b. Methods

b.1 Epidemiological Methods

b.1.1 Urban Community Survey

The studies were carried out in an urban community of North Okkalapa Township. The township comprised of 17 wards, of which 12 were selected to represent the main urban areas of the township. A total of 24 electrical units, two from each of the 12 wards were randomly selected (using random numbers).

The epidemiological study was conducted on a sub-sample of 7 electrical units which were taken randomly from the total sampled units during the dry season. However, in the wet season survey, no sub-sample was taken; instead, all electrical units initially sampled were included for the study.

Study Population

The main objective of the study was to determine the morbidity of acute diarrhoea in children under five years of age. The study included all children in the sampled electrical units who were under five years age at the time of survey. For this purpose, a census was taken prior to the actual survey in each season. All children under five years age in each electrical unit were numbered in a chronological order for identification and for future reference. A total of 407 and 1545 under-five children were studied in the dry and wet seasons respectively.

Survey Methods and Procedures

A standard survey form was designed to obtain formation on the demographic, socio-economic, environmental and other features of each household in 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 subsample were recorded for a recall period of one week on every Monday of the week. However, in the wet season survey, daily recording of diarrhoea occurrence among the family members was carried out from Monday to Friday, and recalls for

Saturday and Sunday were made on the following Monday of the weekend.

The information was collected in both seasons by field staff from the Clinical Research and Epidemiology Research Divisions of the Department of Medical Research, who made daily household visits from Monday through Friday in each of their assigned electrical units. All of these field staff were given a short training for proper information collection and recording. Instruction sheets were also provided to guide them. The definition of diarrhoea used was: "an increase in the frequency of stools (i.e., more than three times/day) end/or an increase in the liquidity of stools. An interval of 24 hours was designated to divide one episode from another (Newell, 1965).

b.1.2 Rural Community Survey

Selection of a suitable rural community for study, although it seemed to be easy, was found to be rather difficult in practice. The foremost difficulty encountered was the definition of a typical rural community. What really constitutes a rural community? On what criteria will a community be chosen as representing typical rural area? After much consideration, it was agreed that a rural community is defined from the following attributes:

(i) major livelihood in the population under study should be by farming;

(ii) source of water supply should not be pipe-water or from tube wells, but water supply should be from surface wells, tanks and/or streams;

(iii) sanitation facilities should be of surface or pit latrine type, or nil,

(iv) type of dwelling should be mostly thatched-roofed, thatched-walled houses;

(v) the area should not be on a main road; and,

(vi) there should be no Rural Health Centre or Station Hospital in the villages studied. There should be a Rural Health Centre within a 50 mile radius of the villages studied.

Besides these criteria, it was also considered necessary to take into account a number of other criteria which were essential

for smooth execution of the study. These were:-

(i) the study area should be so located as to facilitate easy transportation of stool specimens;

(ii) the study area should have an adequate number of under-five children for epidemiological and aetiological study; and

(iii) the villages chosen should not be far apart from one another so that field supervision is not hindered.

With these criteria in view, a number of village tracts within Hlegu, Hmawbi and Pegu townships were visited between March and May, 1982. In the Hlegu township, village tracts within the jurisdiction of Indaing, Lay-daung-kan, Gyogon, Sa-bu-daung and Kale-taw Rural Health Centres were visited. Similarly, village tracts within Shwe-Hlay-Gyi and Myaung-Da-Ga Rural Health Centre Areas in Hmawbi township, and village tracts within Intakaw, Paya-gyi and Pyin-Bon-Gyi Rural Health Centre Areas in Pegu township were visited and feasibility for study considered. Finally, three village tracts in the Intakaw Rural Health Centre area within Pegu township were chosen which met the above criteria. All under-five children residing in the study villages were taken for study. There were 715 under-five children among a population of over 5,000 residing in nearly 490 households in these three village tracts comprising of 15 hamlets/villages.

Training of personnel

Training included explanation and practical exercises. Explanation about the purpose of the survey, the study area, schedules, etc., was done followed by discussion. Oral and written practical exercises were carried cut to see if field staff understood the survey procedures. Constant inspection and supervision was made by one field co-ordinator who accompanied the field workers in turn every day. He checked the work of the workers and ensured that field procedures were followed.

Location of the study area

The study area is situated within the jurisdication of Intakaw Rural Health Centre which is within Pegutownship. The study villages lie along a small road leading from Intakaw to

Htone-gyi. The distances of the study villages from the road ranges from several yards to 1-2 kilometres. These villages are surrounded by paddy fields and orchards. There were 15 villages in the study: Pyin-ma-ngu, Moe-kwe and Kye-phe-la villages belong to Pyin-ma-ngu village tract: Nga-gyi-inn, Paya-noak-to, Ah-nyar-su, Ah-shay-gon and Shan-gon villages to Paya-noak-to village tract, and La-tha-gon, Chan-tha-gon, Tha-byay-gon, Mon-daing, Chaung-ywa, Mai-thi-la-kan and Taung-mo-lone villages to La-Tha-Gon village tract.

Socio-economic aspects

Majority of the people in the study villages were Burmese. Karens made up the second largest ethnic group. Half-casts and other ethnic group were few. Only a small number were illiterate: most mothers were educated up to primary school level, and the majority of fathers had either primary or secondary level education.

Majority of fathers were agricultural or orchard wokers. Most mothers had no occupation. A few mothers worked part-time as cheroot-rollers. The median monthly family income was K 370/-- which was only a little more than that of their urban counterparts in the North Okkalapa study.

Environmental health aspects

About 86% of study households had family size between 3 - 8 persons, and only 14% had family members numbering 9 persons or more.

Their principal drinking water was obtained from dug wells (74%), and only 4% used rain water for drinking.

About 31% had no latrines in their households. Among those households which has latrines, surface latrines were usually used.

Age-sex distribution of study population

Age and sex distribution of under-five children in monsoon and winter surveys are given in Table 4. Male and female children are almost equally distributed in all age-groups. Also, the

Table 4. Distribution of under five years children by age and sex in Indakaw village (Monsoon and Winter Survey)

Survey \ Age in months	Sex	0-5 months	6-11 months	12-23 months	24-35 months	36-47 months	48-59 months	TOTAL
MONSOON	Male	42 (11.3)	43 (11.6)	68 (18.3)	79 (21.2)	74 (19.9)	66 (17.7)	372 (100%)
	F.M	42 (12.2)	51 (14.9)	66 (19.2)	67 (19.5)	62 (18.4)	54 (15.7)	343 (100%)
	TOTAL	84 (11.8)	94 (13.2)	134 (18.7)	146 (20.4)	137 (19.2)	120 (16.8)	715 (100%)
WINTER	Male	27 (7.16)	52 (13.79)	74 (19.62)	72 (19.09)	82 (21.75)	70 (18.56)	377 (100%)
	F.M	26 (7.32)	51 (14.36)	96 (27.04)	58 (16.33)	70 (19.71)	54 (15.21)	355 (100%)
	TOTAL	53 (7.24)	103 (14.07)	170 (23.22)	130 (17.75)	152 (20.76)	124 (16.93)	732 (100%)

NOTE: The numbers in parenthesis represent the percentage in row.

distribution of under-five children by age were quite similar in the two seasons, and the total number of children remained almost the same.

b.2 Bacteriological Methods

Identical bacteriological investigative procedures were employed for monsoon and winter surveys in both urban and rural community studies. Thus, bacteriological results obtained are not only comparable between different seasons in the same (urban or rural) community, but also comparable between different communities for the same seasonal conditions.

Collection of stool specimens

Stools were collected by field staff from the Clinical Research and Epidemiology Research Divisions of the Department of Medical Research. Sterile swab sticks were used for collection, and specimens were transported in sterile bottles containing Gary-Blair transport media. These stool specimens were transported in ice boxes to reach the laboratory for immediate processing within 24 hours after collection from the child by its parent/guardian (N.B. NOT within 24 hours after collection of specimen bottle by the field worker).

In the urban community study, 139 diarrhoea stools and 205 control stools were collected in the winter survey, and 217 diarrhoea stools and 103 control stools were collected in the monsoon survey. In addition, in a hospital-based study of patients draining the same community, 104 and 80 stools, respectively, from diarrhoea and age-matched control patients during the winter survey, and 40 stool samples each from diarrhoea and age-matched control patients during the monsoon survey, were also investigated for bacterial agents.

In the rural community study, 330 diarrhoea and 233 control stool specimens in the monsoon survey, and 171 diarrhoea and 141 control stool specimens in the winter survey were investigated for bacterial agents.

Bacteriological laboratory techniques

All stool swabs were plated directly onto MacConkey's agar, S-S (Salmonella-Shigella) agar, XLD (Xylose-lysine-dextrose) agar, TCBS (Thio-sulphate citrate bile-salts sucrose) agar, and Campy Bap media (see flow chart).

In addition, part of each specimen was enriched in Selenite F broth and alkaline peptone water, and processed by inoculation onto solid media for isolation after appropriate incubation. All these plates were examined for Salmonella, Shigella and Vibrios by standard methods.

Salmonella-like and Shigella-like colonies were further characterized by biochemical and serotypic properties. Vibrio-like colonies were further identified by biochemical, serotypic and salt tolerance properties.

For *Escherichia coli* isolation, 3 lactose-fermenting colonies with typical *E. coli* morphology were picked from among the colonies grown from each stool sample on MacConkey agar. Each colony was individually labelled for reference, and confirmed to by *E. coli* by biochemical characterization. The isolates thus confirmed to be *E. coli* were inoculated in agar slants and maintained as stock cultures for further investigations (eg., serotyping, toxin production).

For identification of Enterotoxigenic *E. coli* (ETEC), the *E. coli* isolate in 1.5 ml trypticase soy broth (BBL) was grown with vigorous agitation at 37°C overnight; the broth was centrifuged at 10,000 rpm for 30 minutes. The supernatant fluid with gentamycin 50 ug/ml was used for both heat-labile (LT) toxin and heat-stable (ST) toxin assays.

For ST assay, the method used was as described by Dean et al. (1972) with slight modification as in WHO protocol (1982, Section 6.3).

For LT assay, Chinese Hamster Ovary Cell assay as described by Guerrant et al., (1974) with modification of Honda et al. (1976) was used. Biken test was done according to the method of Honda et al. (1981).

For identification of Enteropathogenic *E. coli* (EPEC), *E. coli* isolates maintained in agar slants as stock cultures were tested by

slide agglutination employing commercially available *E. coli* antisera (Toshiba Co., Japan).

For preliminary isolation and indentification of *Campylobacter fetus jejuni*, Campy Bap media was used, and the plates were incubated in candle extinction jar at 42°C for 48 hours. The colonies resembling Campylobacter were characterized by Gram reaction, oxidase and catalase tests, motility and growth in air at 37°C.

Yersinia enterocolitica was identified by cold enrichment of the stool specimen in M/15 phosphate buffered saline at 4°C for 3 weeks, the plated onto MacConkey's and S-S agars, and incubated at 25°C. Further biochemical characterization was done on colonies resembling *Y. enterocolitica*.

Since it was not possible to collect and plate stool samples within two hours of defaecation for isolation of Shigella species, plating of stool samples on S-S agar at the field was not done.

b.3 Virological Methods

Collection of stool specimens

Stools were collected in labelled screw-capped containers either directly whenever possible, or otherwise swabs which were then placed in the container and broken off briskly, and then screw-capped. Stool specimens were kept at ambient temperature and brought to the laboratory within the shortest available time, immersing the stool specimens in ice if transport time was prolonged beyond four hours.

Virological laboratory techniques

In the urban community survey, stools (diarrhoea and control) were examined by electronomicroscopy. Owing to defective Electronmicroscope, rotavirus was not detected at all. A subsample of (78 diarrhoea and 34 control) stools were tested by Rotazyme (ELISA) assay for presence of rotavirus.

In the rural community survey, a stratified random sampling (stratified for each age group) of diarrhoea and control stool specimens was made. 166 diarrhoea and 76 control stool samples from the monsoon survey, and 114 diarrhoea and 46 control samples from the winter survey were tested for presence of rotavirus using

RPHA (reverse passive immune haemolysis assay) technique (using Rotacell kits). A random subsample of 77 specimens from the 166 diarrhoea stools of the monsoon survey were also tested by ELISA (WHO ELISA kits for rotavirus) to validate RPHA results.

b.4 Parasitological methods

Stool samples were examined on arrival on the same day under light microscopy for the following parasites:-

Protozoa: *Entamoeba histolytica* trophozoites and/or cysts;
Entamoeba coli trophozoites and/or cysts;
Giardia lambdlia
Trichomonas hominis

Helminths: *Ascaris lumbricoides* ova (fertilized)
Enterobias vermicularis ova
Trichuris trichura ova
Necator americanus ova
Ankylostoma duodenale ova
Strongyloides stercoralis filiform larvae

These investigations were carried out knowing well the existence of limitations of light microscopy and the viability of protozoa on prolonged standing of stools under room conditions.

c. Results

c.1 Urban Community Results

c.1.1 Epidemiological Research Findings

The overall incidence of acute diarrhoea in children under five years were 16.7 and 20.3 per 100 under-five children for winter and monsoon respectively, and the seasonal difference was not statistically significant. The average yearly incidence per child per year for the two seasons were 1.8 and 2.4 (Table 5).

The age-specific incidence for the two seasons showed (in Table 5) higher incidences of diarrhoea in the age groups 0 - 11, 12 - 23 and 24 - 36 months in the wet season, and this was statistically, significantly ($P<0.05$) higher than corresponding rates for the above age-group children in the dry season.

Male children had a higher preponderance of diarrhoea than female children in almost all age-groups, the difference being more evident in the wet season. This difference was not statistically significant in any age-group.

Environmental factors

Table 6 shows the distribution and incidence of acute diarrhoea by drinking water source among the study populations in two seasons. Pipe and tube well water were the principle sources of drinking water in both dry (76%) and wet seasons (95%). Use of rain water during the wet season was increased as expected, but only slightly so (2%). Use of shallow wells and ponds ranged between 2% and 23% in both seasons.

Diarrhoea incidence per 100 household was highest among users of rain water (66.7), followed by pipe (26.4), artesian (19.6) and other sources (16.7) in the dry season. In the wet season, diarrhoea incidence in descending frequency was observed in pipe (35.4), rain water (32.4), artesian (15.9) and other sources (0). An increase in the incidence of diarrhoea in the wet season was observed only among users of pipe water (from 26.4 to 35.4 per 100 households). The reverse was found to be true for users of water from all other sources.

Table 7 shows relationship of diarrhoea incidence to type of domestic animal reared among the study populations in the two seasons. Differences in the incidence of diarrhoea among those who reared animals and those who did not were quite considerable in the dry season (25.0 versus 15.4), but negligible in the wet season (35.2 versus 34.5).

Relationship of diarrhoea with socio-economic status

The incidence of diarrhoea was closely related to family income, family's social class and crowding index. Diarrhoea incidence decreased as the family income improved, and the difference in diarrhoea incidence between lower and higher income groups was statistically significant ($P < 0.01$; Table 8, Figure 4).

Table 5. Incidence[@] of acute diarrhoea by age and sex in two seasons, North Okkalapa, Rangoon, 4 and 5 week periods beginning 4.1.81 and 21.7.81 respectively

(Months)	Dry season (Jan)			Wet season (Jul-Aug)		
	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(312)
48 - 59	15.6(45)	7.9(38)	12.0(83)	12.3(106)	11.4(158)	11.7(264)
Unknown	-	-	-	- (3)	- (1)	- (4)
Total	20.5(205)	12.9(202)	16.7(407)	22.1(763)	18.5(782)	20.3(1545)
Average* yearly incidence	1.8			2.4		

@ Rates per 100 < 5 population 5 population in parentheses

* Episodes per child per year

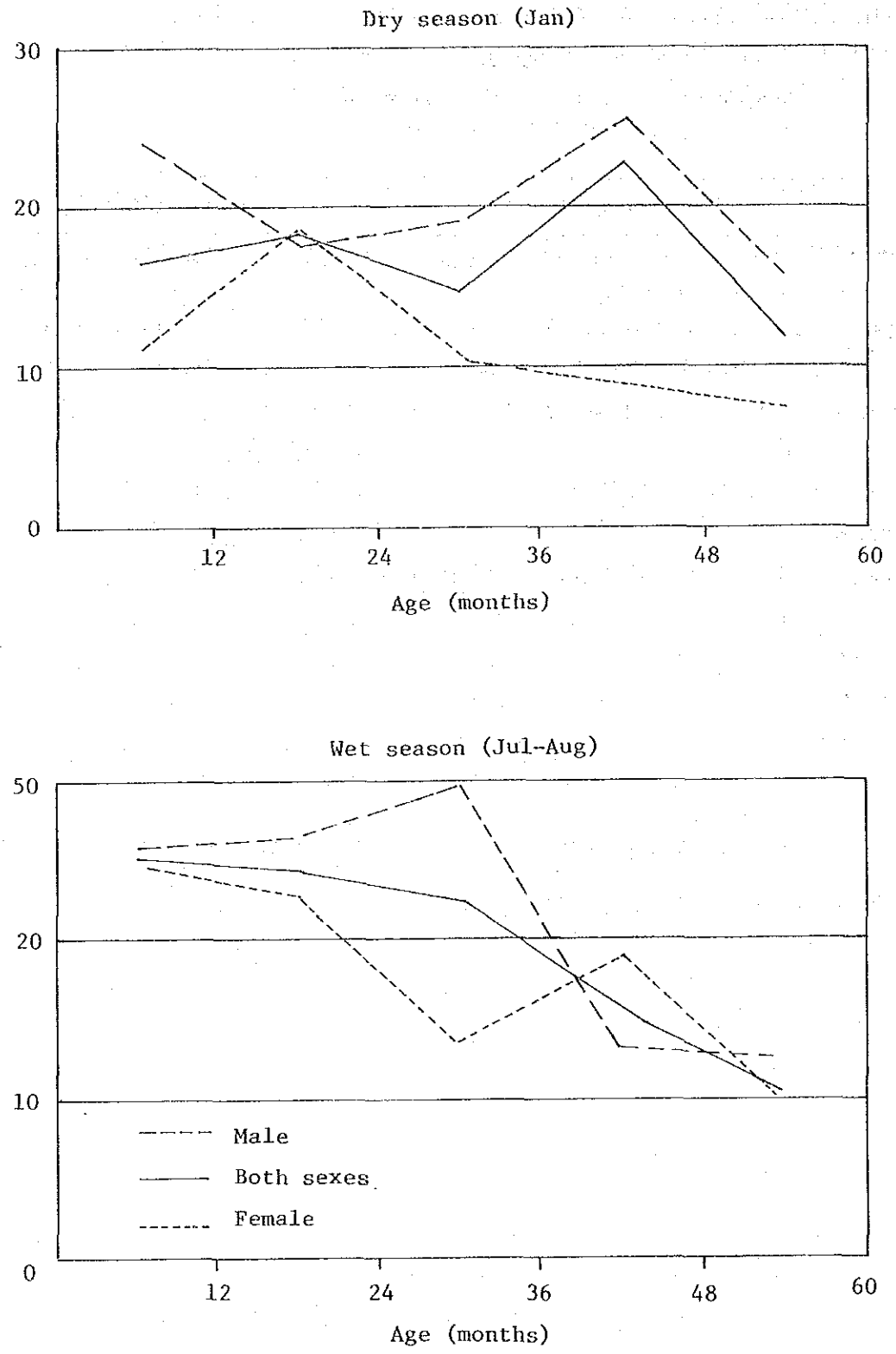

Figure 3. Incidence of acute diarrhoea by age and sex in two seasons, North Okkalapa, Rangoon 4 and 5 week periods beginning 4.1.81 and 21.7.81 respectively

