DMR / JICA Project

Research on Major Arboviral Diseases, Bacterial Enteric Diseases and the Application of its result for the control of these diseases

RESEARCH FINDINGS

1980 - 1982

Introduction

It is my pleasure to introduce the "Research Findings" produced from the DMR/JICA Project "Research on Major Arboviral diseases and Bacterial enteric diseases and the application of its results for the control of these diseases."

This project aims to conduct research on major arboviral and bacterial enteric diseases and apply the results to promote the health care and well being of the people in Burma by effective adequate control measures. Th· Research Findings presented on the five topics investigated have scientific value as well as practical applicability for control of the diseases concerned. The findings also indicate that facilities procured under the Project have been made well use of by the Project personnel.

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CONTENTS

		Page
1.	The epidemiology and etiology of acute diarrhoea in children in an urban community.	1
2.	A new rapid method for detection of dengue virus by the intracerebral inoculation of mosquitoes.	31
3.	Detection of dengue antigen by immunofluorescence in LLC/MK_2 cell cultures.	38
4.	Staining method of infectious focus of dengue virus by enzyme conjugate antiserum.	48
5.	Neutralizing capacity of the sera of dengue haemorrhagic fever patients to Japanese encephalitis virus	53

Title: The epidemiology and etiology of acute diarrhoea in children in an urban community.

Introduction

Diarrhoeas contribute a major part to morbidity and mortality among infants and young children, especially during and around the weaning period. Diarrhoea is still a major problem in many parts of the world, particularly in the developing countries. A high incidence of diarrhoea in early childhood is an old and familiar observation. Age is generally considered as a determinant insofar as it represents exposure of a highly susceptible host to a complicated environment which may differ from area to area and from season to season.

Infectious agents in acute diarrhoeal disease are of many kinds. In addition to the microbiological factors, other factors may also have great importance in casuality, and all are continuously changing. Increasing recognition of this dynamic behavior requires further expansion of the sphere of research activities and epidemiological investigations in the study of diarrhoeal diseases. As such, the more immediate need is for ordinary facts about the natural history of the disease and the major aetiology of the disease under local conditions. Such a knowledge is important to be obtained, because it bears directly on the practical problem of control of the disease.

As such, the present study attempts to determine the extent or incidence of acute diarrhoeal disease in a suburban community and also to define the major etiological agents responsible for acute diarrhoea in children under five years. The ultimate aim of study is to provide useful information on the natural history of the disease in young children which will help in filling the gaps in knowledge

on the practical aspects of the control of disease on a large scale.

Objectives: (1) To identify major aetiological agents of acute diarrhoea in children under 5 years of age.

(2) To make observations on some epidemiological aspects of acute diarrhoea in children with a view to elucidate the mode of transmission of the etiological agents.

Description of study population.

North Okkalapa, a satellite town (with the status of a township in Rangoon Division), is situated 20 Kilometers north of Rangoon city and is bounded by Ngamoeyeik chaung (stream) on the eastern and south-eastern sides and the circular rail-road on the western side, while the northern parts merge into open paddy fields. (See Fig. 1. Map.)

It consists of 17 wards and the total population in 1980 was 1,846,000. The majority of people living there belong to social-economic class III, IV & V (Benjamin 1980) and over 80% of children belong to families where fathers are in IV & V Catagories. Most of the dwellings/houses are thatch-roofed with bamboo walls and only a few are tin-roofed and timber-walled. Large number of people commute to city for work and business.

Most households have no directaccess to any type of water supply, although municipal pipe water is available in common road-side taps and concrete tanks. Water is, therefore, fetched in buckets or drums mounted on three-wheeled cart or trolley. In some wards, there are few artesian wells, a commercial enterprise of some selling water to people who can afford it. Surface wells, lakes or ponds are not commonly used. It is important to note that

whatever the source of water, all water for household use is stored either in earthen jars or pots, steel drums etc, all these being a familiar sight in the wards.

System of excreta disposal was unsatisfactory and there was no regular collection of excreta as was practiced in certain municipal areas in the City. Majority of households had surface latrines while some used pit and indirect pit types and only a few had properly maintained ones. Often the backyards were flooded especially after heavy rains in monsoon and there was hardly any distinctions between surface and pit types.

Population have access to the services of a general hospital including a paediatric unit and two government Primary Health Centre & Secondary Health Centre in addition to a number of private clinics.

SURVEY DESIGN

Community Survey

Out of 17 wards in the townships, 12 wards were included in the study, and only two adjoining electoral units in each ward were randomly selected for the collection of stool samples.

Stool specimens for the identification of the major etiological agents were collected from children under five years, inhabiting in the sampled 24 electoral units during the dry (winter) and wet (monsoon) seasons, except that in the latter survey only 2[illegible] electoral units were included, merely for the reason to have a better coverage of the area with the available field personnel.

For the epidemiological study, a subsample of 7 electoral units were again randomly selected to represent a

30 per cent sample of the total sampled units (i.e., 24 electoral units) during the dry season survey. However, in the subsequent survey (Monsoon survey) all the 21 electoral units were included for the collection of information for epidemiological study.

Study Population

The main objective of the study being to determine the major etiological agents of acute diarrhoea and the incidence of the disease in children under five years of age. The study included all the children of the sampled electoral units, who were less than five years of age at the time of survey. For this purpose a census was taken prior to the actual survey in each season. All the children under five years of age in each sampled electoral unit were numbered in a chronological order, for the purpose of identification and future reference. For the epidemiological study, a total of 407 and 1545 children were sampled in the dry and wet seasons respectively. The difference in the study population for the two seasons was due to the fact that the sampling techniques were not the same for the two surveys. In the dry season, only subsample of 7 electoral units was included for epidemiological study, where as in the wet season all the 21 sampled units were included.

Survey methods and procedures

Epidemiological survey

A standard survey form was designed to obtain information on the demographic features, socio-economic characteristics, environmental sanitation and other features of each household of the sampled community. In addition, a follow-up family record was also designed to collect information on the episode(s) of diarrhoea for each family member of the household. During the dry season, the episodes of diarrhoea from each household of the sub-sample (7 electoral units) were recorded for a recall period of one week on every Monday of the week. However, in the wet season daily recording of occurrence of diarrhoea among the family members was done from Mondays to Fridays and recalls for Saturdays and Sundays was made on the following Mondays of the week end.

The information was collected in both the seasons by the field staff of the Department of Medical Research, who made daily household visit from Friday to Monday in their assigned electoral units for collection of stool samples. All the field staff were given a short training for collection of stool samples and proper recording of the forms. Instruction sheets were also provided to guide them.

Collection of stool samples

During the census survey, which was taken prior to the actual survey in each season, all the households of the sampled electoral units (24 in dry and 21 in wet season) were visited by the field staff and the purpose of the study was explained to the head and elderly persons of the family. Members of the local authorities and voluntary organizations assisted in giving instructions for stool collection. Each household was provided with

a set of stool bottle cups (with enriched media) for the collection of stools from diarrhoea cases as well as from non-diarrhoea children. Stool specimens were collected daily from Mondays to Fridays by the field staff under the supervision and guidance of the field project manager. Transportation of the stool specimens from the field to the laboratory at the Department of Medical Research was made every day before 12:00 noon. Isolation and identification of pathogens was done at the respective laboratories of the department.

The diarrhoea in the present study was defined as an increase in the frequency of defecation (i.e., greater than 3 times daily) and/or in the liquidity of the stool. An interval of 24 hours was designated to divide one episode from the other.

A control was defined as a case of non-diarrhoea child under the age of five years, who is residing in the nearby household of a diarrhoea case.

Hospital study

Hospital-based studies covering the same periods were simultaneously carried out both in Winter and Monsoon, in the pediatric ward of the NOGH. Stool samples were collected from all underfive important children admitted for acute diarrhoea as well as from control cases (admitted for complaints other than acute diarrhoea), matched for sex and age to the nearest month.

Laboratory methods

Established and standard methods were used for isolation of the organisms. Identification and classification of major enteric bacteria responsible for diarrhoea was done according to Edward and Ewing (1979), Bergey (1974), Lennette et al. (1980). Organisms to be specifically isolated included E. coli (ETEC, EPEC), Shigella sp, Salmonella sp, Vibrios, Yersinia and Campylobacter jejuni (WHO 1980).

Rotavirus was examined under electron microscopic after direct negative staining with PTA. Concentration of virus particles using ultracentrifuge was not done.

For E. coli enterotoxins, heat-stable(ST) and heat-labile(LT), were obtained by growing E. coli in casamino acid yeast extract medium overnight in a shaker at 37°C. About 1 ml of the broth culture was treated with Polymyxin B, centrifuged and the supernatant was used as the ST toxin. The remaining broth culture was centrifuged and the cell lysate was next treated with Polymyxin and HEPES solution, centrifuged and the supernatant was passed through millipore filter (.45 membrane filter). The toxic effect of LT was assayed in C.H.O. cell culture (Guerrant et al., 1974, modified by Honda et al., 1976). 3-4 day mice (ddy, ICR strains) were used for detecting ST toxin (Dean et al., 1972). The effect of ST was observed by apparentness of intestinal distention of the suckling mice after intragastric feed.

Culture of E. coli tested for ETEC was also tested for EPEC by using commercial E. coli antisera from Toshiba Co. (Japan). Campylobacter isolation was carried out using Campy BAP media and incubated in a candle extinction jar at 42°C for 48 hours. Preliminary identification is done by the gram stain, oxidase and

motility tests and sensitivity to Nalidixic acid disc and growth.

For Yersinia, the stool swabs were inoculated into 10 ml of M/15 PBS for cold enrichment at 4°C for 3 weeks and plated out in Salmonella-Shigella agar and McConkey agar and incubated at 25°C for 48 hours. The non-lactose fermenting colonies are preliminarily identified by biochemical reactions.

Paired sera from cases of diarrhoea in the hospital were taken and used for counter checking the results of serotyping.

Antibiotic sensitivity of E. coli (both ETEC & EPEC strains) were done according to the method of Kirby-Bauer(1968).

Findings

Epidemiological features

Overall pattern of incidence of diarrhoea

The overall incidence of acute diarrhoea in children under five years of age gave a rate of 16.7 and 20.3 per 100 under five children for dry and wet seasons respectively. However, there is no significant difference in the seasonal incidence of the disease between the two surveys. ($z=1.7037$, $p = <0.09$). The overall attack rates per child per year for children under five years for the two seasons are 1.8 and 2.4. This difference is also not significant (Table 1).

The age specific incidence rates (episode per 100 <5 population) of the two seasons, shows much higher incidences in the age groups 12, 12 - 23 and 24 - 35 months in the wet season. The corresponding rates for the above age groups being 25.2, 24.5 and 22.8 against 16.5, 18.2 and

14.9 for the dry season. As such, young children under 3 years gave a higher incidence in the wet season than the dry season, and this difference was significant ($z = 1.9868$, $p = 0.0233$). Moreover, although the overall attack rate for children under five years does not show significant difference, the attack rate for children under two years show significant difference ($p=0.04$), the attack rate per child per year for under two years children being 2.0 and 2.9 respectively for dry and wet seasons.

With the above differences in the incidence of diarrhoea by different age groups, the pattern of the disease shows marked differences between the two seasons. In the dry season, the incidence remains stationary in different age groups, with a slight increase between the age group 36 - 47 months. The overall incidence rate and the attack rate per child per year for the children under three years of age being 16.4 and 1.9 respectively. However, compared to the dry season, the pattern shows much higher incidence in all the age groups under the age of three years, and as the age advances the incidence decline abruptly. The overall rates of incidence and episode per child per year for the children under three years of age are also significantly higher than the dry season, the rates being 24.1 and 2.9 respectively.

As regards the difference in the incidence of diarrhoea by sex, the males have higher incidence than females in almost all the age groups, the difference being more marked in the dry season. However, this difference is not statistically significant.

Environmental factors

Although the incidence of diarrhoea varied with the different sources of water supply in the two seasons, it was not possible to show the relationship of diarrhoea

with drinking water sources. The only difference observed between the two seasons was that the incidence of diarrhoea increased among the pipe water users in the wet season and the incidence was consistently high in the population using rain water in both the seasons. Similarly, other environmental factors such as type of latrine and rearing of domestic animals have no relation with the incidence of diarrhoea.

Relationship of diarrhoea with socio-economic status

It was observed that the incidence of acute diarrhoea was closely related to family income, father's occupation and crowding index. The incidence decreases as the family income improves and the difference between the lower and higher income groups was statistically significant (P level 0.01 0.001). A similar but less pronounced relationship was noticed with the social class, the incidence of acute diarrhoea being higher in the lower social classes (Social Classes III and IV) (P = 0.05).

Bacteriological findings

The study revealed that the major etiological agents in the January survey were enterotoxigenic E. coli or or ETEC (29.9%) and enteropathogenic E. coli or EPEC (12.0%) and Shigella (3.6%) in the diarrhoea cases in the community and similar rates were found in hospital cases. However, about 5.6% of ETEC and 15.8% of EPEC were recovered from stool samples of controls during the same period. The rates in the hospital cases were relatively higher than of the community. Salmonella (4 out of 104) and V. cholera (1 out of 104) were found in only a few hospital diarrhoea cases. (See Table 3).

The study in July (Monsoon) showed a similar pattern of distribution of major pathogens with a relatively higher rates of isolation for ETEC (32.7%), EPEC (25.4%) and Shigella (13.8%) in the diarrhoea cases from the community. Unlike in January, the monsoon survey showed Campylobacter (6.0%) among diarrhoea cases and 2.9% among controls in the community.

It is interesting to note that major pathogens could be identified in 41.6% of the diarrhoea cases in the community during winter and in 79.36% during the monsoon (See Table 3 & 4). Similarly, even among control cases, major pathogens were isolated in 23.86% during winter and 45.6% during monsoon. An approximately 19% of diarrhoea cases, more than one etiological agent was found, reflecting the polymicrobial nature of diarrhoeal infection.

Distribution of major pathogens isolated from acute diarrhoea in the community showed that infection with ETEC ranked the highest (41.3% of total isolates) followed by EPEC (32.0%) and Shigella (17.4%) while other pathogens like Salmonella, V. cholera and Campylobacter accounted for 1.6, 0.5 and 7.6% respectively (See Table 5). ETEC infection appeared to occur frequently during the first two years of life (45.5 to 46.9% of all pathogens) and in the subsequent years it was found to occur less frequently. EPEC infection, however, was more frequent among children between 2 - 4 years (36.5 to 40.7%) although it was still found in about 1/3 of diarrhoea cases below 2 years of age.

On the other hand, Shigella infection showed an increasing frequency from 11.3% to 36.3% with the increasing age of children (with the exception of 36 - 47 month group) among the diarrhoea cases. This trend was clearly seen inspite of a small number of cases where this organism was isolated (Table 5).

