization step. In the antibody capture system, dilutions of serum up to 10^{-4} give reactions equal in intensity to undiluted serum. Thus, although the dilution of serum tested is unimportant, sera were routinely tested at 10^{-2} dilution. With CSF, where absolute immunoglobulin concentrations are typically 1/100th to 1/1000th that of serum, dilute (10^{-2} or greater) CSF often lacks sufficient immunoglobulin to give full saturation of the test system, so CSF was used at the highest possible concentration (10^{-1} dilution) consistent with the requirement to use sparingly the very limited total volumes available.

On all test plates, 10^{-2} dilutions of negative control, weak positive control, and strong positive control samples were simultaneously run with test samples. Results for JE are expressed in dimensionless MAC ELISA or GAC ELISA units, where,

$$\text{Units} = \frac{A \text{ test} - A \text{ NC}}{A \text{ WPC} - A \text{ NC}} \times 100$$

A test = Absorbance at 492 nm of the test specimen
 A WPC = Absorbance at 492 nm of the weak positive control
 A NC = Absorbance at 492 nm of the negative control.

For the JE MAC ELISA test the WPC was chosen as a specimen with a P/N ratio of 15.0 in the JE MAC RIA system; positive cut off was set at 30 (log = 1.5) JE MAC ELISA units. For the JE GAC ELISA, a positive cut off was arbitrarily set at 10 JE GAC ELISA units. To determine if low level positive reactivity in the JE MAC ELISA could be false positive due to cross reactive dengue IgM antibodies, modified MAC ELISAs were performed using equal concentrations of HA of DEN-2 and JE antigens.

Total IgM and IgG : Total concentrations of IgM in sera and CSF were determined by simple sandwich ELISA using goat anti-human mu chain to sensitize the solid phase and the same antibody conjugated to horse radish peroxidase as the label step.

Absorbance values obtained with dilutions of test specimens were interpolated onto the linear region of the curve (usually 10 ng/ml to 300 ng/ml) obtained using reference sera with known IgM concentrations. Total IgG was similarly measured using reagents of appropriate specificity.

RESULTS : Of the 39 candidates encephalitis cases, 7 were rejected from the group of "g" cases for the reasons shown in Table 2.

JE MAC ELISA antibodies in household contacts : Figure 1 shows the distribution of JE MAC ELISA units in finger tip plasma of "H" cases; 6 specimens are seen to have definitely elevated levels (>100 units). Five H

cases consented to lumbar and vena puncture and are hereinafter referred to as asymptomatic or "A" cases. Distribution of JE MAC ELISA positive H cases in different age brackets is presented in Table 3. Overall 2.2% of household contacts had clearly elevated serum JE MAC ELISA antibodies; all those were children 15 years old or younger.

Date of obtaining initial samples from E cases and from A, R, and F cases is presented in Figure 2.

Clinical diagnoses in R and F cases are presented in Tables 4A and 4B.

Age and sex distribution of E, A, R, and F cases is presented in Figure 3.

Serologic diagnoses of all "E" cases is presented in Table 5.

Group assignments of all E, A, R, and F cases for final analysis of antibody response data are presented in Table 6.

Serum and CSF JE HAI responses are presented in Tables 7 and 8.

CSF white blood cell counts per cubic millimeter are presented in Table 9.

Serum and CSF JE MAC ELISA units are presented in Tables 10 and 11.

Total serum and CSF IgM concentrations are presented in Tables 12 and 13.

Serum and CSF JE GAC ELISA units are presented in Tables 14 and 15.

Serum and CSF total IgG concentrations are presented in Tables 16 and 17.

Mortality among encephalitis cases was strongly a function of admission mental status. Of the total of 39 candidate cases, the proportion dying were as follows: Alert or drowsy, 0/22; light coma, 3/11; deep coma, 4/6.

Summary graphs of all CSF JE antibodies tested in serum and CSF of "E" cases (JE HAI: JE MAC ELISA; JE GAC ELISA) are compared to those of the 25 "R" cases (controls) in Figures 4A, B, C, D and E.

CONCLUSIONS :

JE IgM antibodies in CSF rise rapidly during acute symptomatic infection with (68% positive on admission, 100% by 1 week after admission) and may persist for 6 months or longer (72%).

JE IgG antibodies in CSF rise somewhat more slowly (50% positive on admission, 88% by 1 week, and 100% by 30 days) but always persist for 6 months or longer (100%).