Table 6. Distribution and incidence of acute diarrhoea by drinking water source among the study populations in two seasons, North Okkalapa, Rangoon, 4 and 5 week periods beginning 4.8.81 and 21.7.81 respectively

Drinking Water source	Dry season (Jan)		Wet season (Jul-Aug)	
	% Distribution per household	Incidence per 100 households	% Distribution per household	Incidence per 100 households
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)	- (14)
Total	100.0 (287)	22.3 (287)	100.0 (932)	33.8 (1054)*

@ well, pond

Number of households in parentheses

* includes one unknown

Table 7. Distribution and incidence of acute diarrhoea by the type of domestic animal reared among the study populations in two seasons, North Okkalapa, Rangoon, 4 and 5 week periods beginning 4.1.81 and 21.7.81 respectively

Rearing of Domestic animals	Dry season (Jan)		Wet season (July-Aug)	
	% Distribution per household	Incidence per 100 households	% Distribution per household	Incidence per 100 households
Yes	51.0 (176)	25.0 (176)	59.0 (589)	35.2 (463)
No	49.0 (169)	15.4 (169)	40.9 (408)	34.5 (534)
Unknown	-	-	0.1 (1)	0.0 (1)
Total	100.0 (345)	21.2 (345)	100.0 (998)	34.8 (998)

Number of households in parentheses

Table 8. Incidence of acute diarrhoea by family income, North Okkalapa, Rangoon, 5 week period beginning 21.7.81 (Wet season Jul-Aug)

Income (kyats)	Episodes	Children under 5	Incidence per 100 children
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
Total	314	1,545	20.3

Statistically significant

* $P < 0.001$

** $P < 0.01$

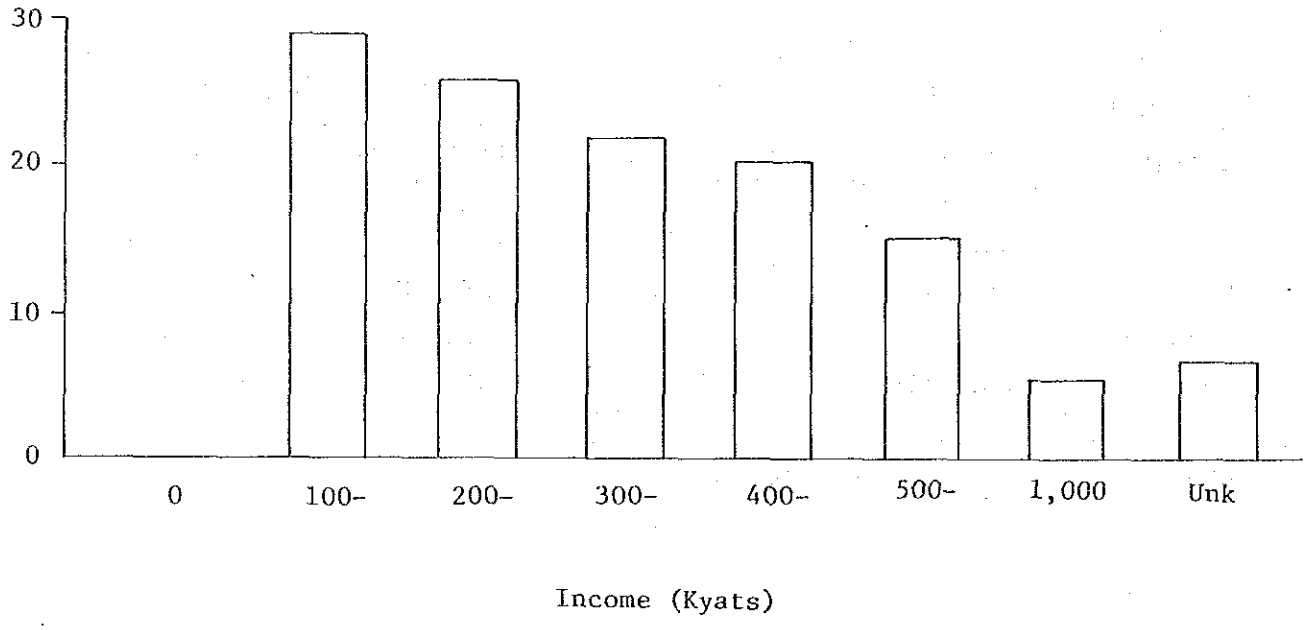

Figure 4. Incidence of acute diarrhoea by family income, North Okkalapa Rangoon, 5 week period beginning 21.7.81 (Wet season-July/Aug)

Table 9. Incidence of acute diarrhoea by fathers' occupation, North Okkalapa, Rangoon, 5 week period beginning 21.7.81 (Wet season Jul-Aug)

Social[@] class	Episodes	Children under 5	Incidence per 100 children
I & II	13	97	13.4
III	107	501	21.4*
IV	77	355	21.7*
V	104	474	21.9*
X	11	111	9.9
Unknown	2	7	28.6
Total	314	1,545	20.3

@ According to occupation (Benjamin, B. 1968)

* Statistically significant $p < 0.05$

I Professional, technical and related workers

II Administrative and managerial workers

III Production and related workers, transport equipment operators, clerical and related workers, service workers and members of armed forces

IV Agricultural, animal husbandary and forestry workers, fisher-man, hunters and sales workers

V Labourers

X Workers not classified by occupation

Table 10. Incidence of acute diarrhoea by crowding index, North Okkalapa, Rangoon, 5 week period beginning 21.7.81 (Wet season Jul-Aug)

Crowding index	Episodes	Children under 5	Incidence per 100 children
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	999	18.2*
6 - 6.9	17	54	31.5
7	21	64	32.8
Unknown	-	4	-
Total	314	1,545	20.3

* Statistical significant ($p < 0.05$)

Table 11. Calculation of expected ETEC isolates in the total study population from the sample ETEC isolates, North Okkalapa, Rangoon, January 1981 (Dry season)

Age (months)	Specimen episodes		Actual episodes in community	Expected* ETEC (+) episodes in community	Total under 5 children	Incidence** per 100
	ETEC (+) episodes	Total episodes				
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	3.5

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

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

Table 12. Calculation of expected ETEC isolates in the total study population from the sample ETEC isolates, North Okkalapa, July-August 1981 (Wet season)

Age (months)	Specimen episodes		Actual episodes in community	Expected* ETEC (+) episodes in community	Total under 5 children	Incidence** per 100
	ETEC (+) episodes	Total episodes				
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	1,545	6.5

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

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

Table 13. Days of diarrhoea experienced and mean duration (days) of diarrhoea of children under 5 years, North Okkalapa, Rangoon, 5 week period beginning 21.7.81 (Wet season Jul-Aug)

Age (Months)	Days with diarrhoea	Mean duration of diarrhoea per sick child	Mean duration of diarrhoea per episodes
0 - 11	240	3.9 (62)[1]	3.0 (80)[2]
12 - 23	227	3.7 (62)	2.8 (80)
24 - 35	241	4.5 (54)	3.3 (73)
36 - 47	121	3.6 (34)	2.4 (50)
48 - 59	85	2.8 (30)	2.7 (31)
Unknown	-	-	-
Total	914	3.8 (242)	2.9 (314)

1 Sick children

2 Episodes

A similar but less marked relationship was noticed between diarrhoea incidence and social class, the incidence of acute diarrhoea being higher in children from families in the lower social classes (i.e., Social Classes III, IV and V, $P < 0.05$) (Table 9).

The incidence of diarrhoea in children also increased in proportion to increased crowding index (Table 10).

Relationship of diarrhoea incidence with ETEC

Table 11 and Table 12 compare the overall diarrhoea incidence with age-specific ETEC positive diarrhoea cases. Expected ETEC isolates for the sampled population were calculated from ETEC isolates obtained from the diarrhoea stool specimens tested. There was a proportional increase in ETEC isolates in the age group 12-23 months during the wet season, which coincided with overall increase of diarrhoea incidence in that age-group. This was not found in the dry season.

Duration of diarrhoea

Table 13 shows days of diarrhoea and mean duration of diarrhoea in children under five years in the wet season. Children under three years had the longest duration (days) of diarrhoea (per sick child and per episode), and they were also more ill from diarrhoea than children three to five years. The mean duration of diarrhoea per sick child and the mean duration of diarrhoea per episode for different age groups range, respectively, from 2.8 to 4.5, and from 2.4 to 3.3.

c.1.2 Bacteriological Research Findings

Major bacterial pathogens were identified in 29.5% of diarrhoea stools in winter, and 56.7% of diarrhoea stools in the monsoon survey (compared to 23.4% and 42.7% from control stools in winter and monsoon respectively) (Table 14).

ETEC were important bacterial pathogens associated with acute diarrhoea in 10 - 12% of stools from diarrhoea patients in the urban community (Table XI), and 8.7 to 12.5% of stools from diarrhoea patients in North Okkalapa General Hospital (Table 15).

EPEC were isolated from diarrhoea and control stools almost equally frequently, in both winter and monsoon surveys (Table 14).

In the urban community, Shigella was an important cause of diarrhoea in monsoon but not in winter (Table 14). Salmonella, Vibrio and Campylobacter were not important pathogens by virtue of very low rates of isolation in the monsoon survey, and no isolation at 11 during the winter survey. Yersinia and *V. parahaemolyticus* were not isolated at all in both winter and monsoon surveys (Table 14).

In approximately 19% of diarrhoea cases, more than one aetiologic agents were isolated (Table 14).

In Table 16 and 17, it was observed that ETEC infection appeared to occur more frequently during the first two years of life (about 81.8% of total ETEC isolates from diarrhoea stools), and less frequently in the subsequent years. EPEC infection, on the other hand, was more frequently observed among children 2 ~ 4 years ago (36% to 40%), and in a third of diarrhoea stools from children under two years (Table 17).

In the urban community study, heat-stable (ST) toxin-producing ETEC were more frequently isolated, followed by heat-labile (LT) toxing-producing ETEC and, lastly, ST-LT toxin producing ETEC (Table 18).

Antibiotic sensitivity testing was done on all ETEC and other pathogens isolated. The distribution of resistance patterns for winter and monsoon surveys are shown in Table 19 and Table 20, respectively. Sulphonamide sensitivity testing was not done because of lack of media and standardized sulphonamide discs. ETEC resistant to tetracycline, chloramphenicol, kanamycin, cephaloridine, etc., were observed more frequently from isolates from the winter survey than from the monsoon survey.

Light microscopy evidence of parasitic infestations was observed in about 17% of diarrhoea and 10% of control stools in the winter survey, and in about 10% of diarrhoea and 6% of control stools in the monsoon survey (Table 14).

c.1.3 Virological Research Findings

Electron microscopy of all diarrhoea and control stools from both winter and monsoon surveys was carried out (139 diarrhoea and 205 control stools in winter, and 217 diarrhoea and 103 control stools in monsoon). Concentration method was not used.

Owing to defective electron microscope, rotavirus was not detected at all in any diarrhoea or control stool specimen. In a previous study carried out in winter (December, 1978) using the same electron microscope, rotavirus was detected in 6 out of 30 (20%) stool samples from diarrhoeic children under two years admitted to Infectious Diseases Hospital.

A random subsample of 78 diarrhoea and 34 control stool samples from the monsoon survey were tested by Rotazyme (ELISA) assay. Rotavirus was detected in about 14% of diarrhoea stools in the monsoon survey (Table 21).

Table 14. Incidence of major etiological agents isolated in acute diarrhoea cases and controls in the urban community

	January		July	
	Diarrhoea	Control	Diarrhoea	Control
No. examined	139	205	217	103
A. Bacterial pathogens				
1. E. coli (ETEC)	17(12.2)	5 (2.4)	22(10.1)	7 (6.8)
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. Samonella	-	-	2 (0.9)	3 (2.9)
5. V. cholera	-	-	1 (0.5)	-
6. V. parahemolyticus	-	-	-	-
7. Yersinia	-	-	-	-
8. Campylobacter	-	-	13 (6.0)	3 (2.9)
Major pathogens	41(29.5)	48(23.4)	123(56.7)	44(42.7)
	- see Virological findings			
B. Rotavirus	-	-	11/78(14.3%)	0/34(0%)
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)	-
Mixed infection	19(10,5)	29(10.2)	11 (5.1)	13(12.0)

Figures in parenthesis denote % of total number examined.

Table 15. Incidence of major etiological agents in acute diarrhoea cases and control in the hospital at North Okkalapa

	January		July	
	Diarrhoea	Control	Diarrhoea	Control
No. examined	104	80	40	40
A. Bacterial pathogenes				
1. E. coli (ETEC)	9 (8.7)	1 (1.3)	5(12.5)	1 (2.5)
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. Samonella	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 bacterial pathogens	26(25.0)	16(20.0)	27(67.5)	13(32.5)
B. Rotavirus	- see virological findings			

Figures in parenthesis denote % of total number examined.

Table 16. Age-distribution of ETEC from urban community and hospital survey

Age (months)	Diarrhoea cases		LT		ST		ST	ST-LT		ETEC		Age (months)	Control		LT		ST		ST-LT		ETEC	
	M	W	M	W	M	W	M	W	M	W		M	W	M	W	M	W	M	W	M	W
0 - 5	35	20	0	0	1 (2.9)	0	0	0	1 (2.9)	0	0 - 5	12	18	2 (16.7)	0	0	0	0	0	2 (16.7)	0
6 -11	24	24	11 (4.2)	1 (4.2)	2 (8.3)	1 (4.2)	1 (4.2)	1 (4.2)	4 (16.7)	3 (12.5)	6 -11	11	35	0	0	0	0	0	0	0	0
12 -23	68	42	4 (5.9)	2 (4.8)	4 (5.9)	1 (2.4)	5 (7.4)	2 (4.8)	13 (19.1)	5 (11.9)	12 -23	35	71	0	2 (2.8)	1 (1.4)	0	0	1 (1.4)	1 (1.4)	3 (4.2)
24 -35	47	24	0	1 (4.2)	1 (2.1)	4 (16.7)	1 (2.1)	0	2 (4.3)	5 (20.8)	24 -35	19	48	1 (5.3)	0	2 (10.5)	0	0	0	3 (15.8)	0
36 -47	31	22	1 (3.2)	1 (4.6)	0	0	0	0	1 (3.2)	1 (4.6)	36 -47	18	16	0	0	0	1	0	0	3	0 (6.3)
48 -59	10	7	1 (10.0)	1 (14.2)	0	2 (28.6)	0	0	1 (10.0)	3 (42.9)	48 -59	11	17	1 (9.1)	1 (9.1)	0	0	0	0	0 (9.1)	1 (5.9)
TOTAL	215	139	7 (3.2)	6 (2.8)	8 (3.7)	8 (5.8)	7 (3.2)	3 (2.3)	22 (10.2)	17 (12.2)	TOTAL	106	205	4 (3.9)	3 (1.5)	3 (2.9)	1 (0.5)	0	1 (0.5)	7 (6.8)	5 (2.4)

M = Monsoon

W = Winter

Figures in parenthesis denote percentages.

Table 17. Distribution of major enteric pathogens (percentages) isolated from acute diarrhoea in the community cases according to age during Monsoon Survey at North Okkalapa.

Age (months)	ETEC	EPEC	Shigella	Salmonella	V. cholera	Campylo-bactor	Total isolates
0-11	17.2 (5)	44.8 (13)	17.2 (5)	3.4 (1)	0	17.2 (5)	29
12-23	3.3 (13)	35.9 (14)	20.5 (8)	0	2.6 (1)	7.7 (3)	39
24-35	6.7 (2)	50.0 (15)	33.3 (10)	0	0	10.0 (3)	30
36-47	5.9 (1)	64.7 (11)	17.6 (3)	5.9 (1)	0	5.9 (1)	17
48-59	12.5 (1)	25.0 (2)	50.0 (4)	0	0	12.5 (1)	8
TOTAL	17.9 (22)	44.7 (55)	24.4 (30)	1.6 (2)	0.8 (1)	10.6 (13)	123

Table 18. Distribution pattern of ETEC toxin production in diarrhoea and control cases in community and hospital study.

Toxin	Winter (January)				Monsoon (July)			
	Community		Hospital		Community		Hospital	
N=	Diarrhoea 139	Control 205	Diarrhoea 104	Control 80	Diarrhoea 217	Control 103	Diarrhoea 40	Control 40
ST	8 (5.8)	1 (0.5)	6 (5.8)	0 0	8 (3.7)	3 (2.9)	3 (7.5)	0
LT	6 (4.3)	3 (1.5)	1 (1.0)	1 (1.25)	7 (3.2)	4 (3.9)	0	1 (2.5)
ST-LT	3 (2.2)	1 (0.5)	2 (1.9)	0 0	7 (3.2)	0	2 (5.0)	0
Total	17 (12.2)	5 (2.4)	9 (8.7)	1 (1.25)	22 (10.1)	7 (6.8)	5 (12.5)	1 (2.5)

Figure in parenthesis denote percentage

Table 19. Antibiotic resistance pattern of isolates from Winter Survey

1. Method: Disc diffusion technique
   Agar used - Brain Heart Infusion Agar

2. Type and concentration of discs used: Showa Japan discs

Pc = Penicillin = 20 U
PcA = Ampicillin = 30 ug
TC = Tetracycline = 200 ug
CM = Chloramphenicol = 100 ug
K = Kanamycin = 50 ug
CER = Cephaloridine = 30 ug

Distribution of Antibiotic Resistance Pattern

Strains isolated	No exam	Pc No.(%) resist	PcA No.(%) resist	TC No.(%) resist	CM No.(%) resist	CL No.(%) resist	K No.(%) resist	CER No.(%) resist
ST	29	25(86)	5(17)	4(14)	6(21)	0	0	1(3)
LT	64	62(97)	20(31)	28(44)	19(30)	0	0	2(3)
ST-LT	6	5(83)	0	0	1(17)	0	0	0
Total ETEC	99	92(93)	25(25)	32(32)	26(26	0	0	3(3)
Shigella	17	12(71)	1 (6)	5(29)	1 (6)	2(12)	0	1(6)
Salmonella	6	2(33)	3(50)	0	0	0	0	1(7)

Table 20. Antibiotic resistance of isolates from Monsoon Survey

1. Method: Disc diffusion technique
   Agar used - Brain Heart Infusion Agar

2. Type and concentration of discs used: Showa Discs

S = Streptomycin = 50 ug per disc
Pb = Aminobenzyl Penicillin = 30 ug per disc
T = Tetracycline = 200 ug per disc
C = Chloramphenicol = 100 per disc
Cr = Cephaloridine = 30 ug per disc
Ka = Kanamycin = 50 ug per disc
Mno = Minocycline = 200 ug per disc

Distribution of Antibiotic Resistance Pattern

Strains isolated	No exam	S No.(%) resist	T No.(%) resist	C No.(%) resist	Pb No.(%) resist	Ka No.(%) resist	Cr No.(%) resist	Mno No.(%) resist
ST	32	5(16)	4(13)	2 (6)	1 (3)	0	0	0
LT	90	15(17)	2 (2)	9(10)	19(21)	2(0)	2(0)	0
ST-LT	12	0	2(17)	3(25)	0	0	0	0
Total ETEC	134	20(15)	8 (6)	14(15)	20(15)	2(2)	2(2)	0
Shigella	35	11(31)	0	0	0	0	0	0

Table 21. Rotavirus detection in monsoon at North Okkalapa(July/August, 1981)

Study Area	Diarrhoea		Control	
	Number of Samples	Number positive for rotavirus(%)	Number of Samples	Number positive for rotavirus(%)
Community Study (North Okkalapa)	78	11 (14.3%)	34	0
Hospital	6	1 (16.7%)	11	1 (9.1%)

c.2 Rural Community Results

c.2.1 Epidemiological Research Findings

Incidence of diarrhoea

The overall incidence of acute diarrhoea in children under five years was 26.5 episodes per 100 children, and 21.3 cases per 100 children in winter, and 56.4 episodes per 100 children and 39.2 cases per 100 children in monsoon (Table 22).