An interesting finding was that Campylobacter was isolated in diarrhoea cases with no predilection for age among under-five children.

An interesting pattern was noted in ETEC toxin distribution. Heat stable toxin (ST) was found in 26 - 28% of all ETEC diarrhoeas in January and 18 - 22% in July. Among control cases, the corresponding values were 33 - 37% (January) and 30% (July) respectively. Heat labile toxin (LT) on the other hand, was found in 65 - 68% of ETEC diarrhoeas in January and 61 - 72% in July. However, the values for control cases were 56 - 66% (January) and 50 - 67% (July). Only a small proportion of ETEC were ST - LT strains (7 - 17%) (See Table 8).

Discussion

Prevalence of diarrhoea and some epidemiological aspects

In spite of the difference in the size of the samples for the two seasons, the sample sizes were sufficiently large to give results which could be analysed with confidence to provide statistically valid results.

The present study revealed that there is no significant difference in the overall seasonal incidence of acute diarrhoea in children under five years of age. This finding contradicts the well known assumption that the diarrhoea incidence in the wet season is far greater (about two or three times) than the dry season. In a hospital based study carried out in the Children's Ward of North Okkalapa General Hospital in 1978, it was reported that the hospital admission rate of children under 12 years was about two and half to three folds more in July than in January (Soe Soe Aye, 1980).

The above hospital based study represents the same community where the present study was undertaken and about 80 percent of the children admitted were under the age of five years. Moreover 55 percent of the children admitted were under two years of age. The general pattern of hospital admissions in various hospital also show much higher admission rates for acute diarrhoea in wet than in dry season (Health Statistics Report, 1978). It could be presumed that the higher admission rates in the wet season might be due to the severity of the episodes rather than the real increase in the overall incidence of the disease in the community. This statement need to be verified by comparing with the results of other population based studies.

The average yearly incidence of 1.8 and 2.4 per child per year (for children under five years of age) for the dry and wet season seem to be higher than the findings of Htaukkyant survey (Thane Toe et al., 1980) as well as the attack rate reported by Bangladesh (ICDDR, B , 1980) (i.e., 1 per child per year under the age of five years). However, compared to the average yearly incidence of 8 episodes per child per year in Costa Rica, reported by Mata et al (1978), our study shows much lower rates.

The present study also pointed out that the age specific incidence rate and the average yearly incidence in younger children was significantly higher in wet than the dry season.

A number of reasons could be thought for the significant increase in the incidence of acute diarrhoea in younger children during the wet season. Firstly, the pattern may reflect the overall increase of the disease in the community, particularly affecting younger children. Secondly, the seasonal difference in the pattern of the disease may be due to changes in the environment marking favourable conditions for transmission

of the disease. Thirdly, the dose and virulence of the pathogens may be increased due to favourable environmental conditions. Fourthly, other pathogens may have been added.

The study carried out in Htaukkyant (Thane Toe, 1980), reported a different pattern of the incidence of diarrhoea in children of a rural community. The peak incidence of diarrhoea in the above study were in the age groups 12 - 23 and 36 to 47 months. As such, it could be stated that the age specific incidence of diarrhoea may differ between rural and urban children, probably because of difference in the environmental conditions as well as the etiological agents.

Another interesting feature observed in the present study was that there is a proportional increase of ETEC isolates in the wet season in the age group 12 - 23 months which coincides with the overall increase of diarrhoea incidence in that age group. The reverse was found to be true in the dry season, as low isolates of ETEC was found in the corresponding age group. This reflects the fact that apart from the various other possible reasons which have been discussed above, ETEC might play an important role in the incidence of diarrhoea in the age group 12 - 23 months.

Major etiological agents

It was found in the present study that percentage of pathogen that could be isolated and identified varied from 41 percent in January (winter) to 79 percent in July (monsoon). However, the pattern of distribution of major pathogens between these two periods was not strikingly different. The fact that less bacterial pathogens (40-50%) could be identified in winter months indicated that non bacterial agents may be responsible for some of the diarrhoeas.

It is highly likely that viruses may be responsible for these non-bacterial diarrhoea cases since rotavirus has already been identified in pediatric gastroenteritis cases admitted to Infectious Diseases Hospital in Rangoon in 1978 (unpublished findings)

Recently, a number of enteric bacteria have been implicated in acute diarrhoea, such as Campylobacter and Yersinia in addition to commoner ones like E. coli, Salmonella and Shigella (WHO, 1980).
Although it has been stated that with present state of advances in medical sciences, it was possible to identify pathogens in up to 70-80 percent of diarrhoea cases, no comprehensive study has been attempted in Burma so far.

Chances of detecting presence of pathogens was greater than before, because of advances in bacteriological isolation techniques and isolation rate of about 80% found in the present study was comparable to those reported by others, (ICDDR reported about 90%). Isolation rates in the present study especially during monsoon was higher for all major pathogens and this seemed to point out the polymicrobial nature of the disease in the community.

The major pathogen or enteric bacteria isolated from stools was found to be E. coli both from the diarrhoea and control cases, and entertoxigenic E. coli or ETEC infection represented 41 to 67% of all pathogens found in diarrhoea cases. It now appears that ETEC diarrhoea is prevalent among children in many developing countries. In Bangladesh, rate of hospitalized ETEC diarrhoea was 8.1/1000 and among South African children, it was found in 19% of diarrhoea cases (Robins-Browne et al., 1980).

Serotypes of EPEC, on the other hand, accounted for about 25-30% of diarrhoea cases and relatively similar rates (20-25%) were found among the controls. Similar findings have been reported in Kenya (Mutanda 1980) where no significant difference between children with diarrhoea and controls with regards to frequency of isolation of EPEC was found. For the time being, it could not be assumed that EPEC strains are responsible for acute diarrhoea in the community. Much remains to be studied, especially the association between serotypes and toxigenic strains; and this aspect also has not been resolved completely in the studies reported elsewhere. In the present study, 17 EPEC strains were found to be associated with toxins production and whether the rest were entroinvasive (EIEC) or not (using Sereny Test) remains to be tested. It has been reported by other workers that certain serogroups ("O" 26, 55, 111, 119, 127 & 128) were found to be commonly associated with out-breaks of infantile enteritis. In the present study nearly all types (26, 28, 44, 86, 112, 114, 119, 124, 125, 177, 143 & 146) have been found. In view of the complexity of serotyping (Using O, H & K) it may not be a practical assay and may not probably help in the mode of transmission study of ETEC. Sera from hospital diarrhoea cases were obtained and used in slide agglutination tests for the isolated E. coli both during the episodes and 2-weeks after. Out of 50 tests, 21 positive tests were obtained with 2-week sera and 17 with both 1st and second sera samples.

Next in importance may be Shigella which was responsible for 13% of all diarrhoea or 17% of all major pathogens isolated among diarrhoea cases in July. Shigella infection was commonly seen in 2-3 year age groups, probably due to or as a result of the children

being left independently on their own to adapt to the environment. Relatively lower rate of isolation was found during January, and the significance of this finding may not be important. In a study in Kenya, Shigella was isolated is about 16.7% of acute GE in early childhood (Mutanda 1980) and it was found in South Africa in about 9% (Robins-Browne et al., 1980).

Other pathogens not encountered in the present study were V. parahemolyticus and Yersinia enterocolitica, although the former organisms have previously been reported in diarrhoea cases admitted to Infection Diseases Hospital in Rangoon. (May Than Su 1979).

Of interest was that Campylobacter jejuni was isolated during monsoon is about 6% of diarrhoea cases, and it also accounted for 4-11% of all pathogens isolated in various groups in 0-5 years. It is becoming clear that Campylobacter infection is world wide and its infectivity has clearly been demonstrated (Oates & Hodgir 1981). Campylobacter had been isolated in 16.9% of all bacterial pathogens in infants and older children in Johannesberg (Mauff & Chapman 1981) and also in 10.9% of GI infections in Sweden (Svedhem & Kaijser 1980) and in about 10% of children 0-9 years with GE in Jakarta, Indonesia (Ringertz et al., 1979). In rural Bangladesh, Campylobacter was found in more than one third of children and higher isolation rates were found in under two years group. (ICDDR,B 1980).
Robins-Browne et al (1980) also found Campylobacter in 6 out of 41 (15%) unselected black South African children. The infection with Campylobacter was generally regarded as mild (majority affected are 20-34 years old) though 50 out of 277 (18%) required hospitalized (Svedhem & Kaijser 1980). But the importance of this organism in Burma remains to be examined. Selective culture policy for this organism and Yersinia might yield higher isolation rate.

It was found that ETEC was more frequently isolated (45-47%) below the age of 2 years and there after less frequently (31-41%). Children under 2 years are said to be

sensitive to E. coli infection because of restricted maternal transfer of E. coli antibodies. However, no greater incidence of ETEC infection was found in children below 6 months, a period when majority of Burmese children are being breastfed supplementary feed not instituted as yet.

An attempt has been made to compare the overall incidence of diarrhoea with the age specific ETEC positive diarrhoea cases. ETEC expected isolates for the sampled population were calculated from the ETEC isolates obtained from the diarrhoea stool specimens tested.

It was interesting to observe that there is a proportional increase of ETEC isolates in wet season in the age group 12 - 23 months, which coincides with overall increase of diarrhoea incidence in that age group (Fig. 1). However, in the dry season, the reverse was found to be true, as low isolates of ETEC was found in the corresponding age group.

The fact that more than 1/3 of ETEC isolated from controls (asymptomatic) showing ST production and 2/3 showing LT seemed to indicate that test for toxin production may not be directively indicative of enterotoxigenic severity. Current tests for identification of enterotoxins are time-consuming and expensive (e.g., Rabbit ileal loop, Infant mouse assay, and Cell cultures). There is an urgent need for more sensitive and rapid tests for both ST and LT and in the circumstances, ELISA and ELLK tests look promising. ELISA technique unfortunately, was not established at the time of the present study and the results of LT from CHO assays could not concurrently be confirmed and evaluated by ELISA. However, a post-survey evaluation done on a small number of samples indicated that CHO positive LT strains gave about ½ positive rates with ELISA technique.

since the interpretation in CHO assay is highly subjective, it may not be a good idea to adopt it as a reference method. ELISA should be useful for study on mode of transmission of ETEC infection since rapid identification of ETEC strains was required.

The fact that rotavirus could not be identified in the present study (both in winter and monsoon) did not exclude the possibility of this virus or other viruses as one of the major etiological agents. Rotavirus has been identified by direct electronmicroscopy in about 30% of the stools from pediatric gastroenteritis cases admitted to the Infectious Diseases Hospital in Rangoon in 1978. Therefore, confirmation of negative finding in direct electron microscopy of stool samples by an appropriate method is required.

Table 1 Incidence of acute diarrhoea (episode/100 population) in the Community.

Age (month)	January			July		
	Male	Female	Total*	Male	Female	Total
0-11	24.2 (33)	10.9 (46)	16.5 (79)	25.5 (157)	24.8 (161)	25.2 (318)
12-23	17.9 (39)	18.4 (38)	18.2 (77)	26.3 (156)	22.8 (171)	24.5 (327)
24-35	19.1 (47)	10.6 (47)	14.9 (94)	29.8 (181)	13.7 (139)	22.8 (320)
36-47	26.8 (41)	9.1 (33)	23.0 (74)	13.1 (160)	19.1 (152)	16.0 (264)
48-59	15.6 (45)	7.9 (38)	12.0 (83)	12.3 (106)	11.4 (158)	11.7 (264)
Unknown	-	-	-	-	-	-
Total	20.5 (205)	12.9 (202)	16.7 (407)*	22.1 (703)	18.5 (782)	20.3 (1545)

Figures - Parenthesis deno e no: of underfive children.

* 30% sub sample of the under-five population under diarrhoea surveillance.

Table 2. Incidence of diarrhoea (episode/100 Household) according to source of water supply and type of latrine in the community.

	January*		July	
	% Distribution per household	Incidence per 100 household	% Distribution per household	Incidence per 100 household
A. Source of water				
Pipe	42.2 (121)	26.4 (121)	90.7 (845)	35.4 (847)
Artesian	33.8 (97)	19.6 (97)	4.7 (44)	15.9 (44)
Rain water	1.0 (3)	66.7 (3)	3.0 (28)	32.4 (74)
Others*	23.0 (66)	16.7 (66)	1.5 (14)	0 (14)
B. Type of latrine				
Surface	6.6 (19)	41.2 (19)	not done	
Pit	23.7 (68)	16.2 (68)		
Indirect pit	67.6 (194)	23.7(194)		
Unknown	2.1 (6)	2.1 (6)		

* Pond/wells figure in parenthesis denote household.

* 30% sub sample of target population surveyed.

Table 3. Incidence of acute diarrhoea in the children (episode/100 children) according to family income, crowding index and occupation of fathers (July only).

	Episodes	No. 5 children	Incidence/100 children
A. Income (Kyats/month)			
0	-	1	-
100	30	102	29.4**
200	72	283	25.4**
300	94	406	23.2**
400	42	204	20.6**
500	69	449	15.4
1000	6	87	6.9
Unknown	1	13	7.7
B. Crowding index (no/house/total rooms)			
0-1-9	68	258	26.4*
2-2-9	85	476	17.9*
3-3-9	71	388	18.3*
4-4-9	34	202	16.8*
5-5-9	18	99	18.2*
6-6-9	17	54	31.5*
7	21	64	32.8
Unknown	-	4	-
C. Father occupation (classification of Benjamin 1968)			
I & II	13	97	13.4
III	107	501	21.4*
IV	77	355	21.7*
V	104	474	21.9*
Unclassified	11	111	9.9
Unknown	2	7	28.6

* $P < 0.05$ ** $P < .001$

Table 4. Incidence of major etiological agents isolated in acute diarrhoea cases and controls in the community.

	January		July	
	Diarrhoea	Control	Diarrhoea	Control
No. examined	139	205	217	103
A. Bacterial pathogens				
1. E. coli ETEC	41(29.5)	14(6.8)	10(32.7)	10(9.7)
2. E. coli EPEC	18(12.9)	38(18.5)	55(25.3)	25(24.3)
3. Shigella	6(4.3)	5(2.4)	30(13.8)	6(3.8)
4. Salmonella	-	-	2 (0.9)	3(2.9)
5. V. cholera	-	-	1(0.5)	-
6. V. parahemolyticus	-	-	-	-
7. Yersinia	-	-	-	-
8. Campylobactor	-	-	13(6.0)	3(2.9)
Major pathogens	65(46.8)	57(27.8)	172(79.3)	47(45.6)
Other isolates	74(53.2)	148(72.2)	45(20.7)	56(54.4)
B. Rotavirus	-	-	-	-
C. Parasites				
1. Ascaris	68(41.5)	131(46.0)	53(24.4)	43(39.8)
2. Others	3(1.8)	6(2.1)	6(2.8)	6(5.5)
D. Protozoa				
1. E. histolytica	16(9.6)	19(6.7)	12(5.5)	4(3.7)
2. Giardia	10(6.0)	12(4.2)	8(3.7)	3(2.8)
3. Others	3(1.8)	-	3(1.4)	-
E. Mixed infection	19(10.5)	29(10.2)	11(5.1)	13(12.0)

Figures in parenthesis denote % of total number examined.