Among patients with asymptomatic JE virus infections or patients with other diseases involving the CNS, serum IgM antibodies with reactivity to JE may be detected. However, these patients always lack detectable CSF JE MAC ELISA antibodies.

In cases of acute encephalitis, specific activity (as measured by AC ELISA units of activity) of IgM and IgG in CSF is invariably higher than that of simultaneously obtained serum, implying local synthesis of JE antibodies in CSF.

In asymptomatic JE infection, IgM anti-JE activity cannot be detected in CSF, implying that little if any local JE antibody synthesis occurs in these cases.

The "antibody capture" solid phase immunoassay approach for testing CSF is a highly effective rapid diagnostic test, clearly superior to existing methods.

Of 26 cases of definite acute encephalitis studied with adequate follow-up during the epidemic season, 23 were due to flavivirus infections (22 JE, one dengue), while three were either definitely (2) or probably (1) due to agents other than flaviviruses.

Four cases of asymptomatic JE virus infection were detected among 267 household contacts of encephalitis patients.

Baseline data was assembled on the clinical, laboratory immunological course of acute JE at the Kamphangphet Provincial Hospital.

Table 1. Outline of study protocol.

<u>Patient Categories for study entry</u>	<u>Definition</u>	<u>Specimens Obtained</u>
Encephalitis ("E") (N = 39)	Suspect viral encephalitis cases (see text)	Venous blood serum & CSF obtained on all patient on admission, day +7, and day +30 If CSF anti-JE positive on day 30, serum and CSF also obtained on day 180.
Household contact ("H") (N = 269)	All persons living under same roof with index E case	Screening finger tip blood serum obtained within 1 wk of admission of index E case to hospital.
Asymptomatic ("A") (N = 5)	"H" cases with high levels of IgM anti-JE in the screening finger-tip blood sample, no history suggestive of recent acute encephalitis and no "contraindications" to lumbar puncture*	Venous blood serum & CSF obtained within 1 wk of screening finger tip sample.
Random ("R") (N = 18)	All patients with a CSF sample submitted to the routine diagnostic laboratory during the study period** and <u>not</u> studied as an "E" case.	Aliquot of submitted CSF retained, Venous blood serum obtained within 24 hours.
Fatal ("F") (N = 10)	All hospitalized patients dying during the study period** <u>not</u> studied as an "E" case.	Heart blood serum

* Normal lumbar skin, no history of bleeding diathesis, age \geq 5 years.

** Study period = 1 July - 1 September 1981.

Table 2. Reasons for exclusion of KE cases from study group.

CANDIDATE

KE CASES (N = 39)

Mental status impaired $\xrightarrow{\text{No}}$ 3 (020, 021, 034; grouped and analyzed with R cases with abnormal CSF)

Yes

36

CSF Pleocytosis (> 5 WBC/mn) $\xrightarrow{\text{No}}$ 3 (005, 013, 016; grouped and analyzed with R cases with normal CSF)

Yes

33

Alternative Diagnosis $\xrightarrow{\text{Yes}}$ 1 (037; bacterial meningitis; grouped and analyzed with R cases with abnormal CSF)

No

32

STUDY KE CASES meeting minimal criteria for presumptive diagnosis of acute viral encephalitis.

Table 3. JE IgM MAC ELISA \geq 100 units on screening of finger tip plasma specimens of household contacts ("H" cases)

<u>Age range</u>	<u># Tested</u>	<u># Positive</u>	<u>% Positive</u>
0-9	19	4	4.4
10-19	50	2*	4.0 (2.0*)
20-29	43	0	0.0
30-39	43	0	0.0
40-49	24	0	0.0
50 +	16	0	0.0

* One case due to dengue-JE IgM cross - reaction.