The average annual incidence of acute diarrhoea by episodes as well as by cases per 100 children were found to be higher in all age groups during monsoon than during winter (Table 22). The incidence in all age groups followed the same pattern in both monsoon and winter surveys, being moderate in the first six months, rising sharply to a peak level in the age-group 6 - 11 months, and declining thereafter to the lowest level in the 48 - 59 months age-group. The peak diarrhoea incidence was consistently at 6 - 11 months age-group in both monsoon and winter surveys, although it has been reported variously at different age-groups by different authors. Our results agree with those of Shaker et al. (1966) who contended that vulnerability was greatest at the age of 4 to 6 months at a time when outside food which was often contaminated was introduced.

Sex-differences in diarrhoea incidence

There was no difference in the incidence of acute diarrhoea between male and female children (Table 23) in both monsoon and winter surveys. Our results agree with those of Shaker et al. (1966), and Saran et al. (1979, 1981).

Socio-economic conditions and diarrhoea incidence

No appreciable difference was found in the incidence of acute diarrhoea when analysed by family size or ethnic groups (Table 24 and Table 25, respectively).

The incidence of diarrhoea did not vary much with monthly family income in the winter survey, although results of the monsoon survey revealed that diarrhoea incidence was highest among the least income group (less than K 200/-- per month), and that

diarrhoea incidence declined as family income increased (Table 26).

Duration of diarrhoea

The mean duration of diarrhoea per sick child ranged between 2.2 days to 3.1 days, and the mean duration of diarrhoea per episode ranged from 1.83 days to 2.29 days, during the winter survey. Corresponding figures for the monsoon survey were: the mean duration of diarrhoea per sick child ranging from 2.5 to 4.0 days, and the mean duration of diarrhoea per episode ranging from 1.9 to 2.6 days. The duration of diarrhoea by case as well as by episode in the winter survey in consistently shorter than that in the corresponding age-groups in the monsoon survey. In both surveys, the youngest age-group suffered the longest duration of diarrhoea and the eldest children had the shortest duration of diarrhoea (Table 27).

Frequency of diarrhoea episodes

66,7% and 81% of children, respectively, in monsoon and winter surveys experienced only one diarrhoea episode during the survey month; 25.7% and 14%, respectively, in monsoon and winter had two diarrhoea episodes, and the remaining 7.5% and 4.5%, respectively, in monsoon and winter had three or more episodes during the survey periods (Table 28). A similar distribution pattern was found in each age-group also.

Feeding practices during diarrhoea

Using a questionnaire survey to enquire into the foods withheld and foods given during a diarrhoea episode, it was found that fruits and pulses were the main items withheld in the monsoon survey, and in the winter survey, pulses and sour edibles were the main items withheld during a diarrhoea episode (Table 29). This difference probably reflected the change in health knowledge affected by the previous survey on the community.

Table 22. Distribution of acute diarrhoea incidence by age in Indakaw Villages; Comparison between Monsoon and Winter Survey

Age (Months)	Number of children under five		Incidence by episodes per 100 children		Incidence by cases per 100 children	
	Monsoon	Winter	Monsoon	Winter	Monsoon	Winter
0-5	84	53	54.8	35.4	36.9	28.3
6-11	94	103	88.3	49.51	61.7	34.95
12-23	134	170	85.1	38.23	53.7	31.17
24-35	146	130	45.2	23.07	33.6	21.53
36-47	137	152	38.7	11.18	27.7	9.21
48-59	120	124	34.2	9.67	26.7	8.06
Total	715	732	56.4	26.5	39.2	21.31

Table 23. Distribution of acute diarrhoea incidence by sex in Indakaw Village, Comparison between Monsoon and Winter Survey

Sex	Children under five		Incidence by episodes				Incidence by cases			
			Monsoon S.		Winter S.		Monsoon S.		Winter S.	
	Monsoon	Winter	No. of episodes	Incidence rate per 100	No. of episodes	Incidence rate per 100	No. of cases	Incidence rate per 100	No. of cases	Incidence rate per 100
Male	374	377	203	54.6	104	27.58	144	38.7	85	22.54
Female	343	355	200	58.3	90	25.37	136	39.7	71	20.00
Total	715	732	403	56.4	194	26.50	280	39.2	156	21.31

Table 24. Distribution of acute diarrhoea incidence by family size: Comparison between Monsoon and Winter Survey

Family size	Children under five		Incidence by episodes				Incidence by cases			
			Monsoon S.		Winter S.		Monsoon S.		Winter S.	
	Monsoon	Winter	No. of episodes	Incidence rate per 100	No. of episodes	Incidence rate per 100	No. of cases	Incidence rate per 100	No. of cases	Incidence rate per 100
3-6	414	413	247	59.7	114	27.6	168	40.6	90	21.79
7-10	279	285	139	49.8	64	22.45	103	39.9	55	19.29
11 and above	22	34	17	77.3	16	47.05	9	40.9	11	32.35
Total	715	732	403	56.4	194	26.5	280	39.2	156	21.31

Table 25. Distribution of acute diarrhoea incidence by ethnic group, Indakaw Village; Comparison between Monsoon and Winter Survey

Ethnic Group	Children under five		Incidence by episodes				Incidence by cases			
			Monsoon S.		Winter S.		Monsoon S.		Winter S.	
	Monsoon	Winter	No. of episodes	Incidence rate per 100	NNo. of episodes	Incidence rate per 100	No. of cases	Incidence rate per 100	No. of cases	Incidence rate per 100
Burmese	521	524	276	54.9	129	24.61	206	39.5	107	20.41
Karen	157	173	93	59.2	57	32.94	60	38.2	42	24.27
Indian	9	9	0	0	1	11.11	0	0	1	11.11
Half-cast	25	26	24	96.0	7	26.92	12	48.0	6	23.07
Total	712*	732	403	56.4	194	26.5	278**	39.2	156	21.31

* Exlude (3) Unknowns

** Exclude (2) Unknowns

Table 26. Distribution of acute diarrhoea incidence by average monthly family income in Indakaw Village, Comparison between Monsoon and Winter Survey

Family Income In Kyats	Children under five		Incidence by episodes				Incidence by cases			
			Monsoon S.		Winter S.		Monsoon S.		Winter S.	
	Monsoon	Winter	No. of episodes	Incidence rate per 100	No. of episodes	Incidence rate per 100	No. of cases	Incidence rate per 100	No. of cases	Incidence rate per 100
0-199	135	114	98	72.6	32	28.07	58	43.0	24	21.05
200-299	212	262	116	54.7	68	25.95	85	40.1	62	23.66
300-399	221	219	116	52.5	55	25.11	87	39.4	41	18.72
400-499	84	83	48	57.2	23	27.71	30	35.7	19	22.89
500	63	54	25	39.7	16	29.62	20	31.7	10	18.57
Total	715	732	403	56.4	194	26.5	280	39.2	156	21.31

Table 27. Table showing days of diarrhoea experienced and mean duration (day) of diarrhoea by age; Comparison between Monsoon and Winter Survey

Age Months	Monsoon Survey					Winter Survey				
	Days with diarr.	No. of sick childr.	No. of episodes	Mean duration of per sick chil.	Mean duration per episodes	Days with diarr.	No. of sick child.	No. of episodes	Mean duration per sick childr.	Mean duration per episodes
0-5	125	31	48	4.0	2.6	49	16	21	3.06	2.23
6-11	215	59	84	3.6	2.6	84	35	49	2.4	1.71
12-23	251	72	114	3.5	2.2	134	53	65	2.54	2.06
24-35	144	51	67	2.8	2.2	64	28	30	2.28	2.13
36-47	128	38	52	3.4	2.5	39	14	17	2.78	2.29
48-59	73	29	38	2.5	1.9	22	10	12	2.2	1.83
Total	936	280	403	3.3	2.3	293	156	194	2.51	2.02

Table 28. Distribution of acute diarrhoea cases by age and frequency of episodes in Indakaw Village, Comparison between Monsoon and Winter Survey

Age in Months \ Frequency of Episodes	Monsoon Survey				Winter Survey			
	One Episode	Two Episodes	Three + Episodes	Total	One Episode	Two Episodes	Three + Episodes	Total
0-5	19 (61.13)	9 (29.04)	3 (9.7)	31 (100%)	12 (70.58)	5 (29.41)	0 (0)	17 (100%)
6-11	38 (65.5)	18 (31.0)	2 (3.4)	58 (100%)	27 (77.14)	4 (11.42)	4 (11.42)	35 (100%)
12-23	43 (60.5)	19 (26.8)	9 (12.7)	71 (100%)	42 (80.75)	8 (15.38)	2 (3.84)	52 (100%)
24-35	37 (74.0)	10 (20.80)	3 (6.0)	50 (100%)	26 (92.85)	2 (7.14)	0 (0)	28 (100%)
36-47	27 (71.1)	8 (21.1)	3 (7.9)	38 (100%)	12 (85.71)	1 (7.14)	1 (7.14)	14 (100%)
48-59	23 (71.9)	8 (25.0)	1 (3.1)	32 (100%)	8 (80)	2 (20.0)	0 (3.1)	10 (100%)
Total	187 (66.8)	72 (25.7)	21 (7.5)	280 (100%)	127 (81.41)	22 (14.1)	7 (4.48)	156 (100%)

Note: The numbers in parenthesis represent the percentage in row for respective survey.

Table 29. Foods with held during diarrhoea Indakaw Village, Comparison between Monsoon and Winter Survey

Foods	Monsoon S.		Winter S.	
	Number	Percentage	Number	Percentage
Fruits	374	46.3	47	5.16
Pulses	142	17.6	266	29.23
Vegetables	104	12.9	52	5.71
Sour edibles	44	5.5	211	23.18
Meat	13	1.6	62	6.81
Potato	11	1.4	74	8.13
Chilli	11	1.4	16	1.75
Milk	9	1.1	5	0.54
Rice	9	1.1	22	2.41
Biscuit	3	0.4	1	0.1
Egg	3	0.4	30	3.29
Others	84	10.4	124	13.62
Total	807	100%	910	100%

	Monsoon	Winter
Note:	Unknown (11)	Unknown (19)
	Nothing with held (12)	Nothing with held (10)

Table 30. Foods given during diarrhoea in Indakaw Village, Comparison between Monsoon and Winter Survey

Foods	Monsoon S.		Winter S.	
	Number	Percentage	Number	Percentage
Soup	176	30.6	36	6.53
Rice (plus oil, salt, jaggery)	168	29.2	250	45.37
Curry	103	17.9	70	12.7
Dried foods	43	7.5	47	8.52
Fried meat	17	3.0	6	1.08
Milk and milk products	13	2.3	11	1.99
Liquid (coffee, rice water)	11	1.9	13	2.35
Biscuits	8	1.4	6	1.08
Vegetables	4	0.7	18	3.26
Others (Boiled rice)	32	5.6	94	17.05
Total	575	100%	551	100%

Note:	All foods given	41	44
	Unknown	17	10
	Nothing but breast fed	6	18

Table 31. Management of acute diarrhoea by severity in Indakaw Village, Comparison between Monsoon and Winter Survey

Treatment Modalities	Monsoon Survey			Winter Survey		
	Mild	Moderate	Severe	Mild	Moderate	Severe
No treatment	57 (10.4)	6 (1.2)	6 (1.2)	30 (5.87)	1 (0.20)	0 (0)
Self treatment (indigenous drugs)	174 (31.8)	35 (7.1)	5 (1.0)	180 (35.23)	41 (8.38)	4 (0.80)
Self treatment (Western Medicine)	157 (28.7)	142 (28.8)	4 (0.8)	232 (45.40)	157 (32.11)	5 (1.01)
Indigenous medical practitioner	17 (3.1)	15 (3.0)	0 (0)	7 (1.37)	7 (1.43)	1 (0.20)
Quacks	4 (0.7)	11 (2.2)	4 (1.0)	13 (2.54)	54 (11.04)	25 (5.03)
Voluntary health worker	47 (8.6)	59 (12.0)	19 (3.9)	18 (3.52)	33 (6.75)	14 (2.82)
Basic health staff	54 (9.9)	147 (29.8)	44 (9)	24 (4.70)	123 (25.15)	66 (13.28)
Qualified doctor	37 (6.8)	78 (15.8)	408 (83.3)	7 (1.37)	73 (14.93)	382 (76.86)
Total	547 (100%)	493 (100%)	490 (100%)	511 (100%)	489 (100%)	497 (100%)

Note: The numbers in parenthesis represent the percentage in column.

Table 32. Usage of oral rehydration salt in acute diarrhoea in Indakaw Village, Comparison between Monsoon and Winter Survey.

Monsoon Survey				Winter Survey			
ORS Never used	ORS packet used	Incomplete formular used	Total	ORS Never used	ORS packet used	Incomplete formular used	Total
407 (82.9)	58 (11.18)	26 (5.3)	491 (100%)	376 (75.35)	111 (22.24)	12 (2.4)	499 (100%)

Note: The numbers in parenthesis represent the percentage in column for respective survey.

As regards foods given during diarrhoea, the majority fed soup, rice, curry and boiled rice during diarrhoea in both monsoon and winter surveys (Table 30). During both surveys, the majority of mothers stated that they would continue regular feeding (breast-fed and/or supplementary foods) during the time their children developed diarrhoea.

Treatment practices during diarrhoea

A questionnaire survey revealed that self-medication using either indigenous drugs or western medicine was the principle treatment modality administered to children with mild diarrhoea. In almost all instances of severe diarrhoea, children were treated by a qualified doctor in hospital or a nearby general practitioner, or by local health assistant or midwife (Table 31).

Regarding use of oralyte (oral rehydration salts) in diarrhoea, 82% of parents in monsoon survey and 75% of parents in winter survey stated that they had never used oralyte in diarrhoea (Table 32). The slight improvement observed in the winter survey was probably due to previous contact with DMR field staff who had demonstrated the use of oralyte for treatment of dehydration in acute diarrhoea during the monsoon survey.

3.2.2 Bacteriological Research Findings

Major bacterial pathogens were isolated in 39.6% and 28.8%, respectively, of diarrhoea and control stools in the monsoon survey, and in 24.6% and 21.3%, respectively, of diarrhoea and control stools in the winter survey (Table 33).

ETEC were the most important causal bacterial pathogen, especially in monsoon where ETEC were isolated more frequently than any other bacterial pathogen. ETEC were isolated significantly more frequently from diarrhoea than control stools in the monsoon survey, also significantly more frequently in diarrhoea stools in the monsoon survey than those in the winter survey. On the other hand, ETEC isolation rates were relatively low from both diarrhoea and control stools during the winter survey. This finding suggests that ETEC is a major bacterial pathogen causing diarrhoea in village children during monsoon.

Table 33. Bacterial and viral agents causing diarrhoea in village children

Pathogen		Monsoon Survey		Winter Survey		Significance
		Diarrhea (n=330)	Control (n=233)	Diarrhea (n=171)	Control (n=141)	
ETEC	LT	26 (7.9) (P 0.05)[a]	8 (3.4)	2 (1.2) (N.S.)[b,y]	2 (1.4)	(N.S.)[c,y] (P 0.05)[d,y]
	ST	35(10.6) (P 0.02)[a]	11 (4.7)	9 (5.3) (N.S.)[b,y]	2 (1.4)	(N.S.)[c,y] (P 0.05)[d,y]
	ST/LT	14 (4.2) (N.S.)[a,y]	4 (1.4)	5 (2.9) (N.S.)[b,y]	2 (1.4)	(N.S.)[c,y] (N.S.)[d,y]
ALL ETEC		75(22.7) (P 0.001)[a]	23 (9.5)	16 (9.4) (N.S.)[b]	6 (4.3)	(N.S.)[c] (P 0.01)[d]
EPEC		42(12.7) (N.S.)[a]	34(14.5)	19(11.1) (N.S.)[b]	20(14.2)	(N.S.)[c] (N.S.)[d]
Salmonella	B	1 (0.3)	1 (0.4)	0	0	
	D_1	0	0	2 (1.2)	0	
	All	1 (0.3)	1 (0.4)	2 (1.2)	0	
Shigella dysenteriae		1 (0.3)	0	1 (0.6)	0	
flexneri		5 (1.5)	2 (0.9)	1 (0.6)	2 (1.4)	
boydii		0	1 (0.4)	1 (0.6)	0	
All		6 (1.8)	3 (1.3)	3 (1.8)	2 (1.4)	
Campylobacter		7 (2.1)	6 (2.6)	2 (1.2)	2 (1.4)	
Vibrio cholerae		0	0	0	0	
Vibrio parahaemolyticus		0	0	0	0	
Yersinia enterocolitica		0	0	0	0	
Total Bacterial Pathogens:		131(39.6)	67(28.8)	42(24.6)	30(21.3)	
Rotavirus: No. examined		166	75	116	44	
No. positive		0	0	17(14.7) (P 0.05)[b,y]	1 (2.3)	(P 0.001)[d,y]

Figures in parenthesis denote percentage.

a = Chi-square test of significance of difference in proportions of patients with positive results between control and diarrhoea children in monsoon survey;

b = Chi-square test of significance of difference in proportions of patients with positive results between control and diarrhoea children in winter survey;

c = Chi-square test of significance of difference in proportions of patients with positive results between control children in monsoon and winter surveys;

d = Chi-square test of significance of difference in proportions of patients with positive results between diarrhoea children in monsoon and winter surveys;

y = Chi-square test using Yates' Continuity Correction

N.S. = not significant

Table 34. Enteropathogenic E. coli serotypes

Serotype	Monsoon Survey		Winter Survey	
	Diarrhoea	Control	Diarrhoea	Control
026 : K60	4	2	2	2
044 : K74	5	3	0	1
055 : K59	1	4	3	2
086a: K61	3	0	2	1
086 : K62	2	0	1	2
0111 : K58	0	1	0	0
0119 : K69	2	3	0	1
0125 : K70	4	1	4	4
0126 : K71	13	8	6	3
0127a: K63	4	3	0	0
0128 : K67	1	2	1	2
0136 : K78	0	0	0	1
0144 : K82	0	1	0	0
0146 : K89	2	1	0	0
ALL EPEC	41	29	19	19

Table 35. Age-specific rate of isolation of ETEC in monsoon survey

Age-group (months)	Number of children		ETEC					
			LT		ST		ST/LT	
	D	C	D	C	D	C	D	C
0 - 5	58	33	5 (8.6%)	1 (3.0%)	0	3 (9.1%)	5 (8.6%)	0
6 -11	53	32	5 (9.4%)	(3.1%)	6 (11.3%)	1 (3.1%)	0	0
12 -23	93	61	5 (5.4%)	3 (4.9%)	18 (19.4%)	3 (4.9%)	4 (4.3%)	3 (4.9%)
24 -35	53	50	5 (9.4%)	0	4 (7.6%)	2 (4.0%)	2 (3.8%)	1 (2.0%)
36 -47	49	34	5 (10.2%)	2 (5.9%)	6 (12.2%)	1 (2.9%)	3 (6.1%)	0
48 -59	24	23	1 (4.2%)	1 (4.4%)	1 (4.2%)	1 (4.4%)	0	0
Total	330	233	26 (7.9%)	8 (3.4%)	35 (10.6%)	11 (4.7%)	14 (4.2%)	4 (1.4%)

b = Sh boydii; f = Sh. flexneri; d = Sh. dysenteriae;

D_1 = Salmonella D; B = Salmonella B.