Table 5. Incidence of major etiological agents in acute diarrhoea cases and control in the hospital.

	January		July	
	Diarrhoea	Control	Diarrhoea	Control
No. examined	104	80	40	40
A. Bacterial pathogens				
1. E. coli ETEC	35(33.6)	6(7.5)	18(45.0)	3(7.5)
2. E. coli EPEC	8(7.7)	9(11.2)	12(30.0)	8(20.0)
3. Shigella	4(3.8)	3(3.7)	2(5.0)	1(2.5)
4. Salmonella	4(3.8)	3(3.7)	1(2.5)	-
5. V. cholera	1(0.9)	-	7(17.5)	1(2.5)
6. V. parahemolyticus	-	-	-	-
7. Yersinia	-	-	-	-
8. Campylobacter	-	-	-	2(5.0)
Major pathogens	52(50.0)	21(26.2)	40(100)	15(37.5)
Other isolates	52(50.0)	59(73.7)	-	25(62.5)
B. Rotavirus	-	-	-	-

Figures in parenthesis denote % of total number examined.

Table 6. Distribution of major enteric pathogens (percentages) isolated from acute diarrhoea in the community cases according to age during Monsoon Survey.

Age (months)	ETEC	EPEC	Shigella	Salmonella	V. cholera	Campylobacter	Total isolates
0-11	45.5 (20)	29.5 (13)	11.4 (5)	2.3 (1)	-	11.4 (5)	44
12-23	46.9 (23)	28.6 (14)	16.3 (8)	-	2.0 (1)	6.1 (3)	49
24-35	31.7 (13)	36.6 (15)	24.4 (10)	-	-	7.3	41
36-47	40.7 (11)	40.7 (11)	11.1 (13)	3.7 (1)	-	3.7	27
48-59	36.4 (4)	18.2 (2)	36.4 (4)	-	-	9.1 (1)	11
Total	41.3(71)	32.0(55)	17.4(30)	1.7(2)	0.6(1)	7.6(13)	172

Figures in parenthesis denote number of case of diarrhoea.

Table 7. Distribution pattern of ETEC toxin production in diarrhoea and control cases in community and Hospital studies.

Toxins	January				July			
	community		Hospital		community		Hospital	
	Dia:	Cont:	Dia:	Cont:	Dia:	Cont:	Dia:	Cont:
ST	12 (27.9)	6 (37.5)	9 (26.5)	2 (33.3)	13 (18.3)	3 (30.0)	4 (22.2)	0
LT	28 (65.1)	9 (56.2)	23 (67.6)	4 (66.7)	52 (72.2)	5 (50.0)	11 (61.1)	2 (66.7)
ST-LT	3 (7.0)	1 (6.2)	2 (5.9)	- -	6 (8.4)	2 (20.0)	3 (16.7)	1 (33.3)
Total	43	16	34	6	71	10	18	3

Figures in parenthesis denote percentage.

Table 8. Distribution of EPEC and ETEC in diarrhoea and control cases from community and and Hospital in January.

	Community			Hospital		
Age group	No. examined	EPEC(%)	ETEC(%)	No. examined	EPEC(%)	ETEC(%)
Dia:						
< 6 mths	26	3(11.5)	2(7.6)	19	0(-)	8(42.1)
6-12	32	6(18.7)	9(28.1)	55	6(10.9)	17(30.9)
12-24	48	7(14.6)	16(33.3)	24	2(8.3)	9(37.5)
24-36	28	3(10.7)	8(28.6)	3	0(-)	1(33.3)
36 mths	32	1(3.1)	8(25.0)	3	0(-)	0(-)
Total	166	20	43	104	8	35
Control:						
< 6 mths	13	4(17.4)	0	16	0(-)	1(6.3)
6-12	51	6(11.8)	4(7.8)	34	6(17.6)	2(5.9)
12-24	94	14(14.9)	5(5.3)	23	2(8.7)	3(13.0)
24-36	64	13(20.3)	4(6.2)	5	0(-)	0(-)
36	53	8(10.1)	3(5.7)	2	1(50.0)	0(-)
Total	285	45	16	80	11	5

Figures in parenthesis denote percentage.

Table 9. Expected ETEC isolates in the total study population from the sample ETEC isolates.

Age (months)	Specimen episodes		Actual episodes in community	Expected* ETEC (+) Episodes in community	Total Under-5 children	Incidence** per 100
	ETEC (+) episodes	Total episodes				
January 0-11	5	15	13	4.3	79	5.4
12-23	1	10	14	1.4	77	1.8
24-35	2	6	14	4.7	94	5.0
36-47	2	12	17	2.8	74	1.9
48-59	-	2	10	-	83	-
Total	10	45	68	15.1	407	3.7
July 0-11	14	56	80	20.0	318	6.3
12-23	28	64	80	35.0	327	10.7
24-35	17	52	73	23.9	320	7.5
36-47	2	28	50	3.6	312	1.2
48-59	8	17	31	14.6	264	5.5
Total	69	217	314	97.1	1545	6.5

* Expected ETEC isolates = $\frac{\text{ETEC (+) specimen episodes}}{\text{Total specimen episodes}} \times$ actual episodes in community

** Incidence = $\frac{\text{Expected ETEC (+) episodes in community}}{\text{Total under 5 children (pop; at risk)}} \times 100$

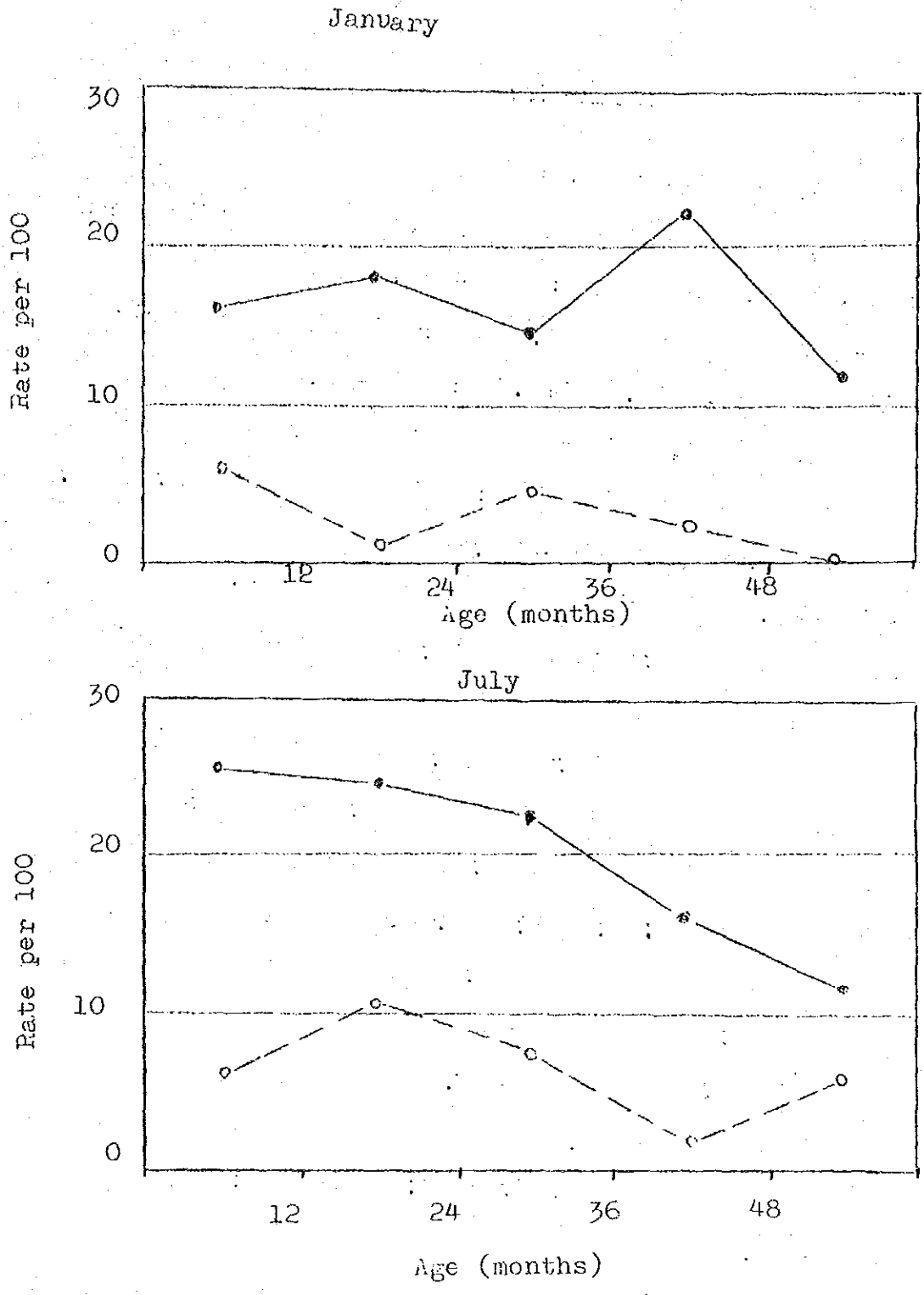
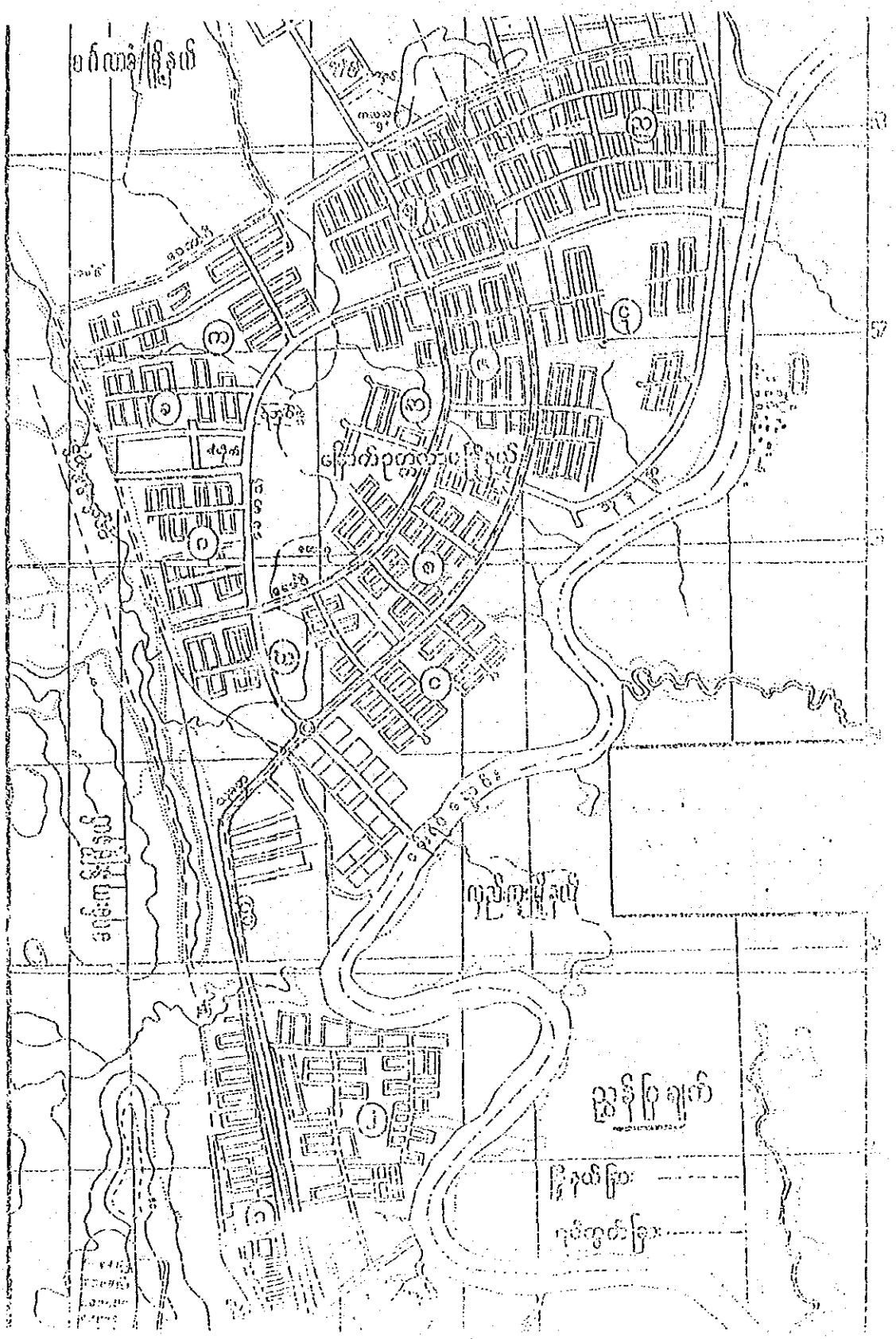


Fig. 1. Comparison of total and ETEC specific diarrhoeas, 1981.



A new rapid method for detection of dengue virus by the intracerebral inoculation of mosquitoes.

Introduction

Dengue is an important virus disease in man and is most difficult to detect and propagate in the laboratory. Among the 4 known serotypes of dengue, two were isolated during world war II by Sabin using the intracerebral inoculation into 1-2 day old mice (1) However by 1960, Hammon and his coworkers succeeded in isolating most of the other serotypes in suckling mice (2) Much difficulty was encountered in using this system since viruses responsible for human diseases were not always pathogenic when inoculated into the brain of 1 - 2 day old mice and many serial passages were required before obtaining seed viruses of high infectivity or antigen titers for characterization of the dengue viruses.

Many workers have been injecting mosquitoes for the last 40 years (3,4 & 5) for possible use as host system for recovery of viruses, and since it was cumbersome large number of insects could not be inoculated within a short period of time. However, Rosen and Gubler have developed the intrathoracic inoculation of mosquitoes, which is relatively simple, inexpensive and sensitive when compared to the previous methods. (6) In this method the presence of dengue viruses was detected in the head-squash preparation by immunofluorescence staining. (7) His studies showed consistent presence of large amount of dengue antigen in some parts of the nervous system, thus suggesting that the brain might be a good source of antigen. Since selection of specimen for isolation of dengue viruses is also an important criteria for the success in detection of viral antigen, choice was laid on the use of mononuclear cells of

DHF patients since they were shown to contain a good source of antigen (Thet Win 1978 unpublished data). We therefore initiated a study of the intracerebral method of inoculation, whether it might yield better and quicker results. The results of this intracerebral inoculation method are herewith reported in this communication.