Table 4A. Age, Sex, and Diagnosis of "Random CSF" (R) cases

<u>CASE #</u>	<u>AGE</u>	<u>SEX</u>	<u>DIAGNOSIS</u>
R01*	25 years	M	Meningitis
R02	12 years	M	Viral gastroenteritis
R03	5 years	M	URI; febrile seizure
R04	10 years	F	Enteric fever
R05	5 years	F	Respiratory infection
R06	8/12 years	M	Post-meningitis hydrocephalus
R07	3 years	F	Post-measles encephalitis
R08*	14 years	M	Lymphoma
R09	25 years	M	Seizure; cause
R10	12 years	M	Fever undetermined origin
R11	2 years	M	Viral gastroenteritis
R12	12 years	M	Dengue hemorrhagic fever with CNS bleed
R13	1 4/12 years	F	URI; febrile seizure
R14	13 years	M	Fever undetermined origin
R15	30 years	F	Meningitis
R16	3 years	M	Pneumonia
R17*	44 years	F	Encephalitis post rabies vaccination
R18*	16 years	M	Meningitis

* = Abnormal CSF as defined by \geq WBC/mm³

Table 4B. Age, sex, and diagnosis of in-hospital fatal (F) cases.

<u>Case #</u>	<u>Age</u>	<u>Sex</u>	<u>Diagnosis</u>
F-002*	6 days	M	Tetanus
F-007	4 days	M	Tetanus
F-008	8 months	M	Mulnutrition
F-010	2 days	M	Pneumonia
F-011	33 years	F	Liver abscess
F-012	1 year	M	Pneumonia
F-013	63 years	F	Stroke
F-015	40 years	F	Cirrhosis, anasarca
F-016	16 years	F	Cardiacarrhythmia
F-017	55 years	F	Asthma

* F-001 = KE 001; F-003 = KE 018; F-004 = KE 024; F-005 = no heart blood; F-006 = KE 026; F-009 = KE 033; F-014 = R 014.

Table 5. Serologic diagnoses by HAI of study KE cases (Total N = 32)

<u>HAI serologic Diagnosis</u>			
Definite flavivirus infection	Four fold or greater rise in serum JE HAI antibody titer	19	(Primary : 001*, 006, 009, 011, 012, 014, 015, 019, 022, 023, 027, 028, 029, 030, 035, Secondary : 008, 025, 031, 032)
Possible flavivirus infection	JE HAI seropositive in both acute and conv sera; <u>no</u> four-fold titer rise	5	(004, 007, 036, 038*, 039)
Definite NOT flavivirus infection	JE HAI seronegative in both acute and conv sera.	2	(002, 017)
No follow-up	Follow-up serum not obtained; either JE HAI positive or negative in acute serum	6	(003*, 010, 018*, 024*, 026*, 033*)

* Fatal case.

Table 6. Group assignments for data analysis

Group assignments for final analysis	Final clinical diagnosis	Admission CSF	HAI JE diagnosis	Initial Protocol Group Assignment			Sub- Total	Totals
				?E	A	R		
I. A.	Acute viral encephalitis	ABN	Definite	19*	-	-	19	
B.	Acute viral encephalitis	ABN	Possible	5*	-	-	5	
C.	Acute viral encephalitis	ABN	Definite	2*	-	-	2	32
D.	Acute viral encephalitis	ABN	?, No F/U	6*	-	-	6	
II. A.	? Other CNS disease	ABN	ND	4**	-	5	9	
B.	? Other CNS disease	NL	ND	3**	-	13	16	25
III.	Asymptomatic (JE Igm +)	NL	? ND	-	5	-	5	
IV.	Other hospital deaths	ND	? ND	-	-	10	10	
Total				39	5	18	10	72

* See Table 5.

** See Table 2.

Table 7. Serum JE HAI Titers^b

Group	Day			
	<u>1</u>	<u>7</u>	<u>30</u>	<u>180</u>
I A (4 x†)	2.3 ± 1.7 (15/19) ^a	6.4 ± 2.3 (19/19)	6.8 ± 1.5 (18/18)	3.8 ± 1.9 (.7/18)
B (No Δ, + → +)	4.4 ± 0.5 (5/5)	4.7 ± 1.3 (4/4)	4.5 ± 1.0 (4/4)	1.7 ± 1.3 (3/4)
C (No Δ, - → -)	0 (0/2)	0 (0/2)	0 (0/2)	-
D (No F/U)	2.4 ± 2.5 (3/5)	-	-	-
II A (Not En, ABM CSF)	4.3 ± 2.8 (8/9)	-	-	-
B (Not En, NL CSF)	3.9 ± 3.1 (12/15)	-	-	-
III Asymp	5.4 ± 1.8 (5/5)	-	-	1.2 ± 1.1 (4/5)
IV Fatal	3.1 ± 3.7 (10)	-	-	-

^a X 1 S.D. (Number \geq 1:10/number tested)

^b Log₂ ($\frac{\text{HAI titer}}{5}$); <1:10 = 0; 1:10 = 1; 1:20 = 2; 1:40 = 3, etc.

