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JAPANESE ENCEPHALITIS VIRUS: EPIDEMIOLOGICAL STUDIES IN MAN IN PENANG ISLAND

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SUMMARY

Human blood donors from Penang Hospital were tested for a period of one year for Japanese encephalitis (JE) virus positive reactors. The presence of specific IgG against dengue virus (DEN) and JE virus were determined by the dot enzyme immunoassay (DEIA) test. Approximately 79% were flavivirus positive while 8.2% had a stronger antibody response to JEV than to DEN. About 6.5% of the total serum tested (8,080) were found to have been exposed to JEV. The plaque reduction neutralisation test (PRNT) titres on 10 sera with higher titres to dengue as compared to JEV showed high correlating dengue PRNT titres and low JEV PRNT titres. However, PRNT on 10% of the sera that were highly positive to JEV than to DEN resulted in only 20% having high titres to JEV as compared to dengue, 62% having high titres to dengue as compared to JEV and 18% having equal titres to JEV and dengue. About 1.3% of the samples tested have shown a likelihood of exposure to JEV.

Key words: Japanese encephalitis virus, dengue virus, man

INTRODUCTION

New virus zoonoses emerge as humans increasingly enter new environments and change the environment in which they live. Some of these zoonoses become established in human populations. Only small changes in the behaviour of virus and host are needed to transform an occasional zoonosis into an established human infection (Bennett and Begon, 1996).

Japanese encephalitis (JE), a rural agricultural disease where ecological conditions facilitate the transmission of the disease is reported to be endemo-epidemic in Asia (Tsai, 1994). In the tropics, the disease occurs sporadically with no seasonal occurrence (Umenai *et al.*, 1985). In Malaysia, infrequent sporadic cases of JE have been documented (Cruikshank, 1951; McCrumb and Traub, 1955; Simpson *et al.*, 1970; Gatus and Rose, 1983; Sinniah, 1989; Kuno *et al.*, 1993). About 44 cases were reported in 1994 and 39 cases were reported in 1995. In Northern Thailand, epidemic encephalitis is unknown. However, epidemic JE recurs every year during the early rainy season with intense transmission to pigs in Southern Thailand (Burke *et al.*, 1986). In a study carried out in Thailand, the incidence rate was 13.5 case per 100,000 children and the ratio of inapparent to apparent case was 351:1 (Rojanasupho *et al.*, 1992).

Serodiagnosis of either JE or dengue by haemagglutination (HI) test was previously found to be inefficient primarily because of the cross-reactivity between JE and dengue viruses (Igarashi *et al.*, 1983). However, the dot enzyme immunoassay (DEIA) and plaque reduction neutralisation test (PRNT) have recently been developed and used in studies of JE and dengue infections in pig and human sera (Cardosa *et al.*, 1988; Cardosa and Tio, 1991; Cardosa *et al.*, 1991; Cardosa *et al.*, 1993).

Since no extensive study on the prevalence of the JE and dengue (DEN) has been reported in Malaysia, a retrospective epidemiological study was carried out with the aim to determine the prevalence of JE infections in humans in Penang Island. This study was also aimed to compare the DEIA and PRNT.

MATERIALS AND METHODS

A total of 8,080 blood samples used in the study were obtained weekly from the Pathology Unit, Penang Hospital after being screened negative for HIV and Hepatitis B antigens for a period of one year from July 1993 till July 1994. The sample population under study included mainly adult blood donors who were civilians, the police, army and students.

The dot enzyme immunoassay (DEIA) as previously described by Cardosa and Tio (1991) was used in the initial screening of the sera. The dengue virus antigens used were a mixture prepared from the supernatants of C6/36 cells that were infected with four dengue viruses type 1 (Hawaii), type 2 (Strain 166811), type 3 (H87) and type 4 (H241) (Cardosa *et al.*, 1988). The JE virus antigen used was of the stock Nakayama strain, maintained in the laboratory

Fifty-five (10%) of the test sera, which showed greater antibody response to JEV than to DEN antigen following DEIA test, were further selected for PRNT (Cardosa *et al.*, 1991) to determine the antibody neutralisation titres to both JEV and DEN. The PRNT was also carried out on 10 sera that showed higher DEIA response to dengue than to JE.

RESULTS

Prevalence to JE and dengue

Out of 8,080 serum samples tested, 79% were found to be flavivirus positive, of which 8.2% were likely to have been exposed to JEV based on the DEIA test (Table 1). Approximately 6.5% of the 8,080 test samples were JE reactive following stronger reaction to JEV than to DEN. These individuals were likely to have been exposed either to JEV only or to JEV and DEN. Higher numbers of sample were positive to JE during the month of July 1993 than other months throughout the study period (Fig. 1).