Materials and Methods

Sixty three samples from non-shock and five from shock of dengue Haemorrhagic Fever patients were collected during the 1980 epidemic season.

Mononuclear cells from the buffy-coat layer were obtained by the Ficoll-hypaque separation method of the heparinized blood. Two to 4 day old Toxorhynchites splendens were inoculated through the dorsal part of the head capsules which lies between the neck and vertex of the mosquito (Fig 1). An approx. volume of 0.17 UI was inoculated by the I.C. route per mosquito. Infected mosquitoes were kept in small cages and held at 32°C incubator with a relative humidity (RH) of 80%. Infected mosquitoes were fed on 10% sugar solution. Presence of viral antigen was observed daily in the head-squash preparations stained with antidengue conjugate by the direct fluorescent antibody technique (DFAT) (Fig 2).

Results

Dengue viral antigen was detected in the head-squash preparation in 13 out of 63 non-shock and 1 out of 5 shock patients as early as 5th post infection day.

Discussion

It is obvious from the data presented that the use of I.C. method to detect dengue viral antigen by the IF method offers an advantage in aiding whether the viruses are present in the sera of DHF patients. However, since the growth and replicability of viruses have not been attempted, it would be difficult to know whether the I.C. infected mosquitoes will help in serotyping by CF. Although it is known that mosquitoes generally are susceptible to infection with arboviruses by parenteral injection than they are by feeding (8) the present study has also shown that dengue antigen was easily detected earlier in the head-squash preparation than when inoculated by the IT route. However, it is evident that more studies should be conducted to determine the sensitivity and reproducibility of this test system.

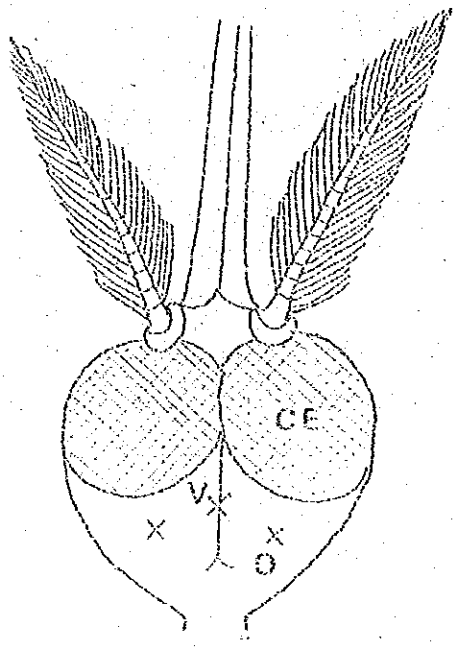
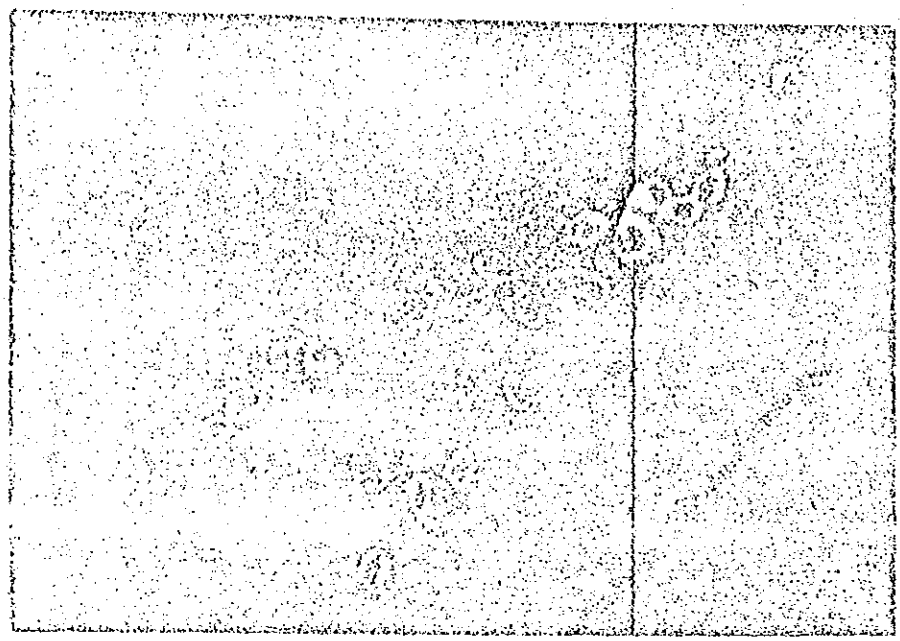


Fig. 1



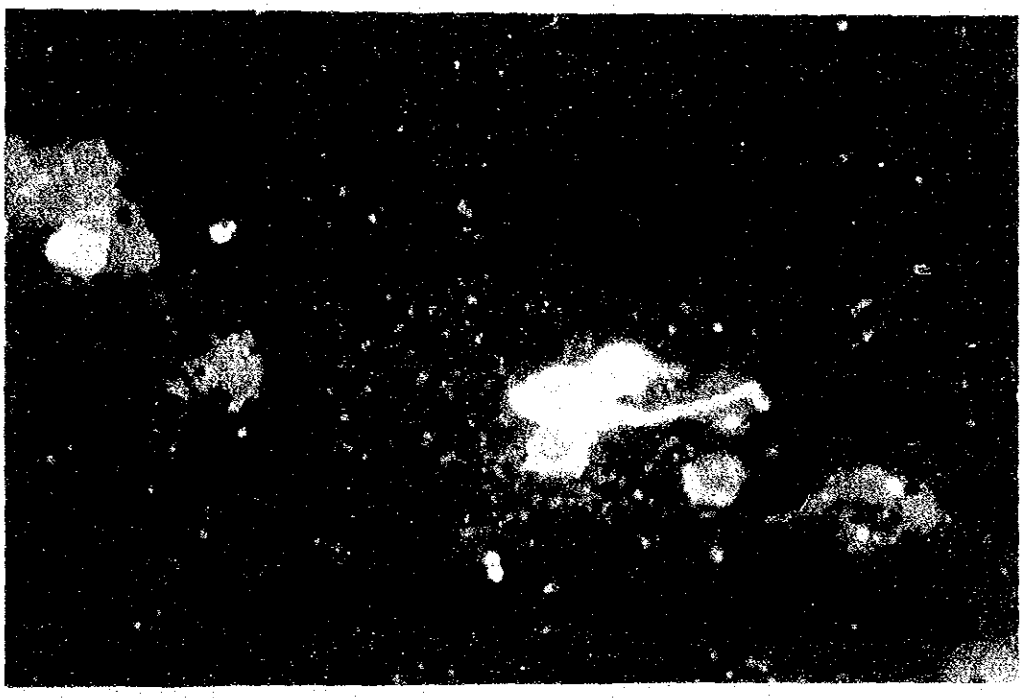


Table 1.

Detection of dengue viral antigens by IF after I.C. inoculation of patient's mononuclear cells.

Degree of illness.	Total # tested	# of Positive specimen	Day of detection after infection
Non shock	63	13	5
Shock	5	1	5

Detection of Dengue antigen in $LLCMK_2$ cell cultures by direct immunofluorescence.

Introduction

Although Dengue Haemorrhagic Fever (DHF) is a major public health problem in South East Asia and Western Pacific regions since the last 3 decades there had been a delay towards the development of sensitive, rapid and simple diagnostic methods. Laboratory diagnosis of DHF is aimed to assist the clinician in his diagnosis in order to facilitate appropriate management and treatment of the patient. Moreover, it also aids in early detection of outbreaks so that prompt control measures can be carried out for the benefit of the community.

Laboratory diagnosis is usually based on (1) isolation or detection of viral antigen from clinical material during the viraemic phase of illness (2) serologic confirmation by the classical haemagglutination inibition test, (1) or by demonstration of specific artibodies by the direct immunofluorescence. Other rapid test system such as Staphylococcal agglutination test (3) and counterimmunoelectrophoresis (4, 5) thin layer immuno assay (6, 7) passive haemolysis in gel (8) immuno adherence haemagglutination (9) and Elisa (10) have also been developed. Assays of various arboviruses in cell monolayers by culture direct or indirect fluorescent antibody have also been reported. (11-12).

Recently reports on the use of arthropod tissue culture system such as Aedes pseudoscutellaris and Aedes albopictus C6/36 have also been published. Presence of the dengue virus was seen by the cytopathic effect (CPE) as early as 4 days after infection and the isolates could be identified by means of the complement fixation test of the tissue culture fluids.

These publications have motivated the present study whereby dengue viruses grown in $LLCMK_2$ although not

producing CPE can be detected by the direct immuno-fluorescence staining method.

MATERIALS AND METHODS

Prototype controls

Mosquitoes infected with dengue 1, 2, 3, 4 and tissue culture adapted JE virus were used as positive controls.

Positive mosquito controls

Toxorhynchites splendens were inoculated intrathoracic with sera from patients admitted to Rangoon Children's Hospital with a clinical diagnosis of DHF. They were proved to contain dengue virus by detection of the dengue antigen by immunofluorescence inthe head-squash preparations after intrathoracic mosquito inoculation (Toxorhynchites splendens). All specimens had been adequately stored at -80°C deep freeze.

Negative mosquito control

Uninfected mosquitoes were processed in the same manner as mentioned above.

Sera

Sera were collected from patients admitted to Rangoon Children's Hospital (1980) with diagnosis of DHF and confirmed serologically by haemagglutination inhibition test in the laboratory. All test sera were stored at -80°C deep freeze.

The inoculum per tube culture was 0.5 ml of a 1:10 dilution in PBS pH 7.2 fortified with 30% inactivated calf serum. All sera were passed through a millipore filter HA 0.45 U after diluting to 1:10 in the diluent.

Preparation of antigen suspension

Both the prototype and positive mosquito controls were homogenized in a Ten Broeck individually using the same diluent as for the serum (Toxorhynchites + 0.5 ml of diluent). The suspension was centrifuged at +4°C, 7000 rpm for 30 mts. The supernatant was diluted to 1:10 in the diluent and passed through millipore filter HA 0.45U.

Preparation of cell cultures

$LLCMK_2$ cells (100,000 cells/1.5 ml) were prepared in test tubes containing coverslips, and by the 3rd day a complete healthy monolayer was formed.
MEM + 10% CS + glutamine pH 7.2 was used as the growth medium, and MEM + 2% CS + glutamine pH 7.2 was used as the maintenance media PBS pH 7.2 was used as the work media.

Infection and adsorption of the cell cultures

On 3rd day of the monolayer $LLCMK_2$ were washed twice with PBS 7.2 and the respective prototype, positive and negative controls and the test serum were added at 0.5 ml per tube and allowed to adsorb in stationary position at 37°C for 2 hrs, tilting every 15 mt to ensure even layering of the virus inoculum and contact on to the cell sheet. After completion of the adsorption the excess virus inoculum was discarded and gently washed with PBS: and filled with 1.5 ml of maintenance media: and incubated at 37°C in stationary position.

Detection of dengue viral antigen by the direct IF method

The infected tubes are harvested in pairs a day 1, 2, 3, 4, 6, 9, 10 and 12 after infections.
The infected coverslips were washed gently in PBS and fixed in cold acetone for 10 mins. After drying it was stained with antidengue conjugate in a humid chamber at 37°C

incubator for 30 mts. The slides were next washed in PBS and mounted in PBS + glycerol (1:1) and observed under the Fluo rescent Microscope (Olympus) equipped with Tungsten light source BH-LHF + bulb 6V, 30W, Barrier filter O-530 + BC-12 exciter filter.

Results

Intracellular dengue viral antigens was detected on the second post infection day for dengue prototype 2 + 3 and on the 3rd post infection day for dengue 4 and only on the 9th day for dengue 1. The number of foci counted in 10 fields under 10x was found to be 6, 4, 5 & 9 for dengue 2, 3, 4 & 1 respectively. These foci increased from 28 - 33 foci by the 4th day for dengue 2, 3 & 4 and for dengue 1 the spread appeared slower and only 8 foci was noted by the 11th post-infection day. The uninfected mosquito control did not show any form of fluorescence (Table 1).

The type of fluorescence of the viral antigen appeared first as a dense mass riding over a pole of the nucleus. It was also interesting to note that recently dividing cells (glass cells) were more commonly infected. By the 4-6th post infection day the dense mass thickens and small fluorescent fragments are seen to burst through the cell wall. Spread of infection to other cells appear radially and generally by the 7-8th post-infection almost 60-70% of the cell sheet appears to be infected in groups (Fig 1 & 2).

Out of the 20 positive mosquito controls which were obtained by inoculation of acute serum from DHF patients collected within 4 days of the onset of illness and containing HI antibodies from the range of 10 - 160, viral antigen was detected by this method in 18 (90%). The geometric mean MID_{50} in Toxorhynchites of the positive controls varied from 3.4×10^6 per ml. (Table 2).

Out of the 18 positive specimens intracellular antigen was detected by DFAT on day 2 in (44%) when the geometric mean MID 50 in Toxorhynchites was found to be between 6.1×10^6 - 9.3×10^7 per ml, on day 3 in 6 (33%) and the geometric mean MID 50 in Toxorhynchites was 6.1×10^5 - 7.1×10^7 per ml, and on day 4 in 4 (22%) and the geometric mean MID 50 in Toxorhynchites was found to be 6.0×10^4 - 7.3×10^6 per ml. Table 3.

Out of the 5 serum isolated the intracellular antigen was detected in 2 specimens. One on day 4 showing 4 foci, and by day 6 increased to 30 foci. For the 2nd positive specimen antigen was detected on day 7; showing 3 foci which increased to 6 and 15 on day 8 and 9 respectively. Table 4.

Discussion

Studies on the mode of detection of intracellular antigen in $LLCMK_2$ cell cultures by the DFAT was established using prototypes, wild strains of dengue virus isolated from the serum of DHF patients.

Similar work have been conducted by other workers using other types of mammalian and arthropod cell cultures. The intracellular antigen was also detected before CPE appeared by the IF technique, using CV (monkey kidney) cell cultures within 22 - 78 hrs with the prototype strain. Using $LLCMK_2$ cell cultures it was found that dengue 2, 3 and 4 antigen was detected within 2-4 days in both the prototype and some wild strains of dengue; however dengue type 1 antigens took 9 days for the detection after infection.

Although the serotypes of the wild and serum isolates have not yet been conducted it appears that they could be either dengue 2, 3 or 4 since the pattern was similar to that of the prototype. Reasons for the specimens which

did not show the presence of viral antigen in the cell culture, but the dengue virus was detected by immuno-fluorescence in the mosquito tissue shows that this system is less sensitive than the mosquito in supporting replication of the virus in $LLCMK_2$ cultures.