Table 36. Age-specific rate of isolation of ETEC in winter survey

Age-group (months)	Number of children		ETEC					
			LT		ST		ST/LT	
	D	C	D	C	D	C	D	C
0 - 55	23	19	0	0	2 (8.7%)	0	1 (4.3%)	1 (5.3%)
6 - 11	45	35	0	1 (2.8%)	2 (4.5%)	1 (2.8%)	1 (2.3%)	0 (5.3%)
12 - 23	55	50	2 (3.6%)	0	4 (7.3%)	0	0	1 (2%)
24 - 35	25	19	0	1 (5.3%)	0	1 (5.3%)	1 (4%)	0
36 - 47	15	10	0	0	0	0	1 (6.7%)	0
48 - 59	8	8	0	0	1 (12.5%)	0	1 (12.5%)	0
Total	171	141	2 (1.2%)	2 (1.4%)	9 (5.3%)	2 (1.4%)	(2.9%)	(1.4%)

b = Sh. boydii; f = Sh. flexneri; d = Sh. dysenteriae;

D_1 = Salmonella D_1; B = Salmonella B.

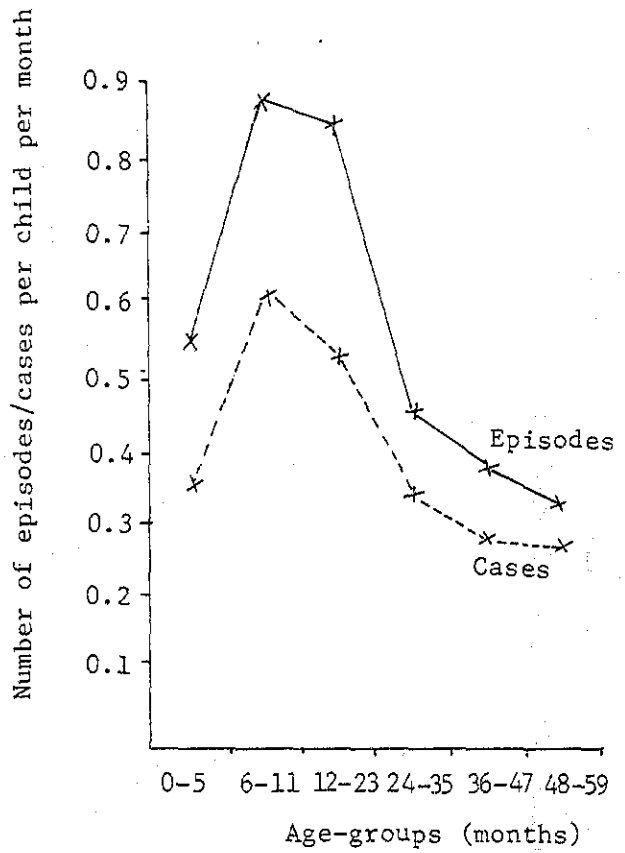

Fig. 5a: Age-specific incidence of diarrhoea in monsoon in Intakaw

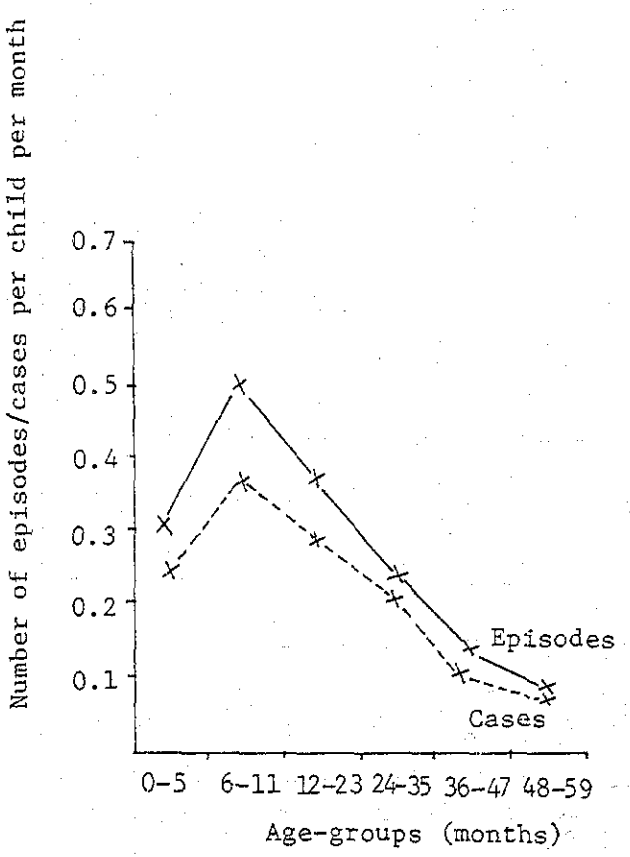

Fig. 5b: Age-specific incidence of diarrhoea in winter in Intakaw

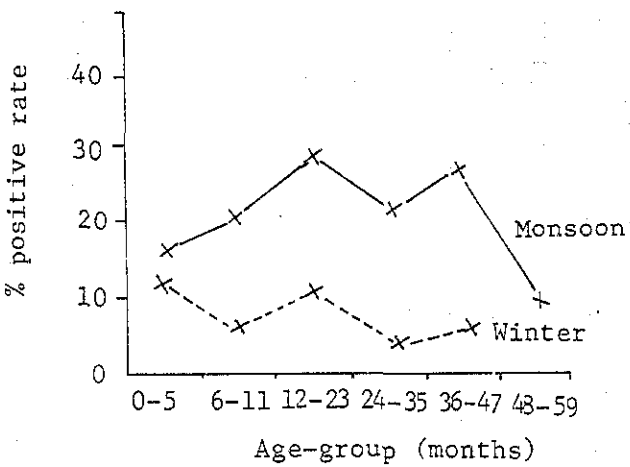

Fig. 6: Age-specific incidence of ETEC diarrhoea in monsoon and winter at Intakaw

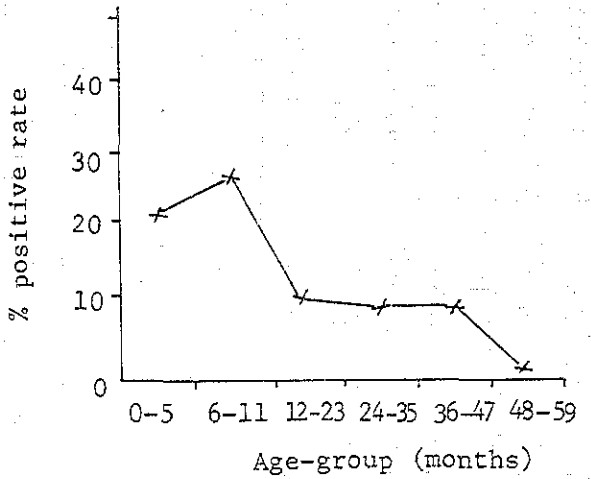

Fig. 7: Age-specific incidence of ETEC diarrhoea in winter at Intakaw

Table 37. Age-specific rate of detection of rotavirus in children at Intakaw

Age-groups (mths)	Monsoon		Winter	
	Diarrhoea	Control	Diarrhoea	Control
0 - 6 months	0/23	0/8	3/13(23.1%)	1/4(25%)
6 - 11 months	0/38	0/13	7/26(26.9%)	0/10
12 - 23 months	0/41	0/19	4/40(10.0%)	0/17
24 - 35 months	0/30	0/18	2/21 (9.5%)	0/7
36 - 47 months	0/19	0/11	1/10(10%)	0%
48 - 59 months	0/15	0/6	0/6	0/2
Total	0/166	0/75	17/116(14.7%)	1/44(2.3%)

Table 38. Incidence of diarrhoea in different seasons in Burma

Survey		Episodes/100 children/month	
		Monsoon	Winter
North Okkalapa	(Urban)	20.3	16.7
Intakaw	(Rural)	56.4	26.3

EPEC were isolated with almost equal frequency from diarrhoea and control stools in both monsoon and winter surveys. The EPEC serotypes were not confined to any particular serotype (Table 34) and it was not possible to specify which serotype was associated with diarrhoea in village children.

Shigella dysenteriae was found in diarrhoea stools only in both monsoon and winter surveys, whereas *Sh. flexneri* and *Sh. boydii* were from diarrhoea as well as control stools. *Sh. sonneii* was not detected at all. No significant differences in isolation rates of Shigellae were observed between diarrhoea and control stools in either monsoon or winter survey.

Salmonella B was found in one case each in diarrhoea and control stools during monsoon. Salmonella D_1 was found in two diarrhoea stool samples, and none in control stools. The rates of isolation of Salmonellae from diarrhoea and control stools in either monsoon or winter survey were not different significantly.

Campylobacter were isolated with almost equal frequency from diarrhoea as well as control stool samples in both monsoon and winter surveys.

Vibrio cholerae, V. parahaemolyticus and *Yersinia enterocolitica* were not detected in any diarrhoea or control stool sample during either monsoon or winter survey.

The age-specific rates of isolation of ETEC in diarrhoea and control stools during monsoon and winter, respectively, are given in Table 35 and Table 36. Figure 5a and 5b show the curves for the age-specific incidences of diarrhoea episodes and cases, respectively, in monsoon and winter surveys. When the curves for the age-specific isolation rates of ETEC in monsoon and winter (Figure 6) were compared with the former curves, it was found that the age-specific curves for the isolation rates of ETEC in monsoon and winter do not conform with corresponding age-specific curves for diarrhoea episodes and diarrhoea cases.

3.2.3 Virological Research Findings

Rotavirus was detected in a significantly larger proportion of children with diarrhoea (14.7%) than control children in winter. No rotavirus was detected from both diarrhoea and control stools

during the monsoon survey by RPHA (Rotacell), and this was confirmed on a random 50% subsample using ELISA (WHO ELISA kits).

The age-specific rates of detection of rotavirus in diarrhoea stools during winter is given in Table 34 and Figure 5. The curve for the age-specific rotavirus detection rates (Figure 7) conforms closely with the age-specific curves for diarrhoea episodes and diarrhoea cases in the winter survey, which suggests that rotavirus is the major pathogen causing diarrhoea in village children during the winter months.

4. Discussion

Incidence of diarrhoea

In spite of the difference in sample-size for the two seasons in the urban community study, the sample sizes obtained were sufficiently large enough to provide results which could be analysed with confidence to afford statistical validity.

The yearly incidence of 1.8 and 2.4 episodes per child per year for the dry and wet season, respectively, in the urban community is higher than that reported in a rural survey at Htauk-kyant (1.06 episode per child per year, Thane Toe et al., 1982), and also higher than the attack rate (one episode per child per year) reported in Bangladesh (ICDDR, B Report, 1980). Our incidence figures were lower than those reported by Mata et al. (1978) in Costa Rica (8 episodes per child per year), however.

On the other hand, children in our village community survey had higher incidences of diarrhoea (in both winter and monsoon) than children in our urban community survey (Table 38), and this difference is statistically significant (P 0.05). In Bangladesh, diarrhoea incidence in rural populations is higher (85.5/100 population, 39% of these being in children under five years; Sunoto, 1982). A survey in an urban community in Indonesia revealed the incidence of diarrhoea as 14.9/100 population/year, 47% being in children under five years; also, it was reported that this urban incidence was lower than that in a semiurban area in Ujung Rudand (Sutoto, Mochtar, et al., 1982).

The reason for a higher incidence in rural community study may be due to differences in aetiologic agents between rural and urban communities, or registering of an undetected epidemic in the rural

community during both study periods, or environmental contamination with diarrhoea pathogens from defecation of adults in woods and orchards (81% practiced this) and of children within the house premises (31% had this habit). These differences are not due to differences in study design or quality control, because the methodology employed in both urban and rural community studies were strictly similar.

Age-specific incidence of diarrhoea

In both urban and rural community studies, the age-specific diarrhoea incidences during the wet season exceeded those during the dry seasons in all age-groups. These differences were statistically significant in the rural community survey (P 0.001), but not so in the urban community study (P 0.2). Further, the age-specific incidences were highest between 6 to 23 months in the rural community in both seasons, whereas in the urban community study, the peak incidence was between 0-11 months and 36-47 months is the wet and dry seasons respectively.

Peaking of diarrhoea incidences in younger age groups, at the time of reduction in material antibodies, indicates the importance of this age group (6 - 23 months) to be considered in any intervention aimed at prevention of acute diarrhoea in children. The peak incidence of diarrhoea was reported by some to be about two to three years of age (Mata et al., 1978), Zijl, 1966, Newell, 1965, Woodward et al., 1974). On the other hand, other studies (WHO, 1979, Shaker et al., 1966) contend that susceptibility to diarrhoea was highest at the age of 4 to 6 months. Such higher incidence in this age group is due probably to introduction of adult-type foods at the time of weaning, most of which, in developing countries, are heavily contaminated with bacterial pathogens.

Seasonality of childhood diarrhoea

In the urban community study, no significant difference in the incidence of diarrhoea was detected between winter and monsoon surveys. In a hospital-based study carried out in the children's ward of North Okkalapa General Hospital (which admitted children from the same community where the present community survey was carried out)

in 1978, the hospital admission rates of children under 12 years was about 2.5 to 3 times more in July than in January (Soe Soe Aye, 1980). The general pattern of hospital admissions in various hospital all over the country also showed much higher admission rates for acute diarrhoea in the wet than in the dry season (Hospital In-patient Reports, Health Information Service, 1979, 1980). It may be presumed that this higher hospital admission rates in monsoon might be due to higher preponderance of more severe episodes rather than due to a real increase in the incidence of diarrhoea in the community.

On the other hand, in our rural community study as well as in another study done in a rural community at Htauk-kyant (Thane Toe et al., 1982), the incidence of diarrhoea was higher in the wet season. Since data from these two village communities are collaborative, it may be inferred that there was indeed a real increase in the number of diarrhoea episodes and diarrhoea cases during monsoon in these villages.

A number of explanations may be given for this increase in the incidence of childhood diarrhoea during the wet season. First, the pattern may reflect an overall increase in infective diarrhoea in the community, particularly affecting younger children. Secondly, the seasonal differences in the pattern of diarrhoea may be due to changes in the environment providing favourable conditions for the transmission of aetiologic agents. Thirdly, dose and virulence of aetiologic agents may be increased due to favourable environmental conditions. Finally, different aetiologic agents may have been responsible in different seasons.

Viral diarrhoea

In the village community study, rotavirus was the major diarrhoea pathogen observed in winter, being detected from 14.7% of diarrhoea stools tested. This rate is higher than the isolation rates of all Enterotoxigenic E. coli (9.4%) together, or that of Enteropathogenic E. coli (5.7%) in the same season. Also, rotavirus was the only pathogen that was significantly more frequently observed in diarrhoea stools than in control stools in the winter months (Table 33). Rotavirus has also been reported to be the most common enteric pathogen in children in Indonesia, Philippines, Thailand, Malaysia, Singapore and Bangladesh (Sunoto, 1982). Among hospitalized

children, the incidence of rotavirus diarrhoea is even higher (84% in Washington D.C. (Brandt et al., 1982); and 45.3% in outpatients in Costa Rican children (Mata et al., 1983)).

Rotavirus was more frequently found in the diarrhoea stools of village children under two years. In a rural area of Kivu Province in Saire, too, rotavirus was found in outpatients with diarrhoea usually among children under two years of age (de Mol, et al., 1983). The age-specific rate of detection of rotavrius diarrhoea during the winter conforms closely to the age-specific curves for diarrhoea episodes and diarrhoea cases in the winter survey, which also supports the fact that rotavirus is the major pathogen causing diarrhoea in our village children during the winter months. This also suggests that the greater occurrence of rotavirus in the environment during the favourable weather conditions in winter is probably confined to children under two years age in whom the local gut immunity to rotavirus has not yet become as protective as in older children.

It is to be remembered however that although the results of studies relating to incidence and seasonal distribution of rotavirus infections in temperate climates are broadly in agreement that rotavirus diarrhoea is more frequent in winter (Kapikian et al., 1976), results obtained from tropical countries show considerable variations. In our urban community study, rotavirus was detected in 14.3% of diarrhoea stools and none from control stools during the monsoon survey. Rotavirus was probably also present in winter too, as evident from another hospital based study in the winter of 1978. In many tropical countries, rotavirus may be detected throughout the year, being commoner during the cooler months (observed in Rhodesia (Cruickshank and Zilberg, 1976), in Vellore, Southern India (Maiya et al., 1977)). In some tropical countries with a high ambient temperature throughout the year, infection may occur more commonly during the rainy season (et. in Northern Nigeria (Dossetor et al., 1979), in Calcutta (Mathan et al., 1977). Other tropical countries in Central and South America do not show well defined seasonal features (eg. in Guatemala (Wyatt et al., 1979). These may represent real differences for in many developing countries bacterial agents may play a more important role in the aetiology of acute diarrhoea than in industrialized countries (Banatvala, 1979). Alternatively,

these differences may reflect language and other difficulties which preclude obtaining specimens at optimum time for diagnosis.

Bacterial agents other than ETEC and EPEC

Y. cholerae, V. parahaemolyticus and *Yersinia enterocolitica* were not important causes of diarrhoea in both urban and rural children. Salmonella and Shigellae too, were relatively infrequently isolated and did not appear to paly a significant causal role in childhood diarrhoea (de Mol et al., 1983).. Campylobacter were isolated with equal frequency in both diarrhoea and control stools in winter as well as monsoon survey, in both communities. However, it is known that campylobacter enteritis tends to affect older persons, usually teenagers and young adults (Cameron et al., 1982), and the relative prevalence of campylobacter in our urban and rural community studies may be underestimated.