PRNT on samples with high response to JE

Following PRNT on samples with higher response to JE than to DEN, only 11 (20%) of those samples still showed higher titres to JEV than to dengue (Table 2). The PRNT values for JEV ranged from a maximum titre of 160 to 40. Thus, there was a slight correlation between PRNT and DEIA when those samples with high DEIA titres to JEV also showed high PRNT titres.

Ten (18%) of the 55 samples with high response to JE following DEIA showed equal titres to JEV and dengue, while the remaining 34 (62%) samples showed greater titre to dengue than to JEV (Table 3).

PRNT on samples with high response to DEN

When 10 samples with high DEIA titres for DEN were further evaluated using PRNT, only two (20%) of the samples showed titres that could really be due to DEN when the PRNT titres were higher to DEN than JE (DEN:JE titres were 160:<10 and >640:40). However, 8 (80%) remaining samples showed higher titres to DEN than to JE (Table 4).

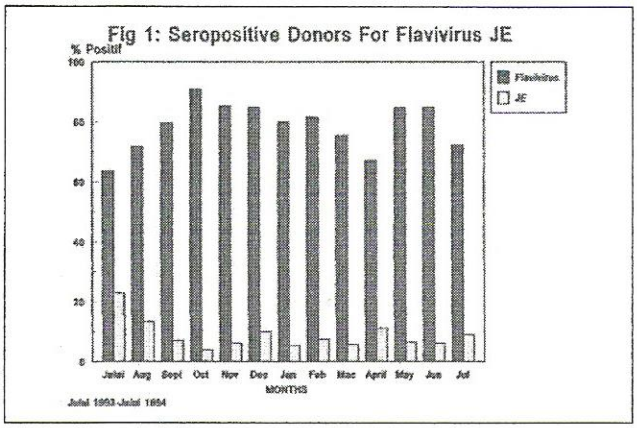

Fig 1: Seropositive donors for flavivirus JE

DISCUSSION

Screening of human sera with DEIA revealed the presence of adult blood donors in Penang Island that were exposed to JE or dengue. The high percentage of flavivirus positive samples (79%) could be due to the circulating dengue and JE flaviviruses in Penang Island (Cardosa *et al.*, 1991). However, only 6.5% were believed to actually being exposed to the JE virus. In a study where the blood from aborigines living in the forest fringe around Kuala Lumpur were tested, 12 out of 54 sera tested (22%) were positive against Japanese

Table 1. Results of DEIA tests on 8,080 serum (1993-1994)

Month	JE>DEN	Total No. of Serum	Total Flavi Positive	% Flavi Positive	% JE>DEN (Total Flavi Positive)	% JE/DEN (Total Sample)
July	79	542	345	63.7	22.9	14.6
August	54	564	405	71.8	13.3	9.6
Sept	44	775	619	79.9	7.1	5.7
Oct	18	501	456	91.0	3.9	3.6
Nov	38	708	605	85.4	6.3	5.4
Dec	65	762	648	85.0	10.0	8.5
Jan	31	715	573	80.1	5.4	4.3
Feb	34	558	456	81.7	7.5	61.
Mac	35	813	615	75.6	5.7	4.3
Apr	42	564	378	67.1	11.1	7.4
May	41	743	630	84.8	6.5	5.5
Jun	24	454	386	85	6.2	5.3
July	25	381	276	72.4	9.1	6.6
Total	530	8080	6392	79.1*	8.3*	6.6*

*Average monthly figures

Table 2. Sera with high PRNT titres to JE

Sample Number	Titres PRNT to JE	DEIA to JE	Titre PRNT DEN	DEIA DEN
999	160	1000	40	1000
1053	160	1000	40	1000
6748	160	2000	10	250
8184	160	2000	10	250
5671	40	2000	<10	250
6669	160	8000	40	250
7350	160	8000	40	1000
4873	40	2000	10	250
6225	160	2000	40	250
1018	40	4000	10	1000
8084	160	2000	40	250

Table 3. Sera having equal titres to JE and dengue

PRNT Titre JE=DEN	DEIA titre 500	1000	2000	4000	8000	Total
40=40	1		2	1	1	5
160=160			1	1	3	5
Total	1		3	2	4	10

Table 4. Sera with high DEIA and PRNT titres for dengue

Serum Number	PRNT to Dengue	PRNT to JE	DEIA to dengue	DEIA to JE
5095	160	10	1000	250
8526	40	10	1000	250
8464*	<10	10	250	250
6245*	<10	<10	1000	250
5772	160	<10	1000	250
8448	160	10	1000	250
5134	40	10	1000	250
8493	>640	40	1000	250
6259	160	40	250	250
7202	40	10	250	250

*sera with low PRNT titre for dengue but great colour reaction in DEIA test to dengue antigen

encephalitis virus while the rest were positive to dengue virus (IMR, 1955). In areas where JEV is endemic high titres may be found in the indigenous population and cross reactions to antigenically related viruses such as West Nile, Murray Valley and St Louis viruses can occur (Gatus and Rose, 1983). A similar study in Sri Lanka involving 44 serum samples, 16 samples showed high titres to JE, 12 samples showed high titres to dengue and 16 had comparable titres to both dengue and JEV (Peiris *et al.*, 1993).