A linear relationship between the virus concentration and number of fluorescent foci was noted in all specimens demonstrating the presence of the intracellular antigen and this could be concluded that one infectious virus is equivalent to a single foci. It seems clear that although this test system could provide results within 2-4 days after collection of the clinical material it is not a sensitive system to detect dengue 1 in a short period, and moreover there is a likelyhood of missing 10% of the test due to inability of $LLCMK_2$ cell cultures supporting growth of the wild strain of dengue viruses.

Table 1.

Detection of intracellular antigen by the DFAT test of the dengue prototypes, infected in $LLCMK_2$.

Prototype controls Positive controls	day of detection of antigen	# of foci in 10 fields under 10x	increase in the # foci
Dengue 1	9	3	8 foci by day 11
Dengue 2	2	6	33 " " " 4
Dengue 3	2	4	30 " " " 4
Dengue 4	3	5	28 " " " 4

Negative control
Uninfected mosquito — Not seen —

Table 2.

Number of specimens showing intracellular antigen and the geometric mean virus titre of the inoculum in Toxorhynchite

Total tested	# showing presence of antigen	# which did not show fluorescence	geometric mean Virus titre per ml
20	18	2	3.4×10^6 per ml.

Table 3.

Day of detection of intracellular antigen and No. of foci and the geometric mean titre in *oxorhynchites.

# of specimens showing intracellular antigen	day of detection	# of foci on	# of foci under 4	Geometric mean virus titre per ml
8-(44%)	2	3-6	25-38	6.1×10^6 - 9.3×10^7
6-(33%)	3	3-7	33-40	6.1×10^5 - 7.1×10^7
4-(22%)	4	2-6	35-48	6.0×10^4 6.3×10^6

Table 4.

Day of detection of intra cellular antigen and No. of foci

# of specimen showing intracellular antigen	day of detection	# of foci on day of detection	# of foci on 6th day	# of foci on 8th day
Total tested 2/5	1 on day 4 1 on day 7	4 3	30 -	6-15 (on day 9)

Staining method of infectious focus of dengue by enzyme conjugate antiserum.

Introduction

The application of cell culture techniques for dengue virus assay, isolation and identification had been developed by some workers, (1) Further development by Yuill and his co-workers (2) facilitated a more specific means of isolation and identification of dengue virus by the virus plaque techniques and ended the study of epidemiology of dengue infections. The use of immunoperoxidase method for serotypic identification of virus within a short time (3-4 hrs) (3, 4) has also been reported. Thus, these different studies motivated to try the use of staining the infectious foci in the cell culture system by the enzyme conjugate antisera so as to provide (1) a rapid diagnostic service (2) see the usefullness of this system as a biological marker and (3) to facilitate easier selection of the dengue infection foci for mechanical processing for electron microscopic studies.

Materials and Methods

Materials

Dengue type 2 (Tr 5751)

Monolayer of Vero cell prepared in Multi-well plate 4 x 6 wells x 2 cm^2.

Eagles' MEM serum free containing 1.5% methyl cellulose.

Anti dengue 2 rabbit serum (HI titre 1:1286).

Peroxidase conjugate anti-rabbit goat serum (Miles)

Phosphate buffered saline containing 0.05% Tween. 20 + 0.5% Bovine serum albumin (PBS/T/BSA).

Kalnovsky's solution.

Method

0.1 ml of each of the ten fold dilution of dengue virus (10^{-1} - 10^{-5} in Hanks solution) is inoculated onto the monolayer of Vero cells and adsorbed at 37°C for 90 mts. (Tilting of the plate is carried out every 15 minutes). To the control well 0.1 ml of Hanks was added. After adsorption the virus inoculation was sucked out using pasteur pipette, and 1 ml of 1.5% Methyl cellulose in Eagle's MEM was added and incubated at 37°C CO_2 incubation. Cell cultures are stained from 18 hrs - 20 hrs post infection after microscopic examination.

Staining

The 1.5% Methyl cellulose in Eagles MEM is sucked out and washed gently twice in PBS without Ca + Mg and fixed with cold methanol at room temperature for 10 mts. Antidengue 2 rabbit serum (1:20 dilution) was added in 0.1 ml amounts in every well and inoculated at 37°C - 30 mts. Next it was washed with cold PBS/T/BSA 3 times at intervals of 15 mts. A 1:50 dilution of Peroxidase conjugate anti rabbit goat serum (Mules) was added in 0.1 ml amounts in all the wells and incubated at 37°C for 30 mts. Next it was washed with cold PBS/T/BSA for 3 times at intervals of 15 mts. Finally, the cells are stained with Kalnovsky's solution (1 ml per well) at room temperature for 30 mts and washed with distilled water. When the plate is dry the enzyme infection foci are easily observed and can be counted.

Results

Dengue 2 virus strain grow well and produce plaques in Vero cell cultures. The number and morphology of plaques are easily recorded. Previous experimental results in relation to the 4 dengue serotypes, concentration of methyl cellulose (1% - 1.8%) and days at which the infectious foci was best seen had been carried out to standardize the optimum conditions (Table 1). It was found that for titration and neutralization test 1.5% methyl cellulose concentration was the best.

Discussion

These findings such as (1) detection of infectious foci under the microscope after 18 - 20 hrs after infection (2) detection of the infectious foci on day 3 by the immunoperoxidase stain (previously dengue plaques are only observed after 7-9 days post infection) was strong evidence for application of this technology for the rapid diagnosis of dengue infection. Another important finding by this method is that by observing the stained infectious foci one can definitely conclude that an infectious virus has invaded the cell and is detected in the intracellular position as seen in the fluorescent antibody technique. However, in the previous system the plaque stain appearance is mainly as a result of cytopathic effect which does not provide a full proof of the invasion of the cell by the virus. All these findings clearly shows the importance of application of this technique as etiological marker for the physical and chemical condition of wild strains of dengue viruses.

Table 1.

Effects of Methyl-cellulose Concentration on the Focus Size of Dengue Viruses

Virus type	Days after inoculation	Concentration of Methylcellulose			
		1%	1.2%	1.5%	1.8%
D-I	3	< 0.2[a)]	< 0.2	< 0.2	< 0.2
	5	0.4-0.6	0.6-0.8 (definite)	0.6-0.8 (definite)	0.4-0.6
	7	1.0-1.6	1.4-1.6	1.2-1.4	1.2-1.4
D-II	3	0.2	0.2	0.2	0.2
	5	0.8-1.0	0.6-0.8 (definite)	0.6-0.8 (definite)	0.6-0.8
	7	1.2-1.6	1.2-1.4	1.6-1.8	1.0-1.2
D-III	3	< 0.2	< 0.2	< 0.2	0.2
	5	0.4-0.6	0.6-0.8 (definite)	0.6-0.8 (definite)	0.6-0.8
	7	1.4-1.6	1.2-1.4	1.2-1.4	1.0-1.2
D-IV	3	0.2	0.2	0.2	0.2
	5	0.6-0.8	0.6-0.8 (definite)	0.6-0.8 (definite)	0.6-0.8
	7	0.8-1.0	0.6-1.0	1.0-1.4	0.6-1.0

a) Focus size (mm)

Neutralizing capacity of the sera of dengue haemorrhagic fever patients to Japanese encephalitis virus.

Introduction

An outbreak of mosquito borne dengue haemorrhagic fever first took place in Burma in 1970 following a few sporadic cases that occured yearly ever since 1965. This has led to yearly monsoon outbreaks up to this day. Studies concerning the etiological agents have revealed that all four serotypes of dengue virus were responsible but dengue 2 was found to be the pre-dominant serotype.

Also small outbreaks of JE have been reported from Northern parts of Burma in 1974, 1975 and 1978, accompanied by high fatality rates. In 1977, JE outbreaks in horses of Ba Htoo Myo yielded a JE virus isolate from the brain of a dead horse. Although no reports of outbreaks occuring elsewhere in the country have been made avail it is of concern whether this disease could infiltrate other parts of Burma in epidemic proportions.

An epidemiological hypothesis stating that a previous infection with one or more types of dengue virus may reduce the clinical manifestations of JE and probably other encephalitis caused by closely related group B arbovirus has been put up and discussed many a times.

Apart from that, previous studies in Thaketa, Rangoon have revealed the presence of JE neutralizing antibodies in 90% of pigs and a low percentage of JE neutralizing antibodies was detected in the humans. Inspite of the presence of pigs which were proven to be amplifier hosts for JE virus (9) and even with the existence of the vector Culex species, JE outbreaks have not been known to have occurred.

A variety of factors may explain the absence of JE viral human infections in Thaketa; such as the zoophilic behaviour of mosquitoes, lack of mosquito human contact, and one other possible factor is that dengue antibodies present in 90% of the population may be protecting against JE infections. In order to understand these factors, an epidemiological study may be required to be conducted. However, at present time a laboratory investigation was carried out to see whether JE antibodies were produced in dengue infections and is presented in this communication..

Materials and Methods

1. Virus

Japanese encephalitis virus Nakayama strain propagated in suckling mouse brain and further passaged in tissue culture to the 7th passage virus was used.

2. Tissue culture

Test tube cultures of Vero cells containing 80,000 cells/ml were prepared using Eagle's Minimum Essential Medium containing 10% calf serum, 3% glutamine, 7.5% sodium bicarbonate and 200 units of Penicillin and Streptomycin as the growth medium. The pH of the medium was 7.2. 1.5 ml of the growth medium was added to each tube. The seeded cells were incubated at 37°C for 4 days to obtain a monolayer.

3. Infectivity titration of JE virus

Ten fold dilutions of JE virus was done using MEM maintenance medium containing 2% calf serum (pH 7.2) as a diluent. 10^{-1} to 10^{-7} dilutions of virus was done.

The tubes containing monolayer cells of Vero were washed twice with phosphate buffer saline pH 7.2 without calcium and magnesium. 0.1 ml of each virus dilution was infected onto the monolayer of Vero cells. The test was done in triplicates. Cell controls were also included. The virus was allowed to adsorb for 1 hour at 37°C and washed with PBS - Ca, Mg. 1.5 ml of MEM maintenance medium was added to each tube and they were then incubated at 37°C.

The infected tubes were checked daily for 10 days for cytopathic effect of JE virus. Virus titres were recorded and 100 $TCID_{50}$ dose of JE virus was also calculated.

4. Serum specimen

Paired serum specimens from clinically diagnosed and HI proven DHF cases were used for the test.

5. Neutralization test of the serum

a. The acute phase and convalescent phase sera of each patient was diluted with MEM maintenance media.
For the acute phase serum - 1/10, 1/40, 1/160 dilutions and for the convalescent were used.
phase - 1/10, 1/40, 1/160, 1/640 dilutions were used.

b. Positive Control

JE mouse immune ascitic fluid 1/10 and 1/40 dilutions were used as positive controls.

c. Negative Control

JE negative serum 1/10 dilution was used as a negative control. All the above diluted sera and JE mouse immune ascitic fluid were inactivated at 56°C for 30 mins in a water bath.

6. JE virus dilution

The previously titrated JE virus was diluted to obtain a 100 $TCID_{50}$ dose using MEM maintenance medium as a diluent.

7. Check titration of JE virus

A JE virus titration was repeated and done simultaneously with each batch of tests.

8. Virus serum mixture incubation

0.3 ml each of all diluted sera and immune fluids and 0.3 ml of 100 $TCID_{50}$ dose of JE virus were added into tubes and incubated in a water bath at 37°C for 1 hour to allow reaction between the virus and the sera.

9. Infection of Vero cells with virus serum mixtures

After incubation for 1 hour at 37°C in a water bath, virus serum mixtures of each serum dilution controls were infected onto the prepared 4 day old

monolayer Vero cell cultures in tubes and incubated at 37°C for 1 hour. The cells were then washed twice with PBS (Ca^{--} Mg^{--}). 1.5 ml of MEM maintenance medium was added into each tube. Cell controls were included. Each test was done in triplicates. All the tubes were incubated at 37°C for 10 days and checked daily for cytopathic effect.

After every four days, all the tubes were washed with PBS Ca^{--}, Mg^{--}. MEM maintenance medium was changed.

Daily results were recorded.

Results

True to the previous knowledge of cross reactions among group B arboviruses, the sera of DHF patients were found to have a neutralizing response to JE virus.

The acute phase serum of the patients were taken within 2-7 days of the onset and the convalescent phase sera after 13-20 days of the onset of the illness. Neutralizing response to JE virus was detected in the sera of 14 patients.

Out of the paired sera of 33 patients that was tested the acute phase sera of 12 patients were positive with 10 at a titre of 1/10 and 2 at a titre of 1/40. Among the 14 positive convalescent phase sera of these patients 1 was positive at 1/10, 11 at 1/40 and 2 at 1/160 (Table 1 & 3).

12 patients did not show a detectable response to JE virus (Table 1) and the remaining 7 demonstrated JE antibodies in low levels, that is at a titre of 1/10 in both the acute phase and the convalescent phase sera (Table 2). Since there was no apparent rise of titre between the acute and the convalescent phase sera it is difficult to interpret in these children.

A question could arise, whether the detectable rise in the JEV NT titre of patients with DHF might not be

the result of dengue infection itself but rather as a result of a previous JEV infection. This was partly answered by the sero conversion of the convalescent sera of 2 patients indicating a dengue stimulus playing for JE neutralizing response of the serum.

Since most of the sera under test have shown a secondary response in the HI test, it was difficult to show many negative acute phase sera becoming positive to JE virus in the convalescent phase sera of DHF patients as the antibody response was already there when the acute phase sera was taken. Moreover, most of the S_1 were taken after 4 days of onset, that is when generally the neutralizing antibodies were considered to have already emitted if there was any reason to do so.

The sera under study were from the same 1980 outbreak and dengue virus was isolated from three patients. It was interesting to note that although the dengue virus was isolated, there was no rise of JE antibody in two patients. Out of the 33 patients studied, 26 were clinically confirmed as dengue grade 3 and 4 (Table 4), in other words with definite haemorrhagic manifestations and sometimes shock the possibility of a misdiagnosis is most unlikely.

Discussion

42% of the sera of DHF patients tested were found to possess a neutralizing response to JE virus by a definite increase to a rise of four fold or more. These findings support the results of Igarashi and his co-workers (10). The percentage positive is lower than their's which was 90%. This could be due to two things. One is due to the use of the plaque reduction neutralization test in their experiment which is well documented to be more sensitive

than the tube neutralization test used in this study. The second point may be due to the difference in the distribution of dengue serotypes and strains in the two different studies.

There have been much controversy on the protective role of the heterologous NT antibody against JEV found in the sera of convalescent cases of dengue haemorrhagic fever.

An extensive epidemiological study was carried out in Chaingmai Valley, Thailand by Grossman and his colleagues (11). It was an area where both dengue and JE existed. Their conclusion denoted that persons under age 20 who have had prior dengue infections are sometimes protected against the development of Japanese encephalitis, though the proportion protected is probably not large.