Enteropathogenic *Escherichia coli* (EPEC) as a causal agent of childhood diarrhoea

EPEC were isolated in almost equal proportions from diarrhoea and control stools in both monsoon and winter surveys in both urban and rural community studies. Thus, EPEC could not be considered important causal pathogens of acute diarrhoea in children in our urban and rural communities.

The serotypes of EPEC in our study were not confined to any particular serotype (although O126:K71 strain was most frequently isolated in both seasons in the rural community). This it was not possible to specify which serotype was a major causal agent.

There is controversy about the value of serotyping *E. coli* isolates from non-epidemic diarrhoea cases using commercially available *E. coli* "O" antisera. The routine slide-agglutination testing of fecal *E. coli* with EPEC antisera is not recommended for several reasons: (i) the clinical significance of the value of EPEC serotyping in non-epidemic diarrhoea is in doubt; (ii) the serotype of *E. coli* is useful only if both "O" and "H" antigens are known (Commerically available EPEC antisera do not test for "H" antigen so that even if an *E. coli* agglutinates with EPEC antisera, one cannot say with certainty that such a strain belongs to an EPEC serotype);

(iii) commercially available EPEC antisera do not detect many of the serotypes frequently found among enterotoxigenic strains; and (iv) serotypes other than those included in the commercial antisera may also be important EPEC serotype. Also, accurate serotyping of *E. coli* is technically difficult, and is probably best done in Reference laboratories. In the present study, serotyping of EPEC with available antisera was done so as to obtain a very general view about the distribution of EPEC in under-five children in the urban and rural communities.

Enterotoxigenic *Escherichia coli* (ETEC) as a causal agent of childhood diarrhoea

ETEC are not important causes of infantile diarrhoea in developed countries with good standards of hygiene (Echeverria et al., 1975; Gurwith and Williams, 1977; Wadstrom, 1978). On the other hand, there is now abundant evidence indicating that ETEC are important causes of diarrhoea in tropical and developing countries, particularly in infants and children under five years age (Guerrant et al., 1975; Nalin et al., 1975; Sack et al., 1977).

In both urban and rural community studies, ETEC were the most important causal bacterial agents of childhood diarrhoea. The isolation rate of ETEC in the urban community were almost the same in monsoon and winter; in the rural community survey, enterotoxigenic *E. coli* were isolated at a significantly higher rate in monsoon than in winter. It is possible that ETEC are either endemic in these particular communities or the monsoon surveys had been conducted at the time of the peak periods of occurrence of ETEC infection.

In the urban community study, 81% of ETEC isolates were from diarrhoea children under two years age. ETEC detection rates declined by the age of four. This may correspond to an increased immunity against ETEC infection as the age increases. No greater incidence of ETEC infection was found in children below six months, a period where majority of these children are being breast-fed, and supplementary feeds are not yet introduced.

The age-specific rates of isolation of ETEC for monsoon and winter (Figure 6) do not conform with corresponding age-specific curves for diarrhoea episodes and cases (Figure 5a and 5b). Thus, it

may be inferred that although ETEC were isolated significantly in diarrhoea children in monsoon, there may be other bacterial pathogens also contributing to the high incidence of diarrhoea episodes and cases during the same season. In addition, it is noteworthy that ETEC was most frequently isolated in the stools of 12 - 24 month age-group in both monsoon and winter; this age group co-incides with the weaning period (6 - 18 months) in these children. It is during weaning that the infant is particularly exposed to the hazards of environmental contamination, especially from food and water.

Summary

Epidemiological features and major bacterial and viral causative agents of acute childhood diarrhoea were studied in an urban community at North Okkalapa (407 children under five) in the winter season (January, February 1981) and on 1545 children during the wet (July, August 1981) season; and in a typical rural community at Intakaw on 715 and 732 under five children during the wet (June, July 1982) and winter (January, February 1983) seasons respectively.

In both urban and rural community surveys, a higher incidence of diarrhoea was observed during the wet season than during winter season.

The major causal bacterial pathogen associated with acute diarrhoea in rural children in the wet season and winter but especially during the wet season was Enterotoxigenic *Escherichia coli* (ETEC, 22.7 per cent and 9.4 per cent respectively) and this was also true for the urban community (10.1 per cent and 12.1 per cent respectively). Other bacteria such as Enteropathogenic *Escherichia coli* (EPEC), Shigella, Salmonella, Vibrio and *Campylobactor jejuni* played relative minor aetiologic roles. *Yersinia enterocolitica* was not detected at all.

The major cause of acute diarrhoea in rural children in winter season was Rotavirus (14.6 per cent) followed closely by ETEC: Rotavirus was not detected at all in wet season in the rural community survey. On the other hand, Rotavirus was detected in wet season (14.1 per cent) as well as in winter season (31.8 per cent) (in another hospital study) in urban children with acute diarrhoea.

ETEC isolated from diarrhoeal patients were frequently resistant (30 per cent) to tetracycline but less frequently (20 per cent) to chloramphenicol, kanamycin, cophaloridine and ampicillin.

The majority of Shigella isolated from patients were frequently resistant to Tetracycline (20 per cent) but less to other antibiotics.

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2-5 Antibiotic Resistance Pattern in Pathogenic Bacteria from Diarrhoeal Cases

Introduction

Emergence of drug-resistant microorganisms is an increasing global problem nowadays. Outbreaks of diseases caused by drug resistant pathogens have been known and documented in many instances.

It is a well known fact that most of the antibiotic resistance among Enterobacteria are R-factor or plasmid mediated (1). Antibiotic resistance is thought to be the result of widespread and indiscriminate use of antimicrobial agents in both men and animals.

This problem is more so common and prominent in developing countries were there is no control and restriction in the aministration of antimicrobial agents. However, the data on antibiotic resistance is rather limited.

This investigation is undertaken in order to locate the presence of any resistant strains of enteric pathogens in the community as well as to review any inappropriate antibiotics still available in open market in Burma.

Antibiotic susceptibility patterns on 75 recently isolated enterotoxigenic *E. coli* and 27 *Shigella* strains have been examined. These bacterial strains are isolated from diarrhoeal children in Rangoon during 1982-1983. Six antimicrobial agents, i.e. Sulphonamide, Tetracyline, Chloramphenicol, Ampicillin, Gentamycin and Trimethoprim-Sulphamethoxazole are tested. They are easily available and commonly used in Burma.

Materials and methods

Bacterial isolates:

Enterotoxigenic *E. coli* were collected from etiological agents study of acute diarrhoea in children in Rangoon during early part of 1983.

Shigella isolates were collected from the same study during 1982-1983.

For Enterotoxigenic *E. coli* isolates, heat-stable toxin detection was done by infant suckling mice assay as described previously by Dean et. al. (2)

For heat-labile toxin assay, Biken test was performed as described by Honda et. al. (3)

For Shigella isolation and identification, procedures according to CDD manual for laboratory investigations acute enteric infections and Ewing's Manual on Identification of Enterobacteriaceae (4). The isolates giving characteristic biochemical reactions were further serotyped using commercially available antisera. All the Shigella isolates were finally tested their invasive property by Sereny Test. (5).

Antibiotic Susceptibility Testing:

Was carried out by disc diffusion technique of Kirby and Bauer Method (6).

Muller Hinton Broth and Muller Hinton agar were obtained from Difco, Detriot, U.S.A. Antibiotic disks were purchased from BBL, Becton Dickinson, U.S.A. The varieties antibiotic concentrations of the disks used were:-

1.	Sulphonamide (Su)	250ug
2.	Tetracycline (Tc)	30ug
3.	Chloramphenicol (Cm)	30ug
4.	Ampicillin (Amp)	10ug
5.	Gentamycin (Gm)	10ug
6.	Trimethoprim + (Sxt) Sulfamethoxazole	1.25ug + 23.75ug

Inoculum size was adjusted to 0.5 No. MacFarland Turbidity tube. All the procedures were followed and performed as described in WHO's guidelines for antimicrobial Susceptibility Test. (7)

Escherichia coli (ATCC 25922), multisensitive standard strain for quality control had been included in every batch testing. Definition of sensitive and resistant was made according to the zone size interpretive chart attached to the reagent.

All isolates given intermediate zones of susceptibility patterns were further evaluated and tested for minimal inhibitory concentration to that drug by agar dilution method as described (8). *E. coli* (ATCC 25922), *E. coli* RP4, and some highly

resistant clinical isolates were included as for both positive and negative controls.

Results

In this study, ETEC as a whole, the highest resistance was shown to sulphonamide (57%), followed by Tetracycline (48%), Chloramphenicol (37%) and Ampicillin (5%) in that order. The ETEC tested were always sensitive to Gentamycin and Trimethoprim-Sulphamethoxazole. It is interesting to note that resistant $ST^{+}LT^{+}$ *E. coli* strains carried same $Su^{r}Tc^{r}Cm^{r}$ resistant patterns. (71%)

Among LT^{+} *E. coli* strains, there were wide scattered distribution of resistant patterns, 63% isolates were resistant to sulphonamide, 32% resistant to Tetracycline, 16% resistant to Chloramphenicol and 5% to Ampicillin. The pattern of multiple drug resistance by ETEC was shown in Table 3. 2 strains of ETEC carried 4 drug-resistance (2.6%), 27 strains of ETEC carried 3 drug resistance (35%), 5 strains of ETEC carried 2 drug-resistance (5.3%) and 12 strains of ETEC carried one-resistance (16%). Only31 (38%) ETEC were sensitive to all six drugs tested.

Among Shieglla species, the highest resistance was shown to Sulphonamide (80%), followed by Tetracycline (37%), Chloramphenicol (30%) and Ampicillin (5%), it was all sensitive to Gentamycin and Trimethoprim-sulfamethoxazole. The pattern of multiple drug resistance of Shigella species was shown in Table 3. Resistance of 4 drugs was carried by 4 strains (14.8%), 3 drugs by 3 strains (17%), and 1 drug by 13 strains (48%). Only 11% of Shigella were sensitive to all drugs, which were all belonged to *Shigella sonnei* serogroup. Resistance to 4 drugs and 3 drugs were distributed among *Shigella flexnerei* serogroups.

Discussion

Although ETEC and Shigella are both factors responsible for acute diarrhoea in tropical countries. However, the role of therapeutic and preventive uses of antimicrobial drugs in ETEC diarrhoea is controversial.

In some parts of the world, e.g. Kenya, Morocco, United States and Bangladesh, ETEC strains found are unusually sensitive to

antibiotics, whereas, reports from Philippines, Thailand, Honduras, ETEC strains are highly resistant (9).

Our results of sensitivity patterns of ETEC were similar to the findings from the authors of Thailand and Philippines (10) except that strains resistant to Gentamycin and Trimethoprim-Sulfamethoxazole were not encountered in this study yet.

It is well understood that Shigellosis is highly infectious disease and Shigella species are notorious for their ability to develop multiple drug resistance. It is also noted that Shigella dysentry has to be treated with specific antimicrobial agents like Ampicillin, Trimethoprim-Sulfamethoxazole, Tetracycline and Nalidixic acid (11). This base line study will provide an information on drug sensitivity patterns of Shigella species isolated in Burma. The Shigella strains tested were shown resistant to Sulphonamide, Tetracycline, Chloramphenicol and Ampicillin with high degree of multiple drug resistance. Only 11% were sensitive to 6 drugs tested. It was interested to note that antibiotic sensitivity patterns on 86 isolates collected during 1971-1972 as reported by Dr. Khin Mya Than gave different picture (12). From their study, the Shigella isolates carried 4-5% resistance to Tetracycline, Chloramphenicol, Ampicillin and 40% to Streptomycin. By comparing to our data, it denotes that probably drug resistant Shigella isolates appear within the last ten years.

Table 39 Resistant pattern of enterotoxigenic E. coli No. (%)

Antibiotic disks / No. of strains used	Su	Tc	Su	Amp	Gm	Sxt
ST^+ = 39	19(49)	18(46)	13(33)	3(8)	0	0
LT^+ = 19	12(63)	6(32)	3(16)	1(5)	0	0
ST^+LT^+ = 17	12(71)	12(71)	12(71)	0	0	0
Total = 75	43(57)	36(48)	28(37)	4(5)	0	0

Table 40. Resistant pattern of Shigella isolates No. (%)

Antibiotic disks / No. strains used	Su	Tc	Cm	Amp	Gm	Sxt
S.flexneri = 16	16(100)	9(56)	6(38)	5(31)	0	0
S.boydii = 2	2(100)	0	0	0	0	0
S.sonnei = 9	6(66)	1(11)	1(11)	0	0	0
Total= 27	24(88)	10(37)	7(26)	5(19)	0	0

Table 41 Multiple drug resistance by ETEC and Shigella

Resistant to	ETEC=75		ST^+=39		LT^+=19		ST^+LT^+=17		Shigella=27	
	No.	%	No	%	No	%	No	%	No	%
One drug	12	16	4	10.2	8	42.1	0	0	13	48.1
Two drugs	5	5.3	3	7.6	2	10.5	0	0	4	14.8
Three drugs	27	36	13	33.7	2	10.5	12	70.5	3	11.1
Four drugs	2	2.6	1	2.5	1	5.2	0	0	4	14.8
Sensitive to all drugs	31	38	18	46.1	6	31.5	5	29.4	3	11.1

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2-6 Plasmid Research

The genetic information coding for production of heat-labile and heatstable enterotoxins has been found to be carried on a family of closely related plasmids. (1)

With the advents of DNA technology, similar, quicker and cheaper micromethods have been developed recently. (2) A large number of isolates can be screened for the presence of plasmid within a short period.

Since Enterotoxigenic *Escherichia coli* is assuming one of the common causes of childhood diarrhoea, it will be both interesting and useful in studying genetic traits of clinical isolates.

This study is initiated to look insight into the plasmid profiles among ETEC strains isolated from children under 2 1/2 years suffering from acute diarrhoea.

Materials and methods

Bacterial strains:

Five enterotoxigenic E. coli producing both ST and LT toxins isolated from Burmese children under 2 1/2 years suffering from acute diarrhoea, were selected for this study. These ETEC isolates were suspended in 20% glycerol with Trypticase soy broth and stored at 80°C deep freezer.

Control strain for plasmid DNA:

E. coli V 517 strain carrying plasmid marker with M.W. 35.8 x 10^6 to 1.4 x 10^6.

Toxin assays:

For ETEC isolates, heat-stable toxin detection was done by infant suckling mice as described preivously by Dean et. al. (3).

For heat-lable toxin assay, Chinese Hamster Ovary cell assay (4). Biken Test (5) and GM^I microtiter ELISA method (6) were carried out.

Antibiotic susceptibility testing:

Was done by disc diffusion technique by Kirby-Bacur method. Media and Discs were obtained commercially. Procedures was carried out as described (7).

Preparation of plasmid DNA:

Plasmid DNA of bacterial cells was isolated and purified as described by Birhoim, H.C. et. al.(2)

Agarose Gel Electrophoresis of plasmid DNA:

The plasmid compositions of these ETEC isolates were examined by Agarose Gel Electrophoresis. A portion (10-12ul) of the DNA preparation was electrophoresed at 20 milliampere constant current for 2 1/2 to 3 hours in a horizontal slab gel apparatus. (gel dimension: 125x85x3mm) with 8 slots (9x9x1.5mm). Agarose gels (0.6% of Wake 1600 agarose) were prepared in Electrophoresis buffer (89 mM Tris, 2.5mM EDTA disodium salts and 89 mM boric acid pH 8.3). After electrophoresis, plasmid DNA was stained with Ethidium bromide (0.5 ug/ml) for 30 minutes and destained for another 30 minutes in distilled water. It was photographed under incident short wave UV light (Atto UV illuminator, Japan) by Asahi Pentax Camera with UV filter using black and white film of Neopan ASA 400. The focal length used was 2.6 with exposure time 4-6 minutes.

Results:

Five strains of ST^{+}-LT^{+} enterotoxigenic E. coli were isolated from children with acute diarrhoea. The distribution of age, sex and antibiotic resistance patterns were shown in Table 1. The plasmid compositions of these five isolated as well as plasmid marker strain V517 were seen in the Fig. 8. All the isolates carried plasmid varying from 2 to 5 numbers with molecular size ranged approximately from greater than $32x10^6$ to $3.7x10^6$ daltons.

Discussion:

Since there is a such heterogenicity existed between five different ETEC isolates. Further characterisation and detailed study

will be necessary inorder to understand the nature and function of each plasmid composition.

Table 42. Sources and characters of Enterotoxigenic Escherichia coli used in this study

Isolate No.	Age & sex of source	Type of entero-toxin	Antibiotic resistance
1. W81/82	1yr, F	$ST^{+}LT^{+}$	all sensitive
2. W91/82	1/2yr, F	$ST^{+}LT^{+}$	$SM^{r}Tc^{r}Cm^{r}Su^{r}$
3. W203/82	12/3yrs, F	$ST^{+}LT^{+}$	all sensitive
4. W261/82	210/12yrs, M	$ST^{+}LT^{+}$	all sensitive
5. W282/82	2/3yr,M	$ST^{+}LT^{+}$	all sensitive

A B C D E F

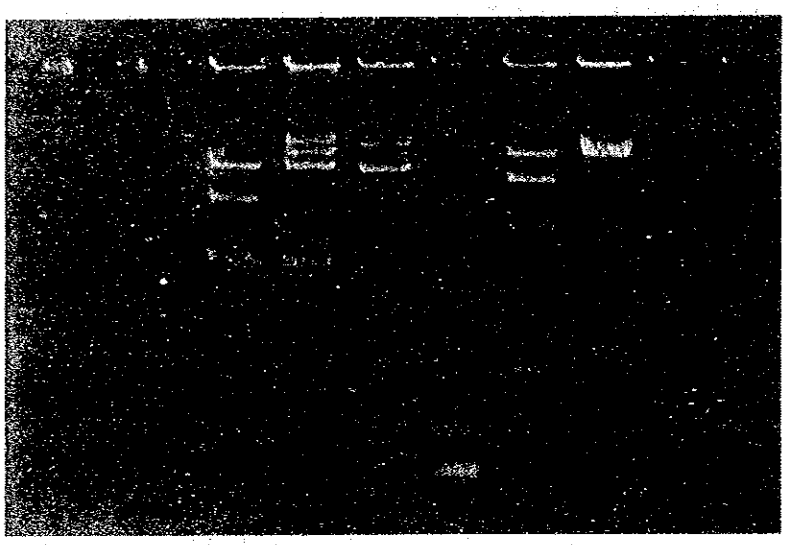

Figure 8. Agarose Gel Electrophoresis of ethanol-precipitated DNA from Escherichia coli lysates of Enterotoxigenic clinical isolates and control E. coli V517 carrying plasmids of known molecular weights. A-W81/82, B-W91/82, C-W203/82, D-V517, E-W261/82, F-W282/82.