Table 8. CSF JE HAI

Group	DAY			
	1	7	30	180
I A	0/18 ^a	2/19 ^b	6/18 ^c	0/18
B	0/5	0/3	0/3	0/4
C	0/2	0/1	0/2	-
D	0/5	-	-	-
II A	1/9 ^d	-	-	-
B	0/16	-	-	-
III	0/5	-	-	-
IV	-	-	-	-

a Number 1:10/Number tested

b HAI titers 1:10, 1:10

c HAI titers 1:10, 1:10, 1:10, 1:20, 1:20, 1:30

d HAI titer 1:40 (Random 008)

Table 9. CSF WBC
 $\bar{X} \pm 1 \text{ S.D. (N)}$

Group	DAY			
	1	7	30	180
I A	87 ± 74 (19)	14 ± 19 (18)	16 ± 13 (18)	1 ± 2 (16)
B	84 ± 54 (5)	19 ± 18 (4)	3 ± 2 (4)	2 ± 2 (4)
C	40 ± 37 (2)	0 (1)	0 ± 0 (2)	-
D	100 ± 69 (6)	-	-	-
II A	247 ± 537 (9)	-	-	-
B	1 ± 1 (16)	-	-	-
III	0 ± 0 (0)	-	-	-
IV	-	-	-	-

Table 10. Log₁₀ serum JE MAC ELISA units^{a,b}

Group	Day			
	1	7	30	180
I A	1.63 ± 0.81 (10/19) ^c	2.70 ± 0.46 (19/19)	2.58 ± 0.42 (18/18)	1.51 ± 0.68 (7/18)
B	2.22 ± 0.75 (4/5)	2.31 ± 0.78 (3/4)	2.16 ± 0.85 (3/4)	1.90 ± 0.66 (3/4)
C	1.00 ± 0.00 (0/2)	1.00 ± 0.00 (0/2)	1.00 ± 0.00 (0/2)	-
D	1.61 ± 0.42 (3/5)	-	-	-
II A	1.04 ± 0.09 (0/9)	-	-	-
B	1.28 ± 0.44 (5/16)	-	-	-
III	2.09 ± 0.63 (4/5)	-	-	1.07 ± 0.10 (0/5)
IV	1.42 ± 0.77 (3/10)	-	-	-

^a Cut - off = 1.5 (30 unit)

^b Anti-log 1.5 = 30, anti-log 2.0 = 100, etc.

^c $\bar{X} \pm 1$ S.D. (N 1.5/N tested)

^d <1 calculated as = 1.00
off scale calculated as = 3.00

Table 11. Log_{10} CSF JE MAC ELISA units^{a,b}

Group	Day				
	1	7	30	180	
I	A	2.04 ± 0.80 (13/19) ^c	2.84 ± 0.36 (19/19)	2.80 ± 0.44 (17/18)	1.90 ± 0.71 (13/18)
	B	2.05 ± 0.94 (3/5)	2.41 ± 0.95 (3/4)	2.41 ± 0.95 (3/4)	1.54 ± 0.63 (2/4)
	C	1.00 ± 0.00 (0/2)	1.00 (0/1)	1.20 ± 0.28 (0/2)	-
	D	1.75 ± 1.05 (4/6)	-	-	-
II	A	1.07 ± 0.16 (0/9)	-	-	-
	B	1.00 ± 0.00 (0/16)	-	-	-
III		1.00 ± 0.00 (0/5)	-	-	-
IV		-	-	-	-

^a Cut-off = 1.5 (30 units)

^b Anti-log 1.5 = 30, anti-log 2.0 = 100, etc.