Muira (12) also mentioned that where there is an epidemic of dengue there will not be an epidemic of JE Gould (12), also found that no epidemic of JE was seen in Bangkok which was notorious for dengue outbreaks, although JE virus was found there. Here again, interference reaction between JE and dengue was suggested.

Animal experiments had been done by many workers (13, 14, & 15) on cross protection between members of group B arboviruses and mary were found to be encouraging. Cell mediated immunity was also suggested as a possible mode of protection.

Oya (16) reported that JEV - NT antibody at a level which was detectable by plaque reduction at a 1:10 serum dilution can inhibit the development of the disease in mice which were inoculated peripherally with 10^4 LD_{50} dose of JEV.

On the other hand, Wisserman et al (17) reported that subjects with pre-existing JEV antibodies developed a low level of type 1 dengue antibody following vaccination with 17D yellow fever vaccine, but that these subjects developed classical type 1 dengue fever when exposed to unmodified type 1 dengue virus.

Also studies on changes in dengue and JE antibody after JE vaccination by Quina et al (18), concluded that the presence of dengue neutralizing antibodies in the pre-vaccination sera did not significantly influence the geometrical mean titre of post vaccination JE N antibody.

Nevertheless, if the neutralizing ability of the DHF patients would contribute towards limiting the spread and the severity of the infection in man (19) by acting as a trigger for a much more effective immune response on encountering with a subsequent JE infection, it may be of much help.

In support of this view, a study on 127 JE encephalitis patients was done by Edelman et al (20). They divided the encephalitis patients into dengue sero positive and dengue sero negative groups and after a one year follow up study suggested that prior dengue infection moderately reduces the morbidity and possibly the mortality of persons hospitalized with JE.

In the present study there were certain limitations which were of a hindrance in securing a firmer inference.

Firstly, instead of carrying out HI test with dengue 2 alone, it would be more informative if HI test was done using all 4 dengue virus antigens.

Secondly, neutralization tests on these sera with all 4 dengue serotypes and JE should be tested simultaneously for the sake of comparison.

Thirdly, a plaque reduction neutralization test instead of the tube neutralization test would help in increasing the sensitivity of the test.

Fourthly, to exclude the presence of pre-existing JE antibodies, to obtain the sera of primary dengue patients in whom the acute phase sera were collected within the first three days of onset and try to demonstrate a sero conversion to JE in the convalescent phase sera.

A clearer picture could be obtained after fulfilling the above requirements.

Despite these limitations, we have demonstrated a rise of JE neutralizing antibodies in clinically and laboratory confirmed cases of DHF in Burma, indicating that Dengue infection can provoke a neutralizing antibody response to JE virus. These results support the evidence reported by other workers.

Table 1

JE heterologous neutralizing response of DHF patients.

Total No. tested	No. of sera showing no neutralizing response	No. of sera showing neutralizing response.
33	12 (36%)	14 (42%)

Table 2

No. of sera demonstrating JE antibodies in low levels without a rise between the acute phase and the convalescent phase sera
7 (21%)

Table 3

JE neutralizing antibody titres of DHF patients

S_1				S_2			
<1/10	1/10	1/40	1/160	<1/10	1/10	1/40	1/160
14	17	2	0	12	8	11	2

Table 4. Dengue 2 HI and JENT antibody in the paired sera of dengue haemorrhagic fever patients.

Sr. No.	Age	Grade	Response	S_1 days after onset	S_2 days after onset	Reciprocal of Dengue 2 HI S_1 titre	Reciprocal of Dengue 2 HI S_2 titre	Reciprocal of JE neut S_1 titre	Reciprocal of JE neut S_2 titre	Virus isolation
1.	6yrs	4	Secondary	8	23	1280	2560	< 10	< 10	
2.	8 "	4	"	4	18	80	2560	< 10	< 10	
3.	1½"	4	"	6	19	1280	1280	< 10	< 10	
4.	3 "	3	"	5	23	320	2560	< 10	< 10	
5.	4½"	3	"	5	19	1280	5120	10	10	+
6.	2½"	3	"	5	18	320	1280	< 10	40	
7.	6½"	3	"	7	14	160	10240	40	40	
8.	5 "	3	"	5	19	5120	5120	40	160	
9.	4 "	3	"	8	19	2560	2560	10	40	
10.	4½"	4	"	6	20	1280	1280	< 10	< 10	
11.	6 "	4	"	6	20	5120	5120	10	40	
12.	9 "	4	"	7	22	2560	2560	< 10	10	
13.	8 "	4	"	4	17	1280	1280	10	10	
14.	7 "	4	"	4	19	160	2560	10	40	
15.	5 "	2	"	3	16	20	320	< 10	< 10	+
16.	5 5/12	3	"	6	21	2560	10240	< 10	< 10	
17.	4 "	3	"	3	18	1280	10240	10	40	
18.	5 "	3	"	3	17	1280	5120	10	40	
19.	3 "	4	"	6	20	1280	2560	< 10	< 10	

Sr. No.	Age	Grade	Response	S_1 days after onset	S_2 days after onset	Reciprocal of Dengue 2 HIS $_1$ titre	Reciprocal of Dengue 2HIS $_2$ titre	Reciprocal of JE neut S_1 titre	Reciprocal of JE neut S_2 titre	Virus isolation
20.	5yrs	3	secondary	7	20	1280	5120	<10	<10	
21.	10 "	3	"	7	13	1280	10240	10	10	
22.	3 "	3	"	3	21	5120	10240	10	10	
23.	4 "	3	"	5	19	2560	10240	10	10	
24.	2½ "	2	"	7	20	20	320	10	10	
25.	4 "	3	"	5	19	1280	1280	<10	<10	
26.	10 "	3	"	4	18	5120	10240	10	10	
27.	10 "	1	"	2	14	80	320	10	40	+
28.	3 "	2	"	4	18	1280	10240	<10	<10	
29.	5 "	2	"	7	20	640	1280	10	40	
30.	2 "	2	"	4	18	1280	1280	10	40	
31.	1 "	2	"	4	18	2560	2560	10	40	
32.	1 1/6	1	primary	4	19	10	640	10	160	
33.	3½"	2	secondary	8	23	40	640	<10	<10	

References

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ပြည်ထောင်စုဆိုရှယ်လစ်သမ္မတမြန်မာနိုင်ငံတော်
ကျန်းမာရေးဝန်ကြီးဌာန
ဆေးသုတေသနဦးစီးဌာန
အမှတ်(၅)ဇီဝကလမ်း၊ ရန်ကုန်မြို့

စာအမှတ်၊ ၃၀၀၁/ဝ/ဧ၃ ရက်စွဲ ။ ၁၉၈၂ ခု၊ ဖေဖေါ်ဝါရီလ (၂၃)ရက်

အကြောင်းအရာ။ ။ <u>ဝမ်းလျှောရောဂါသုတေသနလုပ်ငန်း ဆွေးနွေးပွဲ ဖိတ်ကြားခြင်း ။</u>

ဝမ်းလျှောရောဂါသုတေသနလုပ်ငန်း ဆွေးနွေးပွဲကို အောက်ဖော်ပြပါအစီအစဉ်အတိုင်း ပြုလုပ်မည်ဖြစ်ပါ၍ တက်ရောက်ပါရန် ဖိတ်ကြားအပ်ပါသည်။

ကျင်းပမည့်နေ့	=	(၂၇-၂-၈၂) (စနေနေ့)
ကျင်းပမည့်အချိန်	=	နံနက် ၈း၃ဝ မှ မွန်းလွဲ ၆း၃ဝ နာရီ
ကျင်းပမည့်နေရာ	=	ဇီဝသိပ္ပံသုတေသနအဆောက်အဦး အစည်းအဝေးခန်းမ ဆေးသုတေသနဦးစီးဌာန
ဆွေးနွေးပွဲအစီအစဉ်	=	ပူးတွဲပေးပို့ပါသည်။

(ဒေါက်တာသိန်းတိုး)
ဒုတိယညွှန်ကြားရေးမှူး (သုတေသန)
ဆေးသုတေသနဦးစီးဌာန
(အဖွဲ့ခေါင်းဆောင်၊ ဝမ်းလျှောရောဂါသုတေသ
ပညာရှင်အဖွဲ့)

မိတ္တူကို၊ -

၁။ ညွှန်ကြားရေးမှူးချုပ်၊ ဆေးသုတေသနဦးစီးဌာန

၂။ ညွှန်ကြားရေးမှူးချုပ်၊ ကျန်းမာရေးဦးစီးဌာနသို့သက်ဆိုင်ရာပုဂ္ဂိုလ်များအား ခွင့်ပြုပါရန် မေတ္တာရပ်ခံချက်ဖြင့်)

၃။ ညွှန်ကြားရေးမှူးချုပ်၊ ဆေးပညာဦးစီးဌာနသို့သက်ဆိုင်ရာပုဂ္ဂိုလ်များအား ခွင့်ပြုပါရန် မေတ္တာရပ်ခံချက်ဖြင့်)

၄။ ပါဝင်ဆွေးနွေးမည့်ပညာရှင်များ (Participants)

၅။ တက်ရောက်လေ့လာမည့်ပညာရှင်များ (Observers)

၆။ ဒုတိယညွှန်ကြားရေးမှူး (စီမံ)

၇။ လက်ထောက်ညွှန်ကြားရေးမှူး (စီမံ)

၈။ ဌာနခွဲတာဝန်ခံ၊ စာစောင်ထုတ်ပြန်ရေးဌာနခွဲ သို့ပေးပို့ပါသည်။

ကြ/၂၃-၂။

RESEARCH SEMINAR ON ACUTE DIARRHOEA IN CHILDHOOD (27-2-82)

PROGRAMME

08:30 am	Opening address by Director General	Department of Medical Research
08:45 am	EPIDEMIOLOGY SESSION	
08:45 - 09:10 am	Epidemiology of Acute Diarrhoea in Childhood - Country profile	Disease Control Division, Department of Health Services
09:10 - 09:35 am	Epidemiology of Acute Diarrhoea in Childhood - Review of research done	Departments of Child Health, Institutes of Medicine (1) & (2), Department of Medical Education
09:35 - 10:00 am	Epidemiology of Acute Diarrhoea in Childhood - Urban community study	Epidemiology Research Div., Department of Medical Research
10:00 - 10:10 am	Review of Epidemiology of Acute Diarrhoea in Childhood	Deputy Director (Research) & Group Leader, DMR Scientific Group on Diarrhoea Research, Department of Medical Research
10:10 - 11:10	Discussion by Participants	
	C O F F E E B R E A K	
11:10	AETIOLOGY SESSION	
11:10 - 11:35 am	Aetiologic Agents of Acute Diarrhoea in Childhood	Bacteriology Division, National Health Laboratory, Department of Health Services
11:35 - 12:00 noon	Aetiologic Agents of Acute Diarrhoea in Childhood in Urban Community and Hospitalized Children	Bacteriology Research Div., Department of Medical Research
12:00 - 12:10 pm	Recent advances in Bacterial Diarrhoeas in Childhood	Bacteriology Research Div., Department of Medical Research
12:10 - 12:20 pm	Recent Advances in Viral Diarrhoeas in Childhood	Virology Research Division, Department of Medical Research
12:20 - 13:20 pm	Discussion by Participants	
13:20 pm	L U N C H B R E A K	
14:00 pm	MANAGEMENT SESSION	
14:00 - 14:25 pm	Hospital Management of Acute Diarrhoea in Childhood	Department of Child Health, Institutes of Medicine (1) & (2), Department of Medical Education.
14:25 - 14:50 pm	Other Modalities in Hospital Management of Acute Diarrhoea in Childhood	Paediatric Unit, North Okalapa General Hospital & Paediatric Unit, Infectious Diseases Hospital, Department of Health Services
14:50 - 15:15 pm	Community Management of Acute Diarrhoea in Childhood	Clinical Research Division, Department of Medical Research
15:15 - 15:25 pm	Recent advances in the Management of Acute Diarrhoea in Childhood	Clinical Research Division, Department of Medical Research
15:25 - 16:25 pm	Discussion by participants	
16:25 pm	Closing Address by Director-General,	Department of Medical Research

*HTUH/-

ပါဝင် လွှား ဒေန မည့်ပညာရှင်များ (Participants)

ဆေး သုတေသနဦး စီး ဌာန

၁။ ဆွန်ကြာ ရေး မှူး ချုပ်။

၂။ ဒေါက်တာသိန်း ဝိုး ၊ ခုတိယဆွန်ကြာ ရေး မှူး (သုတေသန)

၃။ ဒေါက်တာမိမိခင် ၊ ခုတိယဆွန်ကြာ ရေး မှူး (သုတေသန)

၄။ ဒေါက်တာသိန်းဝေါင်မြင့် ၊ ခုတိယဆွန်ကြာ ရေး မှူး (သုတေသန)

၅။ ဒေါက်တာ ခေါ်ဟင် အား ၊ ဌာနခွဲ မှူး (ဗက်တီးရီးယား ဗေဒသုတေသနဌာနခွဲ)

၆။ ဒေါက်တာခင်မောင်ဦး ၊ ဌာနခွဲ မှူး (လက် ထွေ့ ဆေးပညာသုတေသနဌာနခွဲ)

၇။ ဒေါ်မာမာညိမ် ၊ ဘဏ်တန်းသုတေသနအရာရှိ (ဗက်တီးရီးယားဗေဒသုတေသနဌာနခွဲ)

၈။ ဒေါက်တာ အေင်ဖျိုးဟန် ၊ သုတေသနအရာရှိ (ကူးဝက် ရောဂါ ဗေဒသုတေသနဌာနခွဲ)

ကျန်း မာ ရေး ဦး စီး ဌာန။

၁။ ဒေါက်တာဦး သောင်း ၊ ဒုဏ်ယဆွန်ကြာ ရေး မှူး (ရောဂါနှိမ်နင်း ရေး)

၂။ ဒေါက်တာ ခေါ်ခင် မေကြည် ၊ ကူး စက် ရောဂါအထူး ကုဆရာဝန်ကြီး (ဗဟိုကူး စက်)

၃။ ဒေါက်တာဖိုး ဖိုး ဆား ၊ က လေး အထူး ကုဆရာဝန်ကြီး ၊ မြောက်ဥက္ကလာပ ဆေး ရုံကြီး

၄။ ဒေါက်တာသိန်း ဝုဝ် ကျော်မြင့် ၊ က လေး အထူး ကုဆရာဝန်ကြီး ၊ ရန်ကုန်က လေးဆေး ရုံကြီး

၅။ ဒေါက်တာမူမူခင် ၊ က လေး အထူး ကုဆရာဝန် ၊ ကူး စက် ရောဂါကု ဆေး ရုံကြီး ။

၆။ ဒေါက်တာ ခေါ်စန်း မြင့် ၊ အဖျိုး သား ဓါတ်ခွဲ ဌာန။

၇။ ဒေါက်တာ ဆောင်ပွန် ၊ အဖျိုး သား ဓါတ်ခွဲ ဌာန။

ဆေး ပညာဦး စီး ဌာန

၁။ ဒေါက်တာဘဝင်ဦး ၊ ပါ မေါက္ခန်ဝင့် ဌာန မှူး ၊ က လေး ကျန်း မာ ရေး ပညာဌာန ၊
ဆေး တက္ကသိုလ် (၁) ၊ ရန်ကုန်မြို့။

၂။ ဒေါက်တာ ခေါ်ဆွန်ဆွန့် ၊ ပါ မေါက္ခန်ဝင့် ဌာန မှူး ၊ အထူးဇီဝ ဗေဒ ဌာန ၊
ဆေး တက္ကသိုလ် (၁) ၊ ရန်ကုန်မြို့။

၃။ ဒေါက်တာဖျိုးမင်း အောင် ၊ ကထိက ၊ က လေးကျန်း မာ ရေး ပညာဌာန ၊ ဆေးတက္ကသိုလ် (၁)
ရန်ကုန်။

၄။ ဒေါက်တာခင် မောင်ကြွယ် ၊ ခုတိယဆွန်ကြာ ရေး မှူး ၊

Special invitees

1" Professor D. Cvjetanovic, Professor of Epidemiology School of Public Health, University of SARAJEVO.