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2-7 Biochemical Research Heat Labile Toxin (LT) of *Escherichia coli*

(a) Purification of Heat Labile Toxin (LT) of Enterotoxigenie *Escherichia coli*

The two virulence factors namely (1) the ability to colonize the small intestines and (2) the production of enterotoxin give the pathogenicity of E. coli which causes acute diarrhoea. It was shown that the heat-labile enterotoxigenic known as LT produced by certain strains of E. coli is a protein remarkably like cholera toxin (Clements & Finkelstein, 1979; Fishman, et al., 1978; Gill, 1976). It contains several B subunits of 11,780 molecular weight (Dallas & Falkow, 1980) and A subunit, estimated as 25,500 (Dollas et al.,1979) to 30,000 (Kunkel & Robertson, 1979) molecular weights. Its subunit number and arrangement was shown by Gill et al., 1981. The hybrid toxin was performed by Takeda et al., 1981 and demonstrated that the hybrid toxins show a similar toxicity to that of the parent toxins from which the A subunits were derived. The immunological and biological similarities of LT and CT were also well established (Clements & Finkelstein, 1978 a, b; Donta, 1974; Evans et al., 1973; Gyles, 1974; Gyles & Barnum, 1969; Honda & Finkelstein, 1979; Honda et al., 1976, 1981 a, b; Richardson et al., 19yy; Smithe & Sack, 1973). Specification of CT and LT was also shown by Honda, whereby, each have a unique antigenic determinant.

MATERIALS AND METHODS

Bacterial strain and culture of cells

Escherichia coli RIMD 526-2; brought by Dr. H. Hayashi; obtained from Dr. Y. Takeda from BIKEN was cultured in 9 litres of CAYE media with vigorously shaking (Takasaki shaking incubator, 280 rpm/min.) at 37°C overnight, caye media contained 2 per cent Casamino acid (Difco); 0.6 per cent Yeast Extract (Difco) 0.25 per cent sodium chloride; 0.871 per cent K_2HPO_4; 0.25 per cent glucose and 0.1 per cent v/v trace salt solution (5% $MgSO_4$; 0.5% $MnCl_2$; 0.5% $FeCl_3$ and 0.001% H_2SO_4). The medium was supplemented with 90 ug of lincomycin hydrochloride per ml of media (UpJohn) to enhance the synthesis of LT (Honda et al., 1981; Levner, 1977)

Preparation of crude LT

Crude LT was prepared from whole cell lysate as it is the richest source of LT (Clements & Finkelstein, 1978). Approximately 9 litres of bacterial culture was centrifuged at 10,000 rpm for 20 mins at 0°C and pellet cells were suspended in approximately 300 ml of 0.01 M Tris (hydroxymethyl) aminomethane (Tris)-hydrochloride buffer (pH 8.6) containing 0.9 per cent sodium chloride. The suspension was sonicated in an ultrasonic disrupter sonicator for 5 mins. The sonicated suspension was spin at 15,000 rpm for 3 hours. The supernatant was then collected and solid ammonium sulphate was added to give 65 per cent saturation. The precipitate was collected by spinning at 10,000 rpm for 30 mins and suspended in about 200 ml of 0.05 M Tris-HCl buffer pH 7.4, containing 1 mM Ethylenediamine-tetraacetic acid; 3 mM sodium azide and 0.2 M sodium chloride (TEAN buffer). It was then dialysed twice against 2 litres of TEAN buffer and used as crude LT preparation.

Purification of LT

LT was partially purified by passing 200 ml of crude LT to a Biogel column (90 x 200 mm) equilibrated with TEAN buffer. The column was washed with 400 ml of TEAN buffer and LT was then eluted with 400 ml of 0.3 M D-galactose solution in TEAN buffer (Fig. 9). Fractions containing LT was assayed by Ouchterlony double gel

diffusion test with anti-purified cholera toxin antisera (DMR); were collected and concentrate to about 3 ml by Amicon PM 10 membrane filtration (Amicon Corp.). The preparation was then applied to a Sephacryl S 200 column (90 x 200 mm) equilibrated with TEAN buffer. The column was eluted with the same buffer and the fractions containing LT were collected, concentrated by Amicon PM 10 membrane filtration and used as purified LT.

Preparation of anticholera toxin

25 ug of purified CT (Sanko Junyaku Co.) in 1 ml of phosphate buffered saline (pH 7.2) was emulsified with an equal volume of Freund's complete adjuvant (Difco). The emulsion was inoculated subcutaneously into young Japanese White rabbits weighing about 1.8 kg each. 2 booster injections were given on day 30 and day 50 with 25 ug of toxin emulsified with Freund's complete adjuvant. The highest dilution of the antisera obtained which give a precipitin line against 30 ug of homologous toxin in Ouchterlony gel diffusion plate was 1: 16; and at that time the rabbits were bled and sera was taken.

Purification of specific immunoglobulins

It was done by ammonium sulphate precipitation method, dialysed against PBS and protein estimation was done as according to the method of Lowry, 1951.

Immunoaffinity column chromatography of Anti CT

Immunoaffinity column chromatography was carried out as 1 g amount of Cyanogen bromide activated Sepharose 4B (Pharmacia) was coupled with 7 mg of purified CT. A 1 ml amount of anti CT was applied to a column of cyanogen bromide activated Sepharose 4 B coupled with CT. The column was washed with PBS (pH 7.2) until the optical density of the elute at 280 nm reached a basal level. The immunoglobulin bound to the column was then eluted with 0.2 M glycine-hydrochloride buffer (pH 2.7) containing. Column was then eluted with 0.8 M glycine-hydrochloride buffer (pH 2.7) containing 0.5 M sodium chloride. After recovery of the specific antisera,

pH was readjusted to about 7.2, as soon as possible. This specific antisera was for the detection of LT throughout the study.

Gradient SDS Polyacrylamide gel slab electrophoresis

This was done as according to the method of Laemali, 1970. The gels were 13.0 per cent acrylamide and electrophoresis at a constant current of 8 MA, overnight. The gels were stained with Coomasie Brilliant blue.

Protein Assay

Determination of protein was done as according to the method of Lowry, et al., 1951.

Ouchterlony test and Immunoelectrophoresis

1.2 per cent of Noble agar and Agarose were used respectively for the detection of specific antigens.

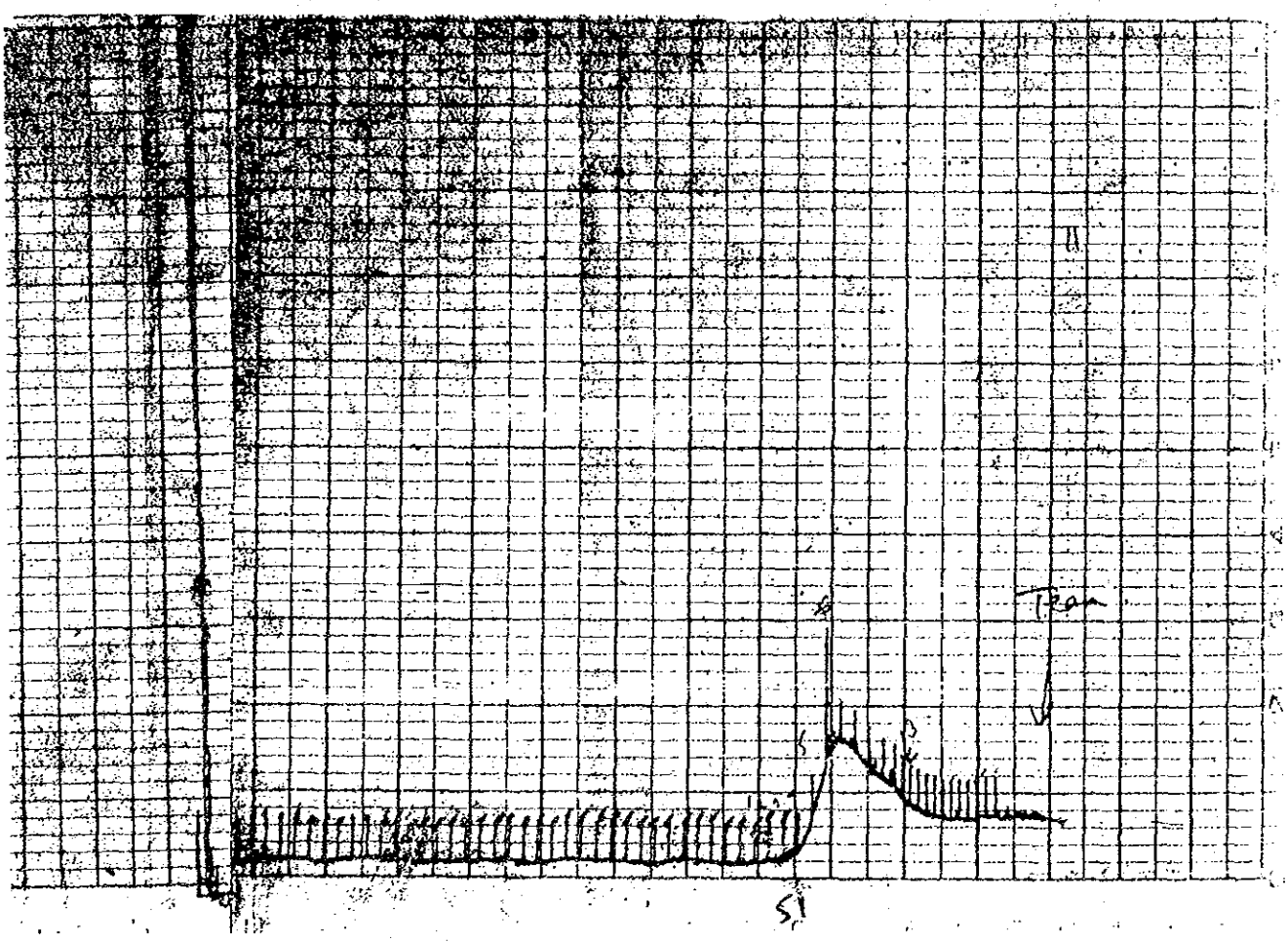


Fig. 9 Separation of partially purified LT after passing through Biogel A 5 %

Chinese Hamster Ovary Cell Assay

It was done as according to the method of Honda et al., 1976, using Eagle MEM (Nissui) and Foetal Calf Serum (Flow).

Biken test

It was done as according to the method of Honda et al., 1981.

Results

Prior to the purification of heat-labile toxin of *Escherichia coli*, anticholera toxin was prepared in Japanese White rabbits using a. pure CT. These were checked by double gel diffusion test. The specific antisera was obtained after affinity chromatography with a titre of 1:16.

Separation of partially purified LT was shown in Fig. 4, by passing the crude LT through BioGel A5M and protein amount recorded at 280 nm wavelength using a UV monitor. The small peak after collecting was then checked by double gel diffusion technique against anti CT was concentrated to about 3 ml by Amicon PM 10 filtration. The preparation was then applied to Sephacryl S 200 column to obtain a pure LT. It was then concentrated and was again checked by double gel diffusion technique against anti CT after affinity column chromatography (Fig. 10). The toxin was then checked by CHO cell assay and the minimum dose to produce cell elongation was found to be about 10 ng/ml.

The purified LT was then checked by SDS gel electrophoresis (Fig. 11) showing the difference in molecular sizes of subunit A when compared with CT (Fig. 12).

Discussion

It is well established that CT and LT are immunologically related. The present study demonstrated also that CT and LT are antigenically related but not identical (Fig. 10). These results suggest that they share a common antigen determinant(s) and, in addition, that each enterotoxin possess its own antigen determinant(s).

By SDS slab gel electrophoresis, the subunit A of CT and LT shows different molecular weights as is found by other investigators.

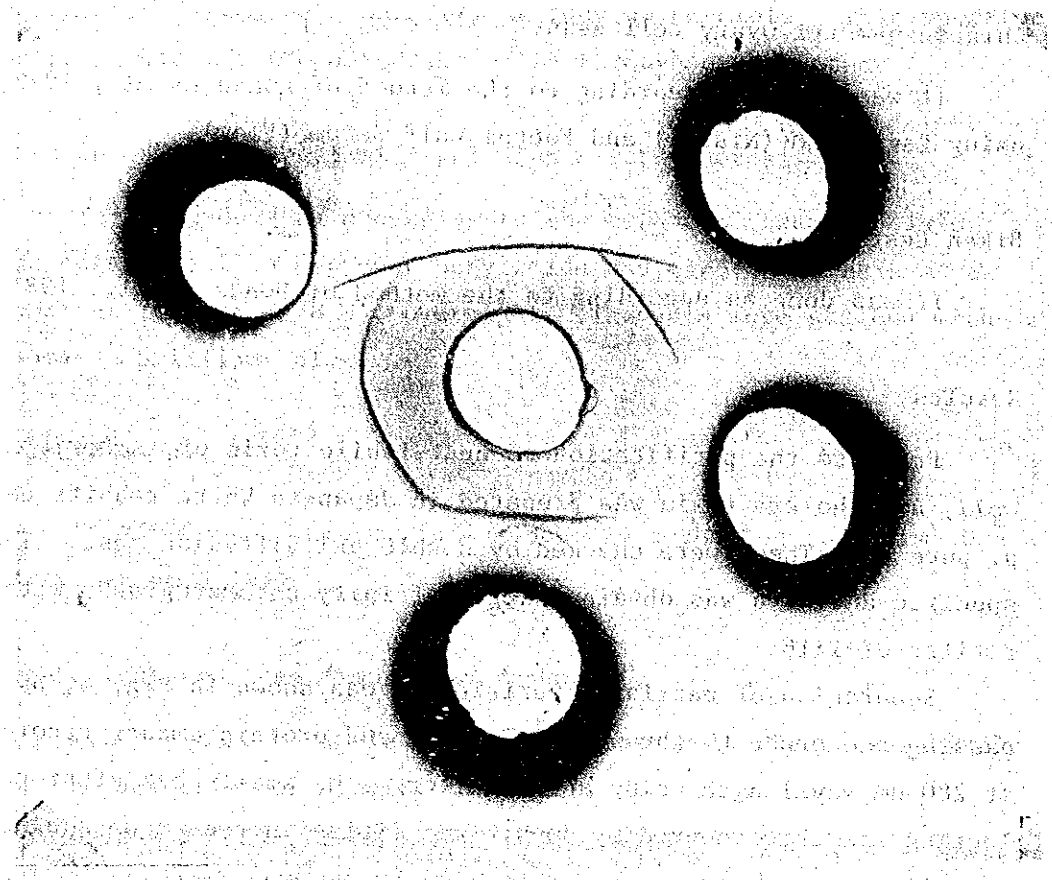
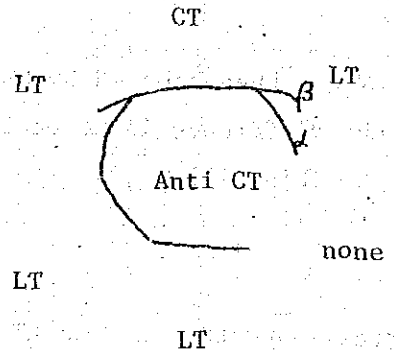
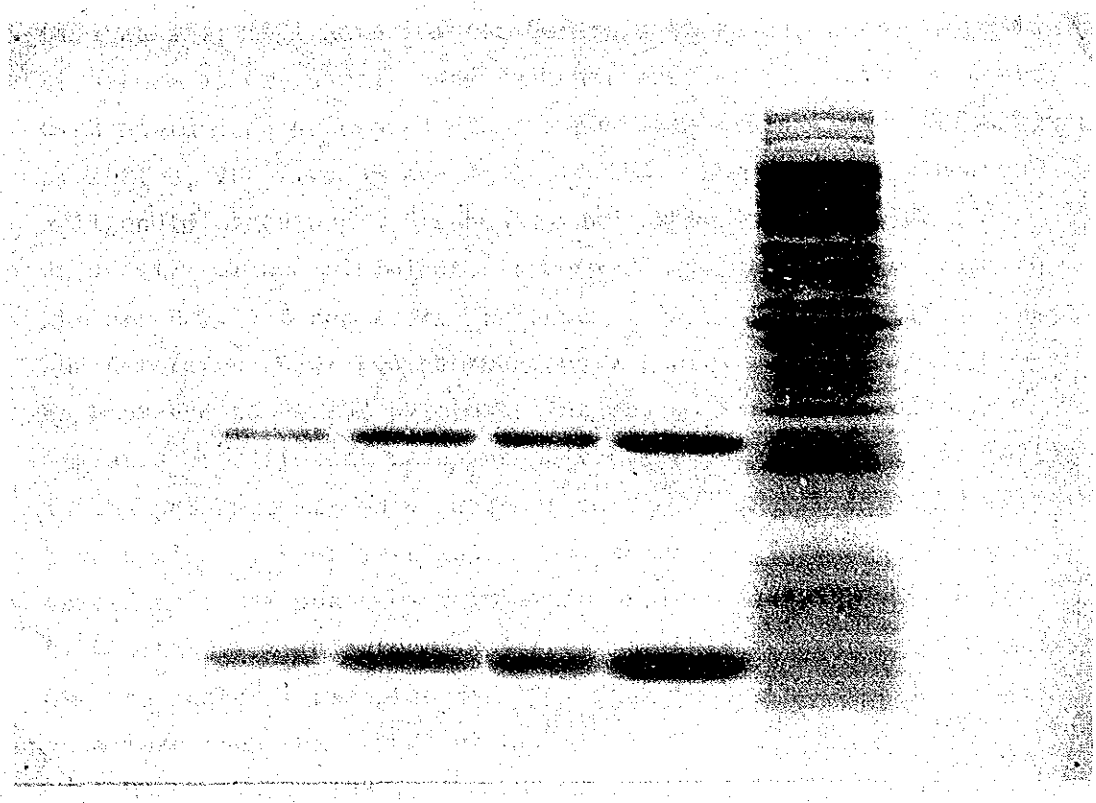


Fig. 10 Ouchterlony double gel diffusion test showing LT and CT are antigenically related but not identical





LT_h1 LT_h-2 LT_h-2 LT_h-2 crude LT

Fig. 11 Preparation of pure LT comparing with crude LT in SUS-PAGE electrophoresis