The DEIA, when compared with the neutralisation and the HI tests was found to be more sensitive than the HI but less sensitive than the neutralisation test when some samples with high titre following DEIA showed low titre following PRNT. The PRNT and DEIA tests were found to give correlating results for almost all samples tested for dengue.

When 10% of the samples with high JE titre than DEN following DEIA were tested further using PRNT, about 20% of the samples still showed higher titre to JEV than to dengue. This could actually be due to JE as there was little cross-reactivity with dengue virus (Cardosa *et al.*, 1991). Although most JE specific sera do not cross-react appreciably with DEN, some cross-reactions can occur when antibody titre is high (Chong *et al.*, 1994). In a study carried out in northern Thailand using neutralisation test, the overall antibody positive rate ranged from 25.8% in the north to 38% in central Thailand. The JE neutralising antibody could be detected in dengue virus infected patients at a low titre. Approximately 90% of the recent dengue infections might cause a slightly definite increase in neutralising antibody titre to JEV (Igarashi *et al.*, 1983).

In the 62% of samples that showed high titres to JE than DEN following DEIA but showed high titres to DEN than to JE following PRNT, there could have been cross-reactions between JEV and DEN. Cross-reactivity was observed with JE virus when dengue titres were correspondingly high, probably due to the effect of immune enhancement and the presence of memory cells from previous infection (Porterfield, 1986). The 'original antigenic sin', reported in sequential dengue virus infections, could be applied to the results in this study where samples showed high titre to JEV but low titre to DEN were due to the initial infection by JEV followed by subsequent dengue infection (IMR, 1955; Kuno *et al.*, 1993).

Following PRNT on the same samples, 18% showed equal titres for JE and dengue probably due to the samples being taken at the time when the titre to both diseases were similar although one of the infections could have occurred earlier.

The presence of circulating JEV in Penang Island was clearly shown with some degrees of cross-reaction between JE and dengue viruses. Control measures need to be formulated, while the strain and pathogenicity of the virus needs to be determined. In Malaysia, an extensive epidemiological study, which includes locality, age group, the various vectors, host and climatic factors involved in the transmission cycle is needed (Vithyalingam *et al.*, 1994).

Immunisation would provide a reasonable and practical way to control JE outbreaks, but the selection of target population may present a problem in countries where JE outbreaks cannot be clearly defined in terms of place of infection and affected age groups (Umenai *et al.*, 1985). Immunisation of pigs could have limitations in the tropical climate where JE virus transmission could take place all year, thus it would be difficult to determine the proper timing for swine immunisation.

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RINGKASAN

VIRUS ENSEFALITIS JEPUN: KAJIAN EPIDEMIOLOGI PADA MANUSIA DI PULAU PINANG

Darah penderma daripada Hospital Pulau Pinang diuji selama tempoh setahun untuk tindakbalas virus ensefalitis Jepun (JE). Kehadiran IgG khusus terhadap virus denggi (DEN) dan JE dibuat dengan melalui kaedah ujian imunoasai titik enzim (DEIA). Lebih kurang 78% adalah positif terhadap flavivirus manakala 8.2% mempunyai gerakbalas antibodi yang lebih kuat terhadap JE daripada DEN. Lebih kurang 6.5% daripada serum yang diuji (8,080) didapati telah terdedAh kepada JE. Titer ujian pengurangan penuetralan plak (PRNT) pada 10 serum dengan titer tinggi kepada denggi berbanding kepada JE masing-masing menunjukkan kaitan tinggi dan rendah titer PRNT denggi dan JE. Bagaimanapun, PNRT pada 10% serum yang teramat positif terhadap JE daripada DEN menghasilkan hanya 20% mempunyai titer kepada JE berbanding kepada denggi, 62% mempunyai titer tinggi kepada denggi berbanding kepada JE dan 18% mempunyai titer setara dengan JE dan denggi. Lebih kurang 1.3% daripada sample yang diuji menunjukkan kemungkinan pendedahan kepada JE.