2" Dr. H. Hayashi, Associate Professor, Okayama University

ဆေး သု တေသန ဦး စီး ဌာန

၁။ ဒါက်တာ ချိန် နွယ် ဦး ၊ ဌာန ခွဲ မှူး ၊ အာဟာရ သု တေသန ဌာန ခွဲ
၂။ ဒါက်တာ မျိုး သိန်း ၊ ဌာန ခွဲ မှူး ၊ ဇီဝ ကမ္မ ဗေဒ သု တေသန ဌာန ခွဲ
၃။ ဒါက်တာ ဖိုး သိန်း ၊ ဌာန ခွဲ မှူး ၊ ဗိုင်း ရပ် ဗေဒ သု တေသန ဌာန ခွဲ
၄။ ဒေါ် သန်း စော ၊ သု တေသန အရာ ရှိ ၊ ကဗီ ဟီး ဗေဒ သု တေသန ဌာန ခွဲ
၅။ ဒါက်တာ မျိုး ခင် ၊ သု တေသန ဆရာ ဝန် ရှိ ၊ လက် တွေ့ ဆေး ပညာ သု တေသန ဌာန ခွဲ

ကျန်း မာ ရေး ဦး စီး ဌာန

၁။ ဒါက်တာ တင် ဦး ၊ ဆေး ရုံ အုပ် ကြီး ၊ ကုား စက် ရောဂါ ကု ဆေး ရုံ ကြီး
၂။ ဒါက်တာ သန်း စိန် ၊ ပြည်သူ့ ကျန်း မာ ရေး စီမံ ချက်
၃။ ဒါက်တာ မြမြအုန်း ၊ က လေး ဆေး ရုံ ကြီး

ဆေး ပညာ ဦး စီး ဌာန

၁။ ဒါက်တာ အောင် ကြွယ် ၊ အတွင်း ရေး မှူး ၊ ပြည်သူ့ ကျန်း မာ ရေး ဝန်ကြီး ဌာန သင် တန်း ကောင်း ။
၂။ ဒါက်တာ ဘွဲ့ နှံ့ ဘွဲ့ နှံ့ ဝေ ၊ က လေး ကုမ္ပဏီ မာ ရေး ဌာန ခွဲ ၊ ဆေး တက္ကသိုလ် (၁) ရန်ကုန် ။

プロジェクト延長の場合のR/Dの準備

本プロジェクト（ビルマ国感染症プロジェクト）は1980年4月10日より1982年4月9日に到る2カ年の期間としてR/Dで締結されていたために、今回のエバリュエーション調査団が第2年次終了に近い1982年2月に派遣されたものである。そしてその評価の成果を日本政府に報告した上で、それから改めて日本政府側とビルマ政府側との間で本プロジェクトを、これで打ち切るのが妥当であるか、あるいは延長が望ましいか、またもし延長ということとなった場合にはその期間はどれだけか……などの検討がなされるという手順が予定された。そして若し延長の場合は出来得れば1982年4月10日より実施出来るようにすることが望ましいということが考慮された。しかし、そのためにはビルマ側からの正式の要請が日本政府に来なければならず、かつそのためには可及的早くビルマ側閣議の了承を得なければならず、そのためには十分な時間的余裕がないために一刻も早くビルマ政府閣議の審議とプロジェクト延長要請の了承を得る必要に迫られた。そのためにわれわれエバリエーション調査団の次の業務として、次のようなJICAエバリュエーション調査団とビルマ政府側との間のMinutes 作製の討議を行った（1982年2月23日午前9：30より）。その結果次のようなMinutes の交換文書が出来上り、同日午後Minutes の署名交換を行った。（出席者：日本側：エバリュエーション調査団全員、本田均書記官、武田慶一JICA所長、ビルマ側：オンタンバツー局長、ジョーテイン副局長）

The Minutes of Discussion between the Japanese Evaluation Team and the Authorities concerned of the Government of the Socialist Republic of the Union of Burma on the Japanese Technical Cooperation for the medical cooperation project.

The Japanese Evaluation Team (hereinafter referred to as "the team") organized by the Japan International Cooperation Agency and headed by Professor Yoshihiro Hamashima, Faculty of Medicine, Kyoto University, visited the Socialist Republic of the Union of Burma from February 17, 1982 to February 24, 1982 for the purpose of reviewing the past activities, and of studying the possibilty of the extension of cooperation period concerning the technical cooperation programme on "Research on major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of these diseases." The team exchanged views and had a series of discussions with the

Burmese authorities headed by Dr. U Aung Than Batu, Director-General of the Department of Medical Research. As a result of the discussions, the team and the Burmese authorities concerned came to a conclusion that it is desirable for the period of the said project to be extended for another two years starting on April 10, 1982.

The team and the Burmese authorities concerned agreed to recommend to each respective Governments the extension of the cooperation period of the said project for another two years.

February 22, 1982

Director-General	Head
Department of Medical Research	The Evaluation Team

本プロジェクトに関しては、すでにＪＩＣＡ側、日本外務省側と、またビルマ政府側も共に引続きあと２カ年の延長が望ましい旨の意向が出されていたがために、その延長の場合のＲ／Ｄをどのようにするか（改めるか）慎重に討議した。その結果、基本的には従来のＲ／Ｄの本文は全然変更しないこと（ビルマ側は、たとえ小さくても変更のあった場合、法務局の了承を得なければならないために閣議了承が４月以降に遅れてしまうことを大いに懸念していた）として次のような案文を提案した。

THE SUPPLEMENTARY DOCUMENT

TO THE RECORD OF DISCUSSIONS BETWEEN THE JAPANESE IMPLEMENTATION SURVEY TEAM AND THE AUTHORITIES CONCERNED OF THE GOVERNMENT OF THE SOCIALIST REPUBLIC OF THE UNION OF BURMA ON THE JAPANESE TECHNICAL COOPERATION FOR THE PROJECT

"RESEARCH ON MAJAOR ARBO-VIRAL DISEASES, BACTERIAL ENTERIC DISEASES AND THE APPLICATION OF ITS RESULTS FOR THE CONTROL OF THESE DISEASES" SIGNED ON 10th APRIL 1980

The Japanese Evaluation Team (hereinafter referred to as

"the Team") organized by the Japan International Cooperation Agency (hereinafter referred to as JICA) and headed by Professor Yoshihiro Hamashima, Faculty of Medicine, Kyoto University visited the Socialist Republic of the Union of Burma from 17th February to 24th February 1982 for the purpose of reviewing the results of the Project as well as working out details of extension of the technical cooperation programme concerning "Research on Major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of these diseases" project in the Socialist Republic of the Union of Burma.

During its stay in the Socialist Republic of the Union of Burma, the Team exchanged views and had a series of discussions with the Burmese authorities concerned in respect of the desirable measures to be taken by both Governments for extension of the above-mentioned Project.

As a result of the discussions, the Team and the Burmese authorities concerned agreed to recommend to their respective Governments the matters referred to in the document attached hereto.

Head of the Japanese
Evaluation Team

Director General
Department of Medical Research
Ministry of Health

THE ATTACHED DOCUMENT

I. COOPERATION BETWEEN BOTH GOVERNMENT

1. The Government of Japan and the Government of the Socialist Republic of the Union of Burma will cooperate with each other in implementing "Research on major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of theses diseases" project (hereinafter referred to as "the Project") for the purpose of extending the biomedical research against these diseases and of developing the function of the Biomedical Research Centre as the central institute undertaking laboratory and other services in support of biomedical research on these diseases in Burma.

   The Project aims to contribute to the control of those diseases with knowledge and experience acquired from the research and thus to promote the health conditions in the Socialist Republic of the Union of Burma.

2. The Project will be implemented in accordance with the Master Plan which is attached as Annex 1.

II. DISPATCH OF JAPANESE EXPERTS

1. In accordance with the laws and regulations in force in Japan, the Government of Japan will take necessary measures through JICA to provide at its own expense services of the Japanese experts as listed in Annex II through the normal procedures under the Colombo Plan Technical Cooperation Scheme.

2. The Japanese experts referred to in paragraph 1 above and their families will be granted in the Socialist Republic of the Union of Burma the privileges, exemptions and benefits within the framework of the Colombo Plan Technical Cooperation Scheme.

III PROVISION OF MACHINERY AND EQUIPMENT

1. In accordance with the laws and regulations in force in Japan the Government of Japan will take necessary measures through JICA to provide at its own expense such machinery equipment and materials necessary for the implementation of the Project as mentioned in Annex III, through the normal procedures under the Colombo Plan Technical Cooperation Scheme.

2. The articles referred to in paragraph 1 above will become the property of the Government of the Socialist Republic of the Union of Burma upon being delivered to the Burmese authorities concerned at the ports and airports of disembarkation, and will be utilized exclusively for the implementation of the Project under the direction of the Co-codinating Committee referred to in paragraph 2 of Clause VI hereunder.

IV. TRAINING OF BURMESE PERSONNEL IN JAPAN

1. In accordance with the laws and regulations in force in Japan, the Government of Japan will take necessary measures through JICA to receive at its own expense the Burmese personnel connected with the Project for technical training in Japan through the normal procedures under the Colombo Plan Technical Cooperation Scheme.

2. The Government of the Socialist Republic of the Union of Burma will take necessary measures to ensure that the knowledge and experience acquired by the Burmese personnel from technical training in Japan will be utilized effectively for the implementation of the Project.

V. MEASURES TO BE TAKEN BY THE GOVERNMENT OF THE SOCIALIST REPUBLIC OF THE UNION OF BURMA

1. In accordance with the laws and regulations in force in the Socialist Republic of the Union of Burma, the Government of the Socialist Republic of the Union of Burma will take necessary measures to provide at its own expense:
   (1) Services of the Burmese counterpart personnel and administrative personnel as listed in Annex IV;
   (2) Land, buildings and facilities in the Biomedical Research Centre and pilot area as listed in Annex V;
   (3) Supply or replacement of machinery, equipment, instrument, vehicles, tools, spare parts and materials necessary for the implementation of the Project other than those provided through JICA under III above;
   (4) Transportation facilities and travel allowance for the Japanses experts for the official travel within the Socialist Republic of the Union of Burma;
   (5) Suitably furnished accommodations for the Japanese experts and their families.
2. In accordance with the laws and regulations in force in the Socialist Republic of the Union of Burma, the Government of the Socialist Republic of the Union of Burma will take necessary measures to meet:
   (1) Expenses necessary for the transportation within the Socialist Republic of the Union of Burma of the articles referred to in clause III above as well as for the installation, operation and maintenance thereof;
   (2) Customs duties internal taxes and any other charges, imposed in the Socialist Republic of the Union Of Burma on the articles referred to in clause III above;

(3) All running expenses necessary for the implementation of the Project.

VI. ADMINISTRATION OF THE PROJECT

1. The Japanese experts will give necessary technical guidance and advice to the Burmese staff associated with the Project pertaining to the implementation of the Project, and the Burmese authorities concerned will be responsible for the administrative and managerial matters pertaining to the Project.

2. For successful implementation of the Project, the Coordinating Committee will be established with the members as listed in Annex VI. The Committee will meet at least once a year. The Functions of the Committee are as follows:

(1) To formulate plan for the Project;

(2) To review the implementation of the Project;

(3) To co-ordinate the activities relating to the implementation of the Project.

VII. CLAIMS AGAINST JAPANESE EXPERTS

The Government of the Socialist Republic of the Union of Burma undertakes to bear claims, if any arises, against the Japanese experts engaged in the Project resulting from, occuring in the course of, or otherwise connected with the discharge of their efficial functions in the Socialist Republic of the Union of Burma except for these arising from The willful misconduct or gross negligence of the Japanese experts.

VIII. MUTUAL CONSULTATION

There will be mutual consultation between the two Governments on any major issues arising from, or in connection with this Attached Deocument.

IX. TERM OF COOPERATION

The duration of the technical cooperation for the Project under this Attached Document will be two years from 10 April 1982, unless otherwise agreed between the two Governments.

ANNEX I. MASTER PLAN OF THE PROJECT

1. Objective

The Project aims to conduct Research on major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of these diseases.

2. Implementation

The Department of Medical Research has overall responsibilities for the Project with the guidance of the Coordinating Committee. The Biomedical Research Centre which is an integrated functional component of the said Department is the executing organ for the achievement of the above mentioned objective.

3. Activities under the Project

Activities under the Project will be carried out at the Biomedical Research Centre premises including the pilot area:

Activities will include the following:

(a) Research on major arbo-viral diseases and the application of its achievement for their control.

(b) Research on major bacterial enteric diseases and the application of its achievement for their control.

(c) To further develop technology of laboratory and other services.

ANNEX II. JAPANESE EXPERTS

1. Team Leader (who may be one of the experts)

2. Experts

In Laboratory technology and Animal care

Bacteriology

Pathology

Virology

Immunology

Other related fields mutually agreed upon as necessary

ANNEX III. ARTICLES TO BE PROVIDED BY THE GOVERNMENT OF JAPAN

Machinery, equipment and materials for the research on major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of these diseases.