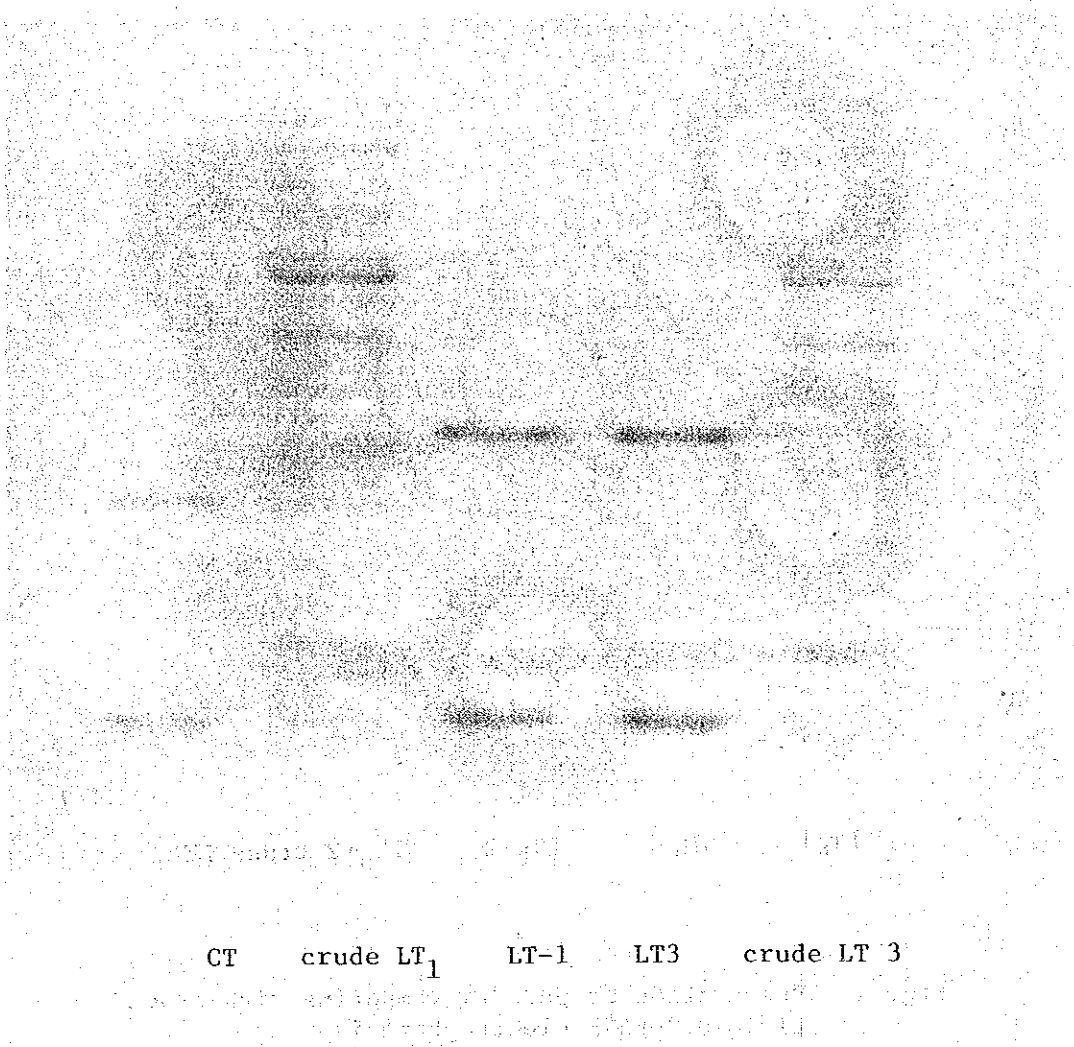


Fig. 12 Comparison of LT and CT by SDS PAGE electrophoresis

Gill et al., 1981 have pointed out that LT have the same subunit structure as CT, namely one A subunit and 5 B subunits. Reaction with bisimidate generated all the possible cross linked derivatives of A5B, B, 2B ..... 5B and A AB ... A5B. The isolated B components coligenoid contained 5 B subunits and showed some tendency to polymerize with a bisimidate it became covalently connected into the set B ... 5B and lesser amounts of 6B .. 6B .. 10B etc. The subunit formulas of two independently prepared samples of LT were both proved to be A5B by cross linking, but their B pentamers migrated at different rates on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate, indicating that they have different conformations. The faster (R) form could be converted to a diffuse slower (C) form by incubating at 50°C or at 37°C with 0.2 M galactose, which is the terminal sugar of ganglioside GM_1, the natural receptor for LT. Cholera toxin resembled the R form more than the C form of LT.

Moss et al., 1982, also showed that LT A and B subunits possess properties similar to the choleragen counterparts. B subunit binds to ganglioside GM_1 and its oligosaccharide; GM_1 on the cell surface may serve as a recepter for LT. The toxin which is not proteolytically 'nicked' during isolation, exhibits increased ADP-ribosyltransferase activity after incubation with trypsin, presumably because the catalytically active component is a free peptide analogous to A, to choleragen. The fact that some LT preparations are unnicked and therefore dependent on a protease as well as thiol for activation, whereas choleragen preparations are nicked and thus only require very similar toxins on target cells.

The specificity of the LT gangliosides interaction, like that of choleragen GM_1 interaction, appears to reside with the B subunt of the enterotoxin and the oligosaccharide moiety of GM_1. GM_1 oligosaccharide but not GD_1 a oligosaccharide or neuramin lactose "blue shifted" the λ mass of LT and its protomere, GM_1 oligosaccharide had no effect on the fluorescence spectrum of the A subunit. Majority of the A subunit of LT was apparently not nicked during isolation.

It will be interesting to determine which fragments of the subunit molecules are necessary for association of their A and B

subunit and whether these fragments of CT and LT really have the same molecular structure and their role of mechanism in the pathogenesis of diarrhoea.

2-7-(b) *Preparation and purification of Anti-LT*

Immunological and biological similarities between cholera enterotoxin (CT) and *Escherichia coli* heat labile enterotoxin (LT) have been demonstrated by many workers (Clements & Finkelstein, 1978 a, b; Dona 1974; Evans et al, 1973; Gyles, 1974; Gyles & Barnum, 1969; Honda & Finkelstein, 1979; Richardson et al., 1977; smith & Sack, 1973). Honda et al, 1981 showed that the use of anti LT showed more specific reactions towards LT producing *Escherichia coli* than anti CT. For further research, the specific antisera is required as there are also immunological nonidentity of heat-labile enterotoxin such as human and porcine ETEC (Honda et al., 1981). Clements & Finkelstein, 1978b, clearly showed that LT is immunologically related to subunits A and B of CT by neutralizing of activities and by double gel diffusion technique. Honda et al., 1981 also demonstrated that the antiserum which reacts with CT and not with LT and vise versa; and isolation of a common antibody against both CT and LT.

MATERIALS AND METHODS

Preparation of antisera against purified LT

25 ug of the purified LT from *Escherichia coli* (BIKEN 536-2, human strain) in phosphate buffered saline, pH 7.2; emulsified with an equal volume of Freund complete adjuvant (Difco) was immunized subcutaneously into each young Japanese White rabbits about 2 kg at the back and hind limbs (Hamashima, 1976). A booster injection was also done as the same way after 45 days. The titre of the sera was checked by Ouchterlony and immunoelectrophoresis, and in CHO cell assay for neutralizing property.

Double gel diffusion test

The double gel diffusion test was done as described by Ouchterlony 1949, with 1.2 per cent Special Noble Agar (Difco) in TEAN

buffer which consists of 0.05 M Tris (hydroxymethyl-aminomethane, 1 mM ethylene diamine tetraacetic acid disodium salt, 3 mM sodium azide and 0.2 M NaCl)(pH 7.40) After the samples were applied, the plates were placed in a humidified chamber at RT overnight. The plates were washed extensively and stained with 0.5 per cent Coomasie brilliant blue in a solution containing 50 per cent methanol and 10 per cent acetic.

Immunoelectrophoresis

It was done by using 1.2 per cent Agarose (Sigma) in veronal buffer pH 8.6. Electrophoresis was run at a rate of 2 mA/cm, as according to the method of Hamashima, 1976. The gels were stained by 0.5 per cent Coomasie Brilliant blue as in the previous experiment.

Neutralizing test using CHO cell assay

The CHO cell assay was carried out by using Eagle MEM (Nissui) in place of F 12 originally described by Guerrnat et al. (1974), and modified by Takeda et al., 1981; using 10 per cent Calf serum (Gibco) and 1 per cent Foetal Calf serum (Flow) for cell culture and 1 per cent Foetal Bovine serum for assay of toxins. Anti LT was administered at the time of assay with 2 fold dilutions.

Titre of Anti LT

It was determined as the method according to Honda et al., 19 , using double gel diffusion test. The antisera was then purified to receive a specific immunoglobulins by ammonium sulphate precipitation and then followed by affinity column chromatography.

Biken test

It was conducted as according to the method of Honda et al., 1981. CAYE Media with glucose, supplemented with lincomycin was used 26 standard strains and 61 unknown strains were used and compared with BIKEN antisera.

Results

The anti LT obtained from individual rabbits were determined by Ouchterlony double gel diffusion test. All the three rabbits showed a good response against purified LT. Fig. 13 and 14 showing different preparations of LT, including before and after passing through Sephacryl and comparing with CT.

The antiserum obtained was then diluted to its optimum dilution as according to Honda et al., 1981 and was used to study in BIKEN test using 26 standard strains from WHO and 61 unknown strains from hospital cases, from Multicentre study. It was found that there was a high correlation among the tested strains.

The antisera so obtained was tested in CHO cell assay for neutralizing and found that it neutralizes well up to the titre of 512; using both CT and LT.

Discussion

Honda et al., reported that the BIKEN test was simple and convenient. When anti LT preparation was used, all the LT strains give a line of identity on Agar (BIKEN) and similar results were obtained with porcine strain, although some of them did not give a definite precipitin line, probably because the precipitating antibody is in anti human LT antiserum was too weak, so does the precipitin between porcine E. coli and anti human LT antisera was weaker than that between human E. coli and anti human antiserum.

Dallas & Falkow (1980) and Spicer et al (1981) also reported similarities and differences in the amino acid sequences of the B and A subunits of CT and porcine LT. The differences probably explain the immunological nonidentity between CT and porcine LT. It will be interesting to know how much difference there is in the amino acid sequence of porcine LT and human LT. It will be also interesting to examine the immunological relatedness of LTs from other animal such as cows, and sheep and to examine that other types of LT could found from diarrhoeal cases.

Honda et al, 1982 reported the usefulness of the BIKEN test for the detection of LT-producing E. coli in a large scale field study. They showed a sharper and clearer precipitin line than did

anti CT when anti LT was used. This was probably because in addition to an antigenic determinant(s) common to both LT and CT, LT and CT each have specific antigenic determinant. Some strains which gave positive results with BIKEN test only might have been producing either B subunit alone or nontoxic cross reacting material(s). Further study is necessary to elucidate the nature of the product(s). Some of the strains might produce an enterotoxin with different antigenic determinant(s).

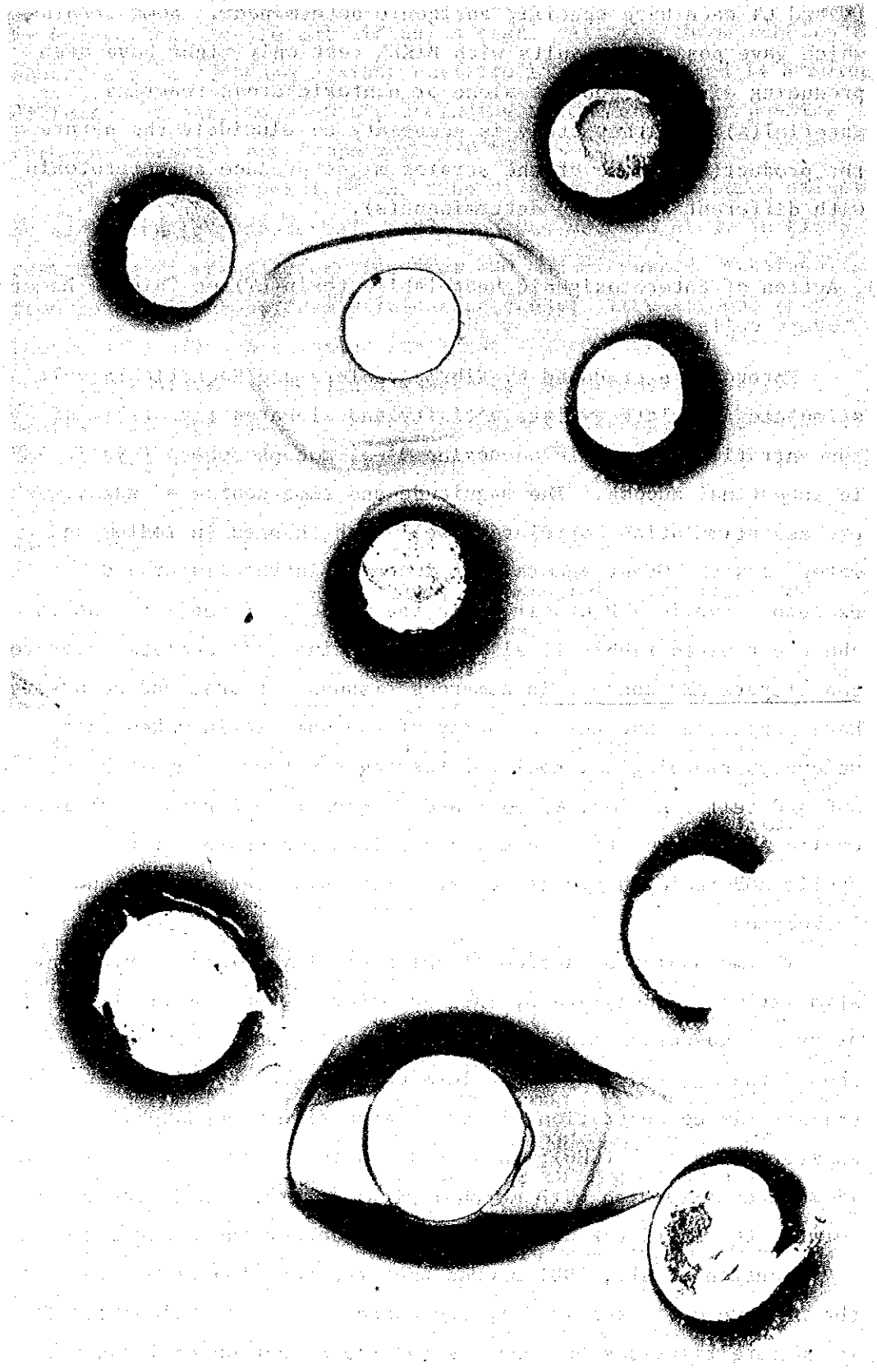
2-7-(c) Action of Enterotoxigenic heat-labile toxin(LT) on Chinese hamster ovary cells

Enterotoxin produced by Vibric cholera and Escherichia coli stimulate adenylate cyclase activity and elevates intracellular concentrations at cyclic adenoxine 3'-5'-monophosphate (cyclic AMP) in intestinal mucosa. The magnitude and time course of adenylate cyclase stimulation correlation well with changes in medium and water fluxes. Other agents that increase intracellular and exogenous cyclic AMP itself mimic the affects of these toxins in short circuited rabbit ileal mucosa. Noting that cholera enterotoxin can elevate AMP content in numerous tissues. Bourne and coworkers have suggested that this property of the same toxin makes it a unique pharmacological tool for testing hypothesis involving cyclic AMP and cell function. *E. coli* and *V. cholera* enterotoxin stimulate cyclic AMP accumulation in thyroid elices and cholera toxin induces cyclic AMP mediated events in fat cells, platelets, liver and leukocytes.

The mechanism of action of antigenically related toxins involves stimulation of adenylate cyclase and elevation of intracellular level of cyclic adenosine monophosphate. Guerrant and coworkers, 1974 showed that *E. coli* and V. cholera enterotoxins could elevate intracellular concentration of cyclic adenosine monophosphate in Chinese Hamster Ovary cell (CHO); these changes in cyclic nucleotide metabolism were correlated with morphological alterations in the cells forming the basis for a simple assay to detect these enterotoxins.

Houston et al., 1981 showed that the addition of mytomycin to the culture media resulted in increased amounts of Salmonella Toxin in culture filtrates but had the reverse effect on cell sonic

Fig. 13 and 14. Checking of anti-LT



extracts. They suggested that the increased amounts of Salmonella toxin culture filtrates caused by mitomycin C were due to cell lysis, resulting the release of intracellular toxin rather than to an increase in the synthesis of Salmonella toxin. Clements and Finkelstein, 1979 demonstrated the heat-labile enterotoxin (LT) has been isolated in homogeneous form with high specific activity from three sources: cell free supernatant, NaCl extract and whole cell lysates of an enterotoxigenic *Escherichia coli* strain. They reported that the whole cell lysate is the richest source of LT. Evans et al., 1974 also demonstrated a rapid release of enterotoxin by using Polymyxin B induction. Incubation of cells derived from older (18hr) cultures with polymyxin caused the release of both low (20,000) and high molecular weight forms of enterotoxin. They concluded that either the 20,000-dalton form of heat labile enterotoxin is not released by E. coli under in vitro growth conditions or that enterotoxin released in this form is rapidly destroyed or inactivated.

The purpose of this investigation was to study the different preparations of enterotoxin produced by enterotoxigenic *E. coli* with regard to its release of enterotoxin, for monitoring the biological activities and antigenic presence of the toxin. This is also part of the work to study the peptide profiles of enterotoxigenic *Escherichia coli* for the determination of antigenic similarities and variations.

Materials and methods

Chinese Hamster Ovary Cell Assay (CHO)

The established cell line from BIKEN is maintained in Eagle MEM (Nissui) containing glutamine and bicarbonate. 10 per cent Calf serum (Flow) and 1 per cent Foetal Calf Serum (Gibco) are also supplemented. For an assay, the cells were grown in 25 cm^2 Corning tissue culture flask for 3 days. Just before assay, the culture fluid was decanted and washed three times with PBS pH 7.2. The cells were trypsinised by trysene versene (Dr Mi Mi Khin) and keep the flask up for 2-3 mins. The cells were then adjusted to 50,000/ml in MEM containing 1 per cent Foetal Calf Serum. 0.25 cells were dispensed into each Lab Tek Chamber IMiles)

or 0.2 ml into each microtitre wells (Falcon). Approx. 0.02ml of toxin to be investigated was added to each well including both positive and negative standard controls. The slides/plates were then kept at 37°C overnight in a Carbon dioxide incubator (14-16 hrs). The slides were recorded negative when spindle shape is less than 10 per cent of the coli population;one plus when morphology of the cells changes to 20 to 40 per cent and is recorded two plus when morphological alteration is more than 40 percent. The spindle shape (elongation is defined as bipolar and three times longer than width. Lab Tak slides were stained with Giemea and the cells counted.

Preparation of crude enterotoxigenic Escherichia coli toxin

Method 1. Trypticase Soy broth (TSB, BBL)

E. coli (LT) producing strain (536.2) was cultured in 1.5 ml of trypticase Soy Broth (TSB, BBL) in a universal container (28 ml). The culture was shaken well in Takasaki shaking incubator (280 rpm) overnight at 37°C. The broth culture was spinning at 10,000 rpm in a refrigerated centrifuge (Tomy) for 30 mins. The supernatant was collected in vials containing 50 ug/ml of gentamycin.

Method 2. Trypticase Soy Broth (TSB) with 0.6 per cent Yeast Extract (Difco)

F. coli (LT) producing strain (536.2) was cultured in 1.5 ml of trypticase Soy broth (TSB, BBL) containing 0.6 per cent Yeast Extract (Difco) in a 28 ml Universal container. The culture was allowed to stand stationary for 48 hrs at 37°C. The broth culture was spinned at 10,000 rpm in a refrigerated centrifuge (Tomy) for 30 mins. The supernatant was collected in vials containing 50 ug/ml of gentamycin.