^c $\bar{X} \pm 1$ S.D. (N > 1.5/N tested total)

<1 calculated as 1.00; off scale calculated as = 3.00

Table 12. Log_{10} Total Serum Igm

Group	Day			
	1	7	30	180
I A	3.31 ± 0.14 (19) ^a	3.37 ± 0.23 (19)	3.36 ± 0.19 (18)	3.24 ± 0.30 (18)
B	3.37 ± 0.11 (5)	3.30 ± 0.19 (4)	3.21 ± 0.19 (4)	3.31 ± 0.27 (4)
C	3.28 ± 0.14 (2)	3.27 ± 0.22 (2)	3.21 ± 0.26 (2)	-
D	3.24 ± 0.17 (6)	-	-	-
II A	3.33 ± 0.31 (9)	-	-	-
B	3.32 ± 0.20 (16)	-	-	-
III	3.17 ± 0.22 (5)	-	-	3.39 ± 0.30 (5)
IV	3.00 ± 0.16 (10)	-	-	-

^a $\bar{X} \pm 1$ S.D. (Number tested)

Table 13. Log₁₀ Total CSF Igm

Group	Day			
	1	7	30	180
I A	0.87 ± 0.37 (18/18) ^a	0.76 ± 0.48 (17/18)	0.79 ± 0.50 (17/18)	-0.04 ± 0.34 (8/17)
B	0.82 ± 0.57 (4/5)	0.82 ± 0.46 (4/4)	0.20 ± 0.13 (4/4)	-0.29 ± 0.24 (1/4)
C	0.08 ± 0.25 (1/2)	-0.30 (0/1)	-0.37 ± 0.45 (0/2)	-
D	0.66 ± 0.22 (5/5)	-	-	-
II A	0.63 ± 0.95 (7/9)	-	-	-
B	-0.28 ± 0.66 (4/16)	-	-	-
C	-	-	-	-
III	-0.88 ± 0.16 (0/5)	-	-	-
IV	-	-	-	-

^a $\bar{X} \pm 1$ S.D. (N > 1 $\mu\text{g}/\text{ml}/\text{total N}$)

Table 14. Log_{10} Serum GAC ELISA units

Group	Day			
	1	7	30	180
I A	1.07 ± 0.26 (4/19) ^a	1.66 ± 0.49 (15/19)	1.96 ± 0.30 (18/18)	1.33 ± 0.46 (9/18)
B	1.02 ± 0.05 (1/5)	1.15 ± 0.18 (2/4)	1.14 ± 0.16 (2/3)	1.00 ± 0.00 (0/4)
C	1.00 ± 0.00 (0/2)	1.00 ± 0.00 (0/2)	1.00 ± 0.00 (0/2)	-
D	1.11 ± 0.25 (1/5)	-	-	-
II A	1.27 ± 0.40 (6/9)	-	-	-
B	1.28 ± 0.46 (7/15)	-	-	-
III	1.44 ± 0.62 (2/5)	-	-	1.00 ± 0.00 (0/5)
IV	1.12 ± 0.48 (2/10)	-	-	-

^a $\bar{X} \pm 1$ S.D. (Number >1.0/number tested)

<1.0 calculated as 1.00; off scale calculated as 2.50

Table 15. Log₁₀ CSF JE GAC units

Group	Day			
	1	7	30	180
I A	1.15 ± 0.20 (9/19) ^a	2.04 ± 0.47 (16/18)	2.30 ± 0.15 (18/18)	2.11 ± 0.42 (18/18)
B	1.35 ± 0.22 (4/5)	1.51 ± 0.50 (3/4)	1.67 ± 0.62 (2/7)	1.26 ± 0.40 (2/4)
C	1.00 ± 0.00 (0/2)	1.00 (0/1)	1.00 ± 0.00 (0/2)	-
D	1.20 ± 0.34 (2/6)	-	-	-
II A	1.22 ± 0.40 (5/9)	-	-	-
B	1.19 ± 0.26 (7/16)	-	-	-
III	1.40 ± 0.58 (2/5)	-	-	-
IV	-	-	-	-

^a $\bar{X} \pm 1$ S.D. (Number >1.00/number tested)

<1 calculated as 1.00; off scale calculated as = 2.50

Table 16. Log_{10} Total Serum IgG

Group	Day				
	1	7	30	180	
I	A	4.16 ± 0.18 (18) ^a	4.11 ± 0.20 (19)	4.04 ± 0.24 (18)	4.07 ± 0.14 (18)
	B	4.06 ± 0.26 (5)	4.10 ± 0.15 (4)	4.15 ± 0.16 (4)	3.99 ± 0.12 (4)
	C	3.92 ± 0.26 (2)	4.00 ± 0.06 (2)	3.83 ± 0.18 (2)	-
	D	3.99 ± 0.08 (5)	-	-	-
II	A	4.16 ± 0.19 (9)	-	-	-
	B	4.22 ± 0.22 (16)	-	-	-
III		4.17 ± 0.19 (5)	-	-	4.13 ± 0.18 (5)
IV		4.18 ± 0.16 (10)	-	-	-

a $\bar{X} \pm 1$ S.D. (Number tested)