ANNEX IV. BURMESE PERSONNEL

1. Director-General
2. Deputy Directors/Assistant Directors
3. Head, Researchers and Laboratory Technicians

   In Immunology and Immunochemistry

   Bacteriology

   Epidemiology

   Virology

   Pathology
4. Infectious Diseases control personnel
5. Other Technical supportive personnel from maintenance including instruments and equipment maintenance personnel
6. Administrative personnel in various categories

ANNEX V. LIST OF LAND, BUILDINGS AND FACILITIES IN THE BIOMEDICAL RESEARCH CENTRE AND PILOT AREA

1. Land
2. Buildings
   (A) Administrative building
      (a) Director's Room
      (b) Team Leader's Room
      (c) Expert's Room
      (d) Office
   (B) Laboratory
   (C) Animal House
3. Facilities
   (A) Store Room
   (B) Garage
   (C) Shower and Washing Room
   (D) Other necessary facilities
4. Pilot Area

Note: The site of pilot area for the application of the achievement of the research for the control of major arbo-viral diseases, bacterial enteric diseases will be chosen by the Coordinating Committee.

ANNEX VI. COMPOSITION OF THE COOPDINATING COMMITTEE

Deputy Minister, Ministry of Health Chairman

Director-General, Department of Medical Research	Vice-Chairman
Japanese Team Leader	Member
Japanese Experts	Member
Deputy Directors/Assistant Directors	Member
Heads of Division	Member
Representative of the Department of Health	Member
Official of the Japanese Embassy	Observer

N.B. One of the members of the Committee will act as Secretary.

The Japanese Evaluation Team and Director-General, Ministry of Health have jointly formulated, for reference to the supplementary Document to the Record of Discussions Between the Japanese Implementation Survey Team and the authorities concerned of the Government of the Socialist Republic of the Union of Burma on the Japanese Technical Assistance for the "Research on major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of these diseases", the Tentatively Estimated Scale of the Project as annexed hereto.

Head of the Japanese Evaluation Team	Director General Department of Medical Research Ministry of Health

ANNEX: TENTATIVELY ESTIMATED SCALE OF THE PROJECT

Number of the Japanese Experts: about 20 man/month per year

Amount of Machinery, Equipment: about 40 million yen per year
and Materials

Total amount : about 100 million yen for two years.

Note: The above-mentioned scale is subjected to conditions that necessary budget will acquired for the implementation of the Project.

供与機材、備品の運営状況

本プロジェクトにもとづく技術協力として日本政府よりビルマ生物医学研究センターに供与された膨大な機材設備備品の使用状況ならびにその管理運営面について実地に検べた結果、一、二の小さなトラブルを除いては概ね理想的な運営がなされていることが確認された。

なかでも各部局の研究進展がうまくいっている大きな理由の一つに、良質の水が使用出来るようになったということである。研究する上で、水質によるデータの影響は想像以上に大きいものであるが、この生物医学研究センターの屋上に設置された大型水槽の水（水道水と地下水混合）は、ビルマ国内では最良質（大腸菌数がもっとも少く、有機、無機物質が極微量）のものであり、実験に必要な純水や、蒸溜水の良質のものが使用出来ていることは理想的である。

一、二の小さいトラブルとは、①各室に設けられた冷房装置の排水管の不備のため、とくに高湿季節には、床に溢れて精密器械の破損を招いている。このような現象は日本では考えも及ばないことであるが、これが熱帯高湿地方特有のことであるので、対策が必要である。

②動物センターの焼却爐。これは明らかに場所設定と工事ミスである。焼却爐の床コンクリートが雨季では低いので（乾季に造設されたもの）、雨季には溢れた水が床上１５cmまで溜るので、ガス管、油バーナーなど１年で完全腐蝕して、現在使用不能。

専門家指導上の障害

日本人派遣専門家は、超一流のエキスパート（それだけに皆さん物凄く多忙の人）を送っているのであるし、現地では指導を目的として赴任しているのであるが、肝腎の携行機材が使えない（未着、遅着が多い）という、もっともあってはならないことが、しばしば発生している。これは以後、絶対このようなことがないよう強く改善を要求する。携行機材は専門家の到着前に、すでに現地で、税関もクリアされ、直ちに使えるような早目の配慮がなければならない。

6. 結　　論

調査団は今回の調査を通じて以下に述べるような評価結論を得た。

1. 本医療協力は実施調査の際に締結されたR / Dにもとづいて順調に進展していることを認める。とくに生物医学研究センター内での活気ある研究成果は国際医療協力のすぐれたモデルとなり将来のより一層の発展が約束されているものと評価した。また同センターにおいて度重なる国際会議などが開催されている事実も、協力国としても喜ばしい状態と考えられる。これらの素晴しい成果は過去2カ年の短時日に長足の進歩をみたものと思われたが、未だすべて緒についた許りのものであり、さらに本プロジェクトによる協力の延長されることが望まれる。
2. 本プロジェクトの期間中は一層の、日本人専門家の派遣（年20 man month）ならびに可及的多くのビルマ人研修生の日本への受入れが望まれる。日本人専門家派遣は極めて大切な要素であり英語会話による抜群の指導力を有する人の派遣が望ましい。
3. 今後の供与機材には、重要なスペアパーツの送付も考慮に入れる必要がある。

THE SUPPLEMENTARY DOCUMENT

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The Japanese Evaluation Team (hereinafter referred to as "the Team") organized by the Japan International Cooperation Agency (hereinafter referred to as JICA) and headed by Professor Yoshihiro Hamashima, Faculty of Medicine, Kyoto University visited the Socialist Republic of the Union of Burma from 17th February to 24th February 1982 for the purpose of reviewing the results of the Project as well as working out details of extension of the technical cooperation programme concerning "Research on Major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of these diseases" project in the Socialist Republic of the Union of Burma.

During its stay in the Socialist Republic of the Union of Burma, the Team exchanged views and had a series of discussions with the Burmese authorities concerned in respect of the desirable measures to be taken by both Governments for extension of the above-mentioned Project.

As a result of the discussions, the Team and the Burmese authorities concerned agreed to recommend to their respective Governments the matters referred to in the document attached hereto.

Head of the Japanese
Evaluation Team

Director General
Department of Medical Research
Ministry of Health

THE ATTACHED DOCUMENT

I. COOPERATION BETWEEN BOTH GOVERNMENT

1. The Government of Japan and the Government of the Socialist Republic of the Union of Burma will cooperate with each other in implementing "Research on major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of these diseases" project (hereinafter referred to as "the Project") for the purpose of extending the biomedical research against these diseases and of developing the function of the Biomedical Research Centre as the central institute undertaking laboratory and other services in support of biomedical research on these diseases in Burma.

   The Project aims to contribute to the control of those diseases with knowledge and experience acquired from the research and thus to promote the health conditions in the Socialist Republic of the Union of Burma.

2. The Project will be implemented in accordance with the Master Plan which is attached as Annex I.

II. DISPATCH OF JAPANESE EXPERTS

1. In accordance with the laws and regulations in force in Japan, the Government of Japan will take necessary measures through JICA to provide at its own expense services of the Japanese experts as listed in Annex II through the normal procedures under the Colombo Plan Technical Cooperation Scheme.

2. The Japanese experts referred to in paragraph 1 above and their families will be granted in the Socialist Republic of the Union of Burma the privileges, exemptions and benefits within the framework of the Colombo Plan Technical Cooperation Scheme.

III. PROVISION OF MACHINERY AND EQUIPMENT

1. In accordance with the laws and regulations in force in Japan the Government of Japan will take necessary measures through JICA to provide at its own expense such machinery equipment and materials necessary for the implementation of the Project as mentioned in Annex III, through the normal procedures under the Colombo Plan Technical Cooperation Scheme.
2. The articles referred to in paragraph 1 above will become the property of the Government of the Socialist Republic of the Union of Burma upon being delivered to the Burmese authorities concerned at the ports and airports of disembarkation, and will be utilized exclusively for the implementation of the Project under the direction of the Co-ordinating Committee referred to in paragraph 2 of Clause VI hereunder.

IV. TRAINING OF BURMESE PERSONNEL IN JAPAN

1. In accordance with the laws and regulations in force in Japan, the Government of Japan will take necessary measures through JICA to receive at its own expense the Burmese personnel connected with the Project for technical training in Japan through the normal procedures under the Colombo Plan Technical Cooperation Scheme.

2. The Government of the Socialist Republic of the Union of Burma will take necessary measures to ensure that the knowledge and experience acquired by the Burmese personnel from technical training in Japan will be utilized effectively for the implementation of the Project.

V. MEASURES TO BE TAKEN BY THE GOVERNMENT OF THE SOCIALIST REPUBLIC OF THE UNION OF BURMA

1. In accordance with the laws and regulations in force in the Socialist Republic of the Union of Burma, the Government of the Socialist Republic of the Union of Burma will take necessary measures to provide at its own expense:

(1) Services of the Burmese counterpart personnel and administrative personnel as listed in Annex IV;

(2) Land, buildings and facilities in the Biomedical Research Centre and pilot area as listed in Annex V;

(3) Supply or replacement of machinery, equipment, instrument, vehicles, tools, spare parts and materials necessary for the implementation of the Project other than those provided through JICA under III above;

(4) Transportation facilities and travel allowance for the Japanese experts for the official travel within the Socialist Republic of the Union of Burma;

(5) Suitably furnished accommodations for the Japanese experts and their families.

2. In accordance with the laws and regulations in force in the Socialist Republic of the Union of Burma, the Government of the Socialist Republic of the Union of Burma will take necessary measures to meet:

(1) Expenses necessary for the transportation within the Socialist Republic of the Union of Burma of the articles referred to in clause III above as well as for the installation, operation and maintenance thereof;

(2) Customs duties internal taxes and any other charges, imposed in the Socialist Republic of the Union of Burma on the articles referred to in clause III above;

(3) All running expenses necessary for the implementation of the Project.

VI. ADMINISTRATION OF THE PROJECT

1. The Japanese experts will give necessary technical guidance and advice to the Burmese staff associated with the Project pertaining to the implementation of the Project, and the Burmese authorities concerned will be responsible for the administrative and managerial matters pertaining to the Project.

2. For successful implementation of the Project, the Coordinating Committee will be established with the members as listed in Annex VI. The Committee will meet at least once a year.

The Functions of the Committee are as follows:

(1) To formulate plan for the Project;

(2) To review the implementation of the Project;

(3) To co-ordinate the activities relating to the implementation of the Project.

VII. CLAIMS AGAINST JAPANESE EXPERTS

The Government of the Socialist Republic of the Union of Burma undertakes to bear claims, if any arises, against the Japanese experts engaged in the Project resulting from, occuring in the course of, or otherwise connected with the discharge of their official functions in the Socialist Republic of the Union of Burma except for those arising from the willful misconduct or gross negligence of the Japanese experts.

VIII. MUTUAL CONSULTATION

There will be mutual consultation between the two Governments on any major issues arising from, or in connection with this Attached Document.

IX. TERM OF COOPERATION

The duration of the technical cooperation for the Project under this Attached Document will be two years from 10 April 1982, unless otherwise agreed between the two Governments.

ANNEX I. MASTER PLAN OF THE PROJECT

1. Objective

The Project aims to conduct Research on major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of these diseases.

2. Implementation

The Department of Medical Research has overall responsibilities for the Project with the guidance of the Coordinating Committee. The Biomedical Research Centre which is an integrated functional component of the said Department is the executing organ for the achievement of the above mentioned objective.

3. Activities under the Project

Activities under the Project will be carried out at the Biomedical Research Centre premises including the pilot area:

Activities will include the following:

(a) Research on major arbo-viral diseases and the application of its achievement for their control.

(b) Research on major bacterial enteric diseases and the application of its achievement for their control.

(c) To further develop technology of laboratory and other services.

ANNEX II. JAPANESE EXPERTS

1. Team Leader (who may be one of the experts)

2. Experts

In Laboratory technology and Animal care

Bacteriology

Pathology

Virology

Immunology

Other related fields mutually agreed upon as necessary

ANNEX III. ARTICLES TO BE PROVIDED BY THE GOVERNMENT OF JAPAN

Machinery, equipment and materials for the research on major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of these diseases.

ANNEX IV. BURMESE PERSONNEL

1. Director-General

2. Deputy Directors/Assistant Directors

3. Heads, Researchers and Laboratory Technicians

   In Immunology and Immunochemistry

   Bacteriology

   Epidemiology

   Virology

   Pathology

4. Infectious Diseases control personnel

5. Other Technical supportive personnel from maintenance including instruments and equipment maintenance personnel

6. Administrative personnel in various categories

ANNEX V. LIST OF LAND, BUILDINGS AND FACILITIES IN THE BIOMEDICAL RESEARCH CENTRE AND PILOT AREA

1. Land

2. Buildings

(A) Administrative building

(a) Director's Room

(b) Team Leader's Room

(c) Expert's Room

(d) Office

(e) Others

(B) Laboratory

(C) Library

(D) Animal House

3. Facilities

(A) Store Room

(B) Garage

(C) Shower and Washing Room

(D) Other necessary facilities

4. Pilot Area

Note: The site of pilot area for the application of the achievement of the research for the control of major arbo-viral diseases, bacterial enteric diseases will be chosen by the Coordinating Committee.

ANNEX VI. COMPOSITION OF THE COORDINATING COMMITTEE

Deputy Minister, Ministry of Health	Chairman
Director-General, Department of Medical Research	Vice-Chairman
Japanese Team Leader	Member
Japanese Experts	Member
Deputy Directors/Assistant Directors	Member
Heads of Division	Member
Representative of the Department of Health	Member
Official of the Japanese Embassy	Observer

N.B. One of the members of the Committee will act as Secretary.

The Japanese Evaluation [redacted] Team and Director-General, Ministry of Health have jointly formulated, for reference to the supplementary Document to the Record of Discussions between the Japanese Implementation Survey Team and the authorities concerned of the Government of the Socialist Republic of the Union of Burma on the Japanese Technical Assistance for the "Research on major arbo-viral diseases, bacterial enteric diseases and the application of its results for the control of these diseases", the Tentatively Estimated Scale of the Project as annexed hereto.

Head of the Japanese
Evaluation Team

Director General
Department of Medical Research
Ministry of Health

ANNEX: TENTATIVELY ESTIMATED SCALE OF THE PROJECT

Number of the Japanese Experts: about 20 man/month per year

Amount of Machinery, Equipment and Materials: about 40 million Yen per year

Total amount :-about 100 million yen for two years.

Note: The above-mentioned scale is subjected to conditions that necessary budget will acquired for the implementation of the Project.

THE SUPPLEMENTARY DOCUMENT

TO THE RECORD OF DISCUSSIONS BETWEEN THE JAPANESE IMPLEMENTATION SURVEY TEAM AND THE AUTHORITIES CONCERNED OF THE GOVERNMENT OF THE SOCIALIST REPUBLIC OF THE UNION OF BURMA ON THE JAPANESE TECHNICAL COOPERATION FOR THE PROJECT-

"RESEARCH ON MAJOR ARBO-VIRAL DISEASES, BACTERIAL ENTERIC DISEASES AND THE APPLICATION OF ITS RESULTS FOR THE CONTROL OF THESE DISEASES" SIGNED ON 10th APRIL 1980

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