Table 17. Log₁₀ Total CSF IgG

Group	Day			
	1	7	30	180
I A	1.82 ± 0.20 (17) ^a	1.85 ± 0.18 (18)	1.89 ± 0.30 (18)	1.31 ± 0.33 (17)
B	1.92 ± 0.35 (5)	1.45 ± 0.51 (4)	1.28 ± 0.20 (4)	1.00 ± 0.30 (3)
C	1.34 ± 0.08 (2)	1.15 (1)	0.97 ± 0.30 (2)	-
D	1.73 ± 0.32 (5)	-	-	-
II A	1.98 ± 0.55 (9)	-	-	-
B	1.43 ± 0.40 (16)	-	-	-
III	1.13 ± 0.11 (5)	-	-	-
IV	-	-	-	-

^a $\bar{X} \pm 1$ S.D. (Number tested)

FIGURE 1. JE MAC ELISA ANTIBODIES IN SERA OF HEALTHY HOUSE HOLD CONTACTS OF ENCEPHALITIS PATIENTS.

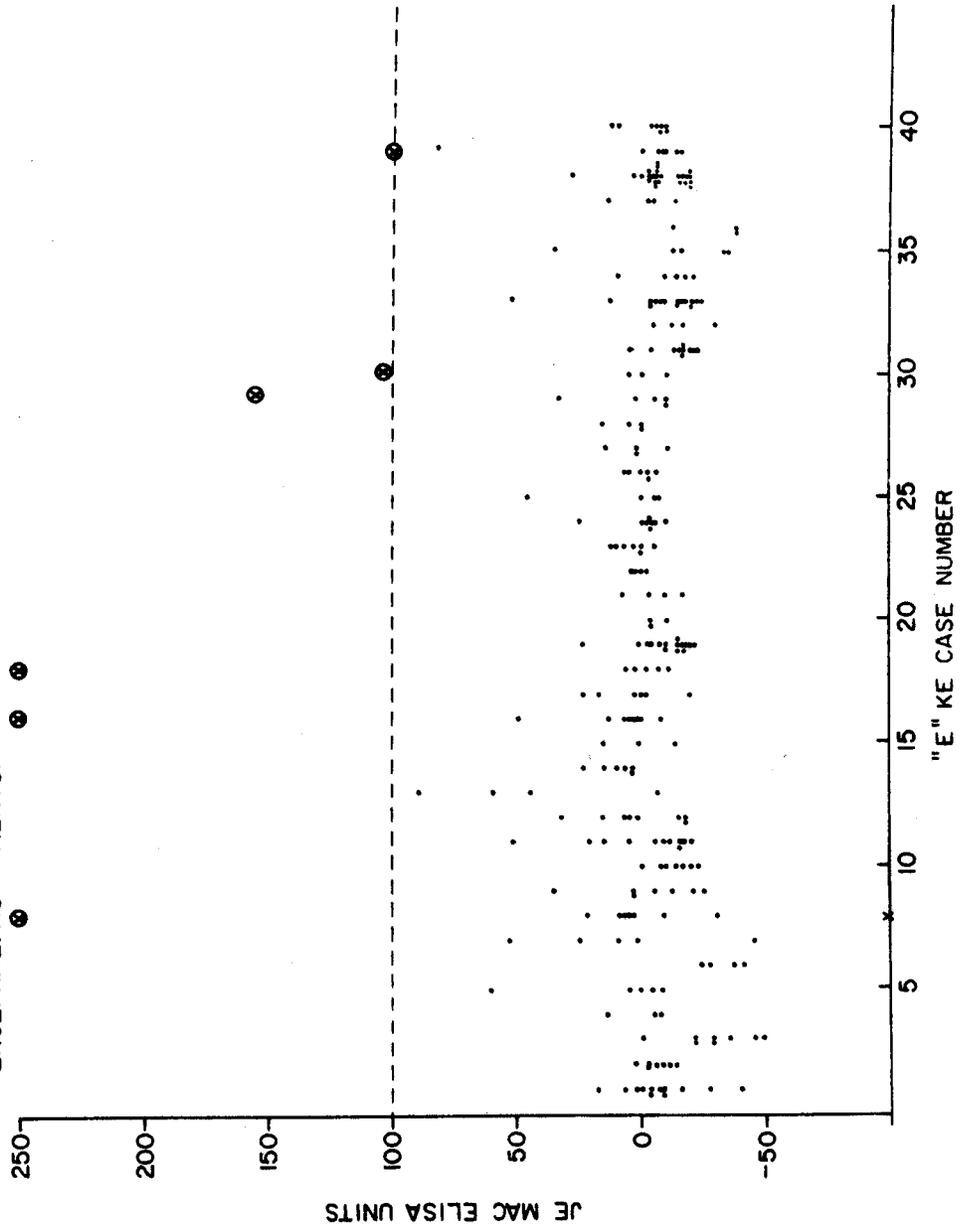


FIGURE 2. DATE OF FIRST SERUM OR CSF SAMPLE FROM ALL PATIENTS STUDIED

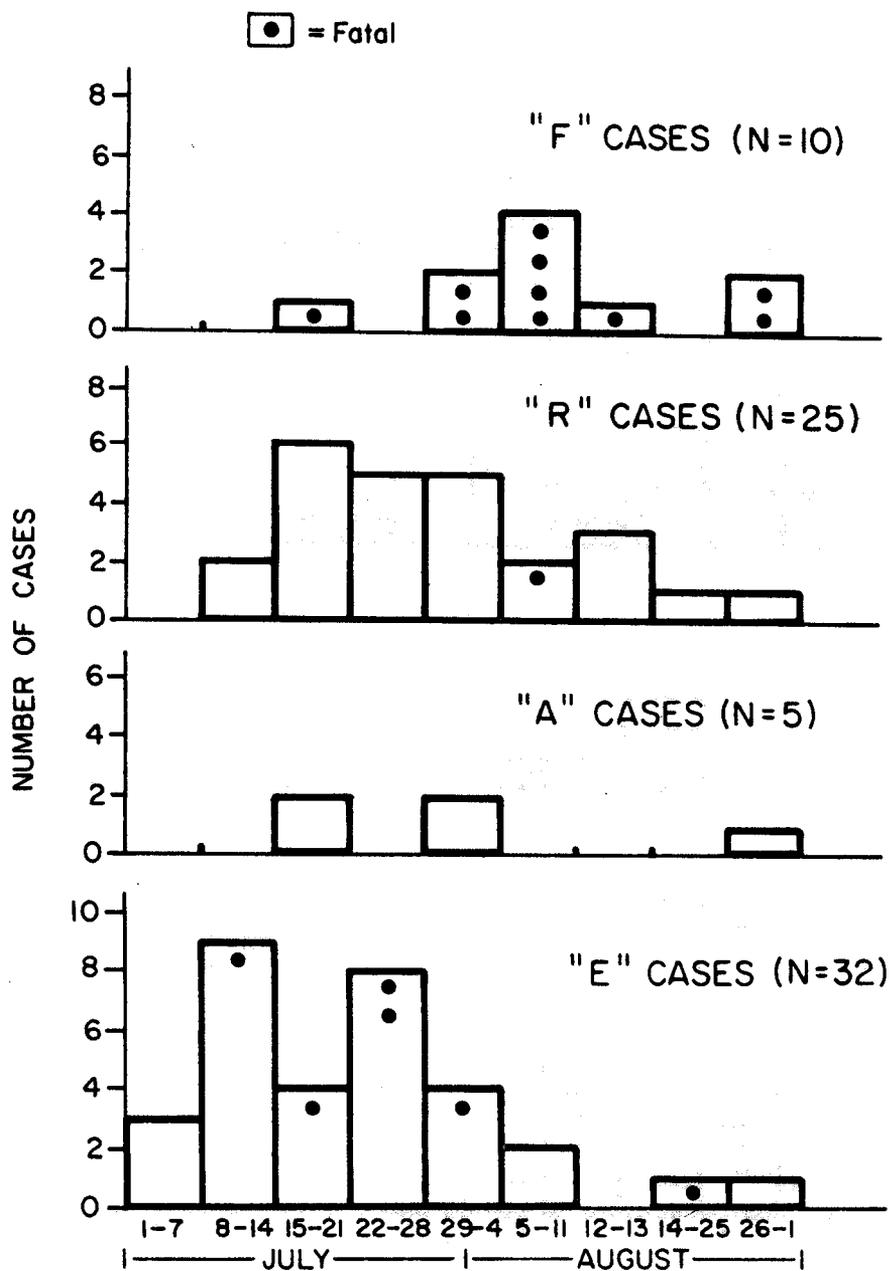
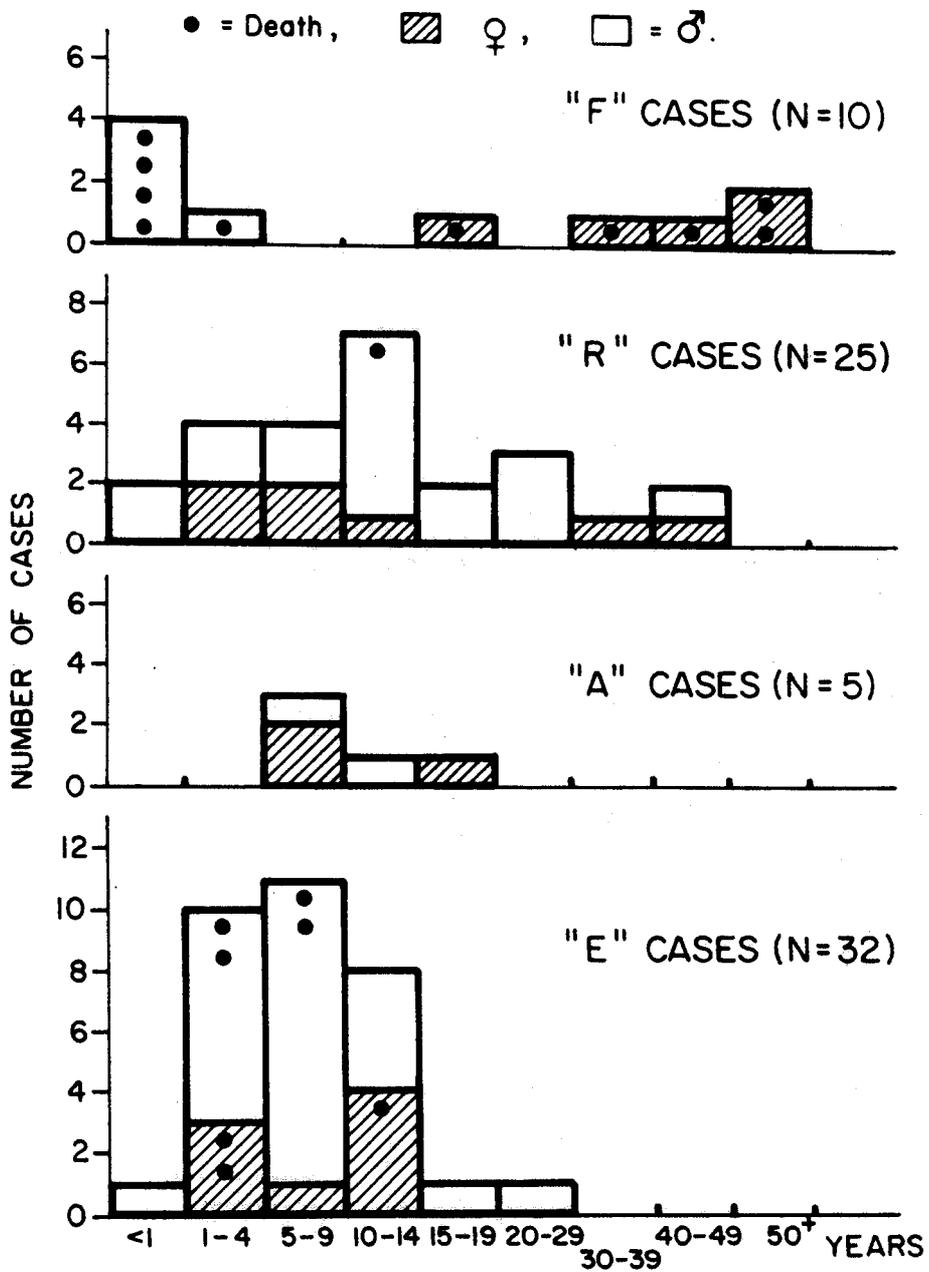


FIGURE 3. AGE AND SEX OF ALL PATIENTS STUDIED



JE MAC ELISA