Method 3. CAYE media and treated with Polymyxin B sulphate

E. coli (LT) producing strain 536.2 was cultured in 5 ml of CAYE media (Cassamino acid 2 per cent; yeast extract 0.6 per cent, sodium chloride 0.25 per cent, disodium phosphate 0.871 per cent, glucose 0.25 per cent and trace salt 0.1 per cent). The culture was shaken well in Takasaki shaking incubator overnight at 37°C.

The broth culture was spinned at 3,000 rpm for 20 mins at 4°C; treated with Polymyxin B sulphate (Pfizer) 50,000 Upper ml) for 30 mins and spinned again at 10,000 rpm for 30 mins in a refrigerated centrifuge.

Method 4. CAYE media and sonication

E. coli (LT) producing strain (536.2) was cultured in a 5 ml of CAYE media as described elsewhere and supplemented with 90 ug/ml of lincocin (Upjohn) to enhance the synthesis of LT production, as described by Takeda et al., 1981. The cells were pelleted by centrifugation at 10,000 rpm for 30 mins at 4°C. The pelleted cells were then suspended in about 0.18 ml of 8.01 M tris (hydroxymethyl) aminomethane (Tris)-hydrochloride buffer (pH 8.6) containing 0.9 per cent sodium chloride. The suspension was sonicated in an ultrasonic (Tomy) using microtip for 2 mins. The supernatant was recovered by spinning at 10,000 rpm for 30 mins.

Results

The present paper explained that the action of *E. coli* toxin on CHO cells under phase contrast microscopy (10x) and after staining with Giemsa (10X) and (40X) respectively.

Fig. 15 shows the sensitivity of toxin against CHO cells, responding that CHO cell assay is capable of detecting as little as 0.01 ng of cholera toxin and 10 ng of LT. This assay was based on the capacity of enterotoxin to increase the adhesiveness of proliferation of CHO cells, causing them to accumulate on adherant monolayers.

Fig. 16-17 shows morphological changes of CHO cells before and after by P/B treatment.

Fig. 18-19 showing morphological changes with time intervals, resulting that the cells are transformed into round and swollen and loses their bipolar ends making the cells to degenerate or back to its normal state and then division takes place; which also depends upon the type and titre of the toxin.

Fig. 23 shows the morphological changes of CHO cells, using different preparations of toxins. 18 strains of E. coli were used

and preparation was done three times. It was found that the whole cell lysate obtained by sonication shows the richest source of toxin in most cases yielding up to the titre of 10^{-11} dilutions, resulting that they were detected by Ouchterlony double gel diffusion test whereby other preparations fail to do so.

Fig. 24 & 25 shows the effect of various toxin sera on CHO cell assay respectively.

Discussion

Guerrant et al., showed that the initial studies showed that CT (lug/ml) produced an elevation of intracellular cyclic AMP in each of 18 different mammalian cell lines. CHO cells showed the morphological responses maximal at 24 hr after CT exposure with cells grown in 1 per cent foetal calf serum. Since serum has been shown to inhibit CT effect on Hela cells (Davies et al., 1973); Guerrant et al., examined the effects of foetal calf serum upon ECT 334 stimulated cells. They showed 50 and 95 per cent reduction in ECT 334-stimulated cyclic AMP levels by 1 and 10 per cent foetal calf serum respectively. The elongation, polarization and loss of knoblike projections of CHO cells are striking by 24 hrs after exposure to a 0.08 dilution of ECT.

Clements & Finkelstein, 1979, showed that the LT enterotoxin has been isolated in homogeneous form with high specific activity from 3 sources (1) cell free supernatant (2) sodium chloride extract and (3) whole cell lysate of an ETEC strain and found that the whole cell lysate is the richest source of LT.

Evans et al., 1974 reported that polymyxin B caused the rapid release of enterotoxin with a molecular weight of approximately 20,000. Incubation of cells derived from 18 hrs culture with polymyxin caused the release of both low 20,000 and high molecular weight forms of enterotoxins and concluded that either the 20,000 dalton forms of LT enterotoxin is not released by E. coli under in vitro growth conditions or that enterotoxin released in this form is rapidly destroyed or inactivated.

ETEC have been associated with severe human diarrhoea in numerous parts of the world. Although these E. coli isolates include countless different serotypes immunological studies have

been demonstrated only one antigenic form of heat-labile ECT. The LT is predominantly intracellular: relatively little is found extracellular or in extracts of the cells. It appears that toxigenic *E. coli* may resemble that *V. cholera* mutant with regard to a relative insniliyy to transport intracellular toxin. The relevance of this observation, if any, to the relatively mild and shortlived diarrhoea generally elicited by ETEC is deserving of further study. Certainly, wild type *V. cholera* strains which secrete orders of maginitude less cholera enterotoxin than 569 B in vitro are equally virulent in vivo. Likewise, the pertinence, to the lower severity of E. coli diarrhoea, showed that the activity of E. coli LT benefits from proteolytic processing is not immediately cleared. There appears to be ample proteolytic activity in the succus entericus to activate LT in the gut, and preparations with markedly different activities in other assays were found to be equally active in rabbit ileal loops without experimental trypsin activation. It should be noted that proteolytic activities of LT was reported earlier (Rappaport, 1978). However, in that study, as the LT was not homogenous, it is not clear whether the LT was activated directly or whether the effect was mediated via digestion of other associated protein, thus freeing LT. Both the explanation may be applicable.
Incubation and different methods of toxin preparation. It was found that these are important variables in the CHO cell assay system. (Annex - Research Findings)

2.8 Training Received

(a) One laboratory technician received training in Japan in basic bacteriological laboratory technology related to research.

(b) Three Japanese Experts came to the Division for varying periods and collaborated with Burmese Scientists. They also gave training to scientists and technicians on various aspects of Research and the development of bacteriological technology.

(c) A course on study of gas liquid chromatography was conducted by a visiting Japanese Expert for Senior Burmese Scientists in DMR.

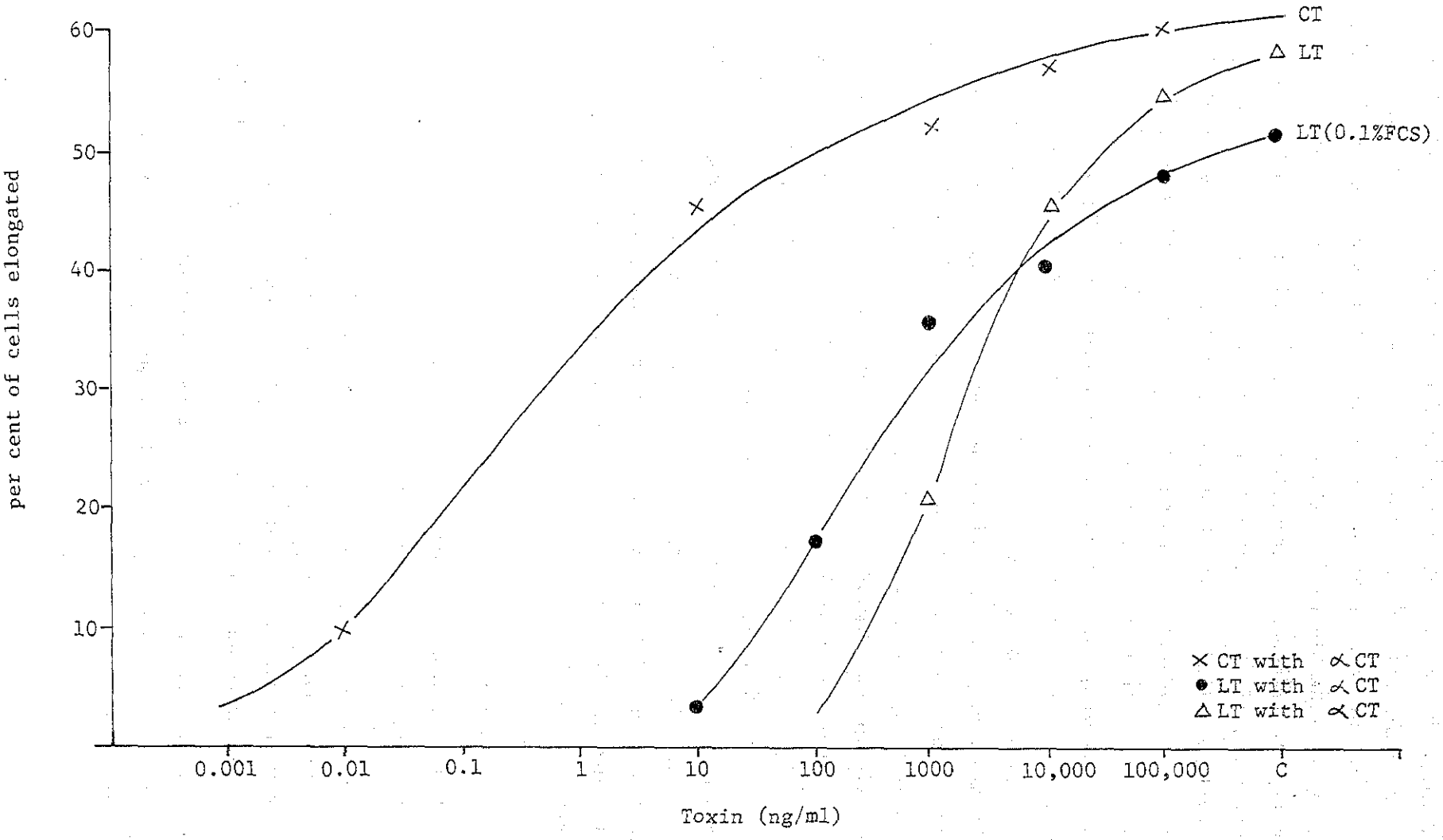


Figure 15 Effect of cholera and ETEC (LT) toxin in CHO cell assay.

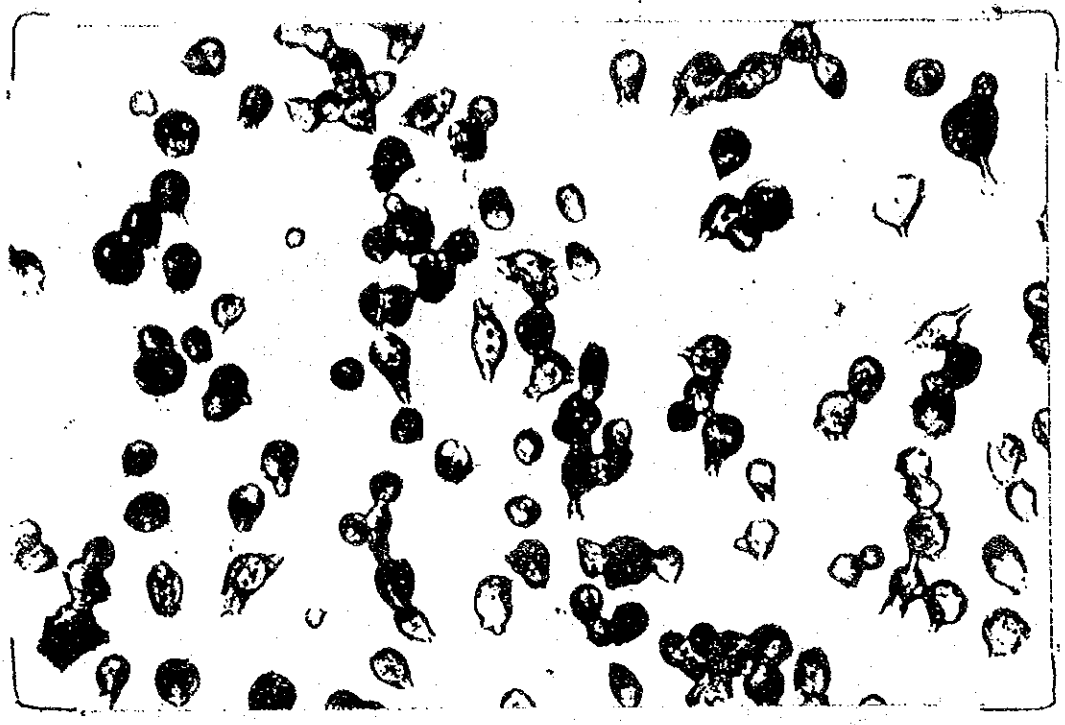
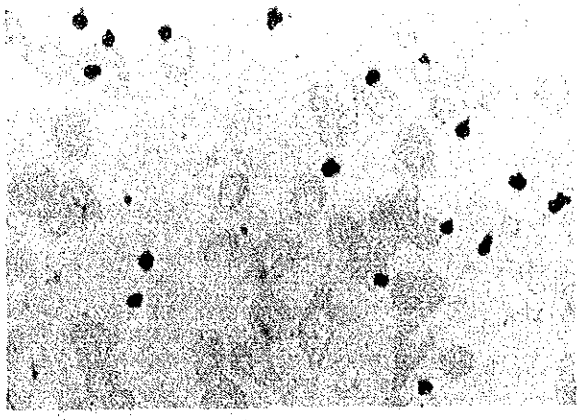


Fig. 16

P/B

Fig. 17



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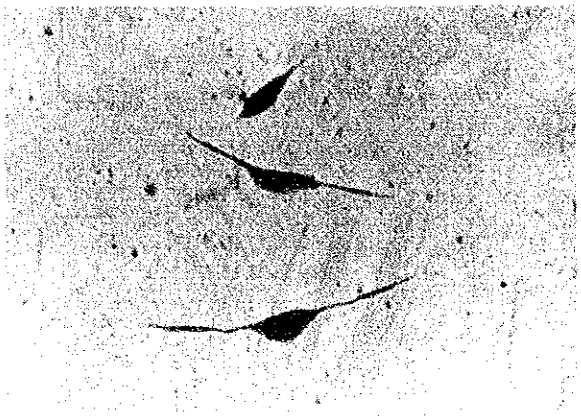

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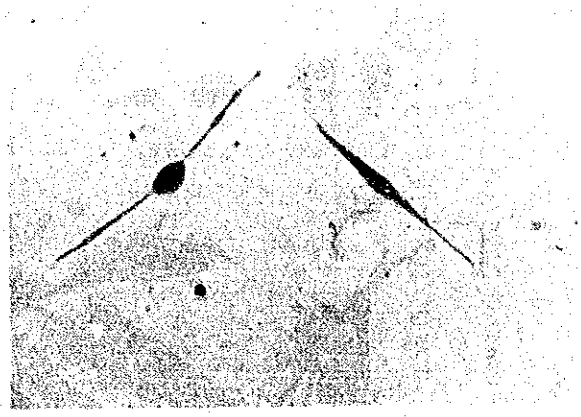

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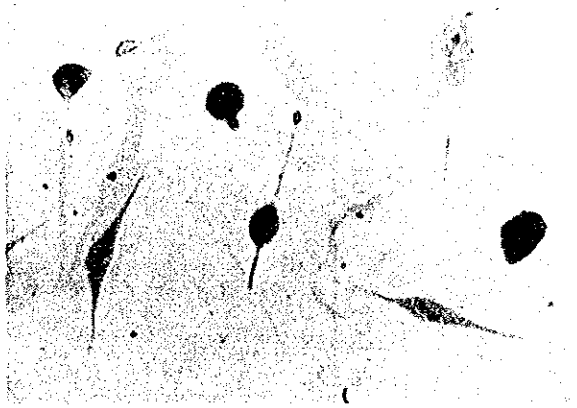

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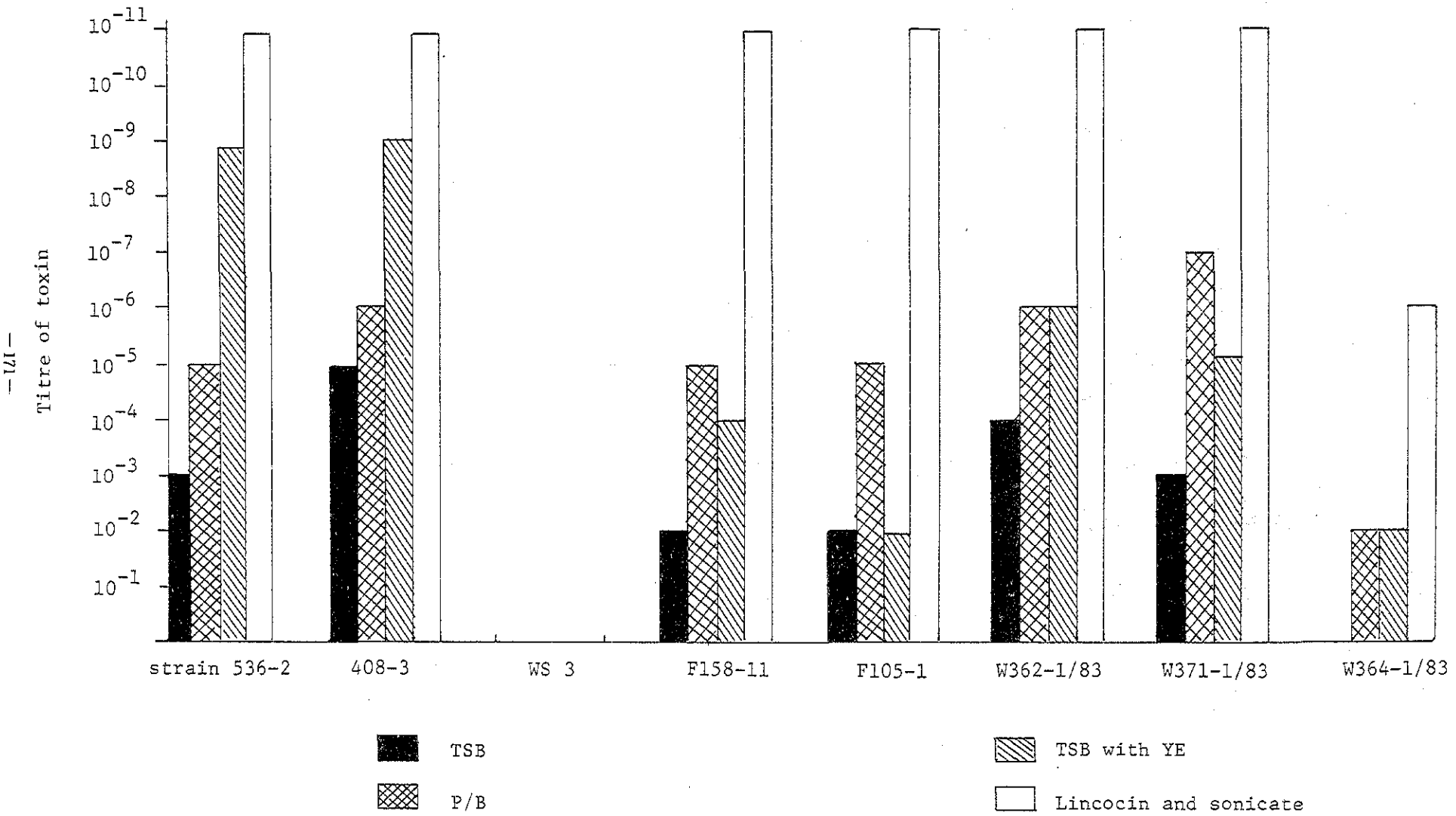
Fig. 23 Effect of toxin preparation in CHO cell assay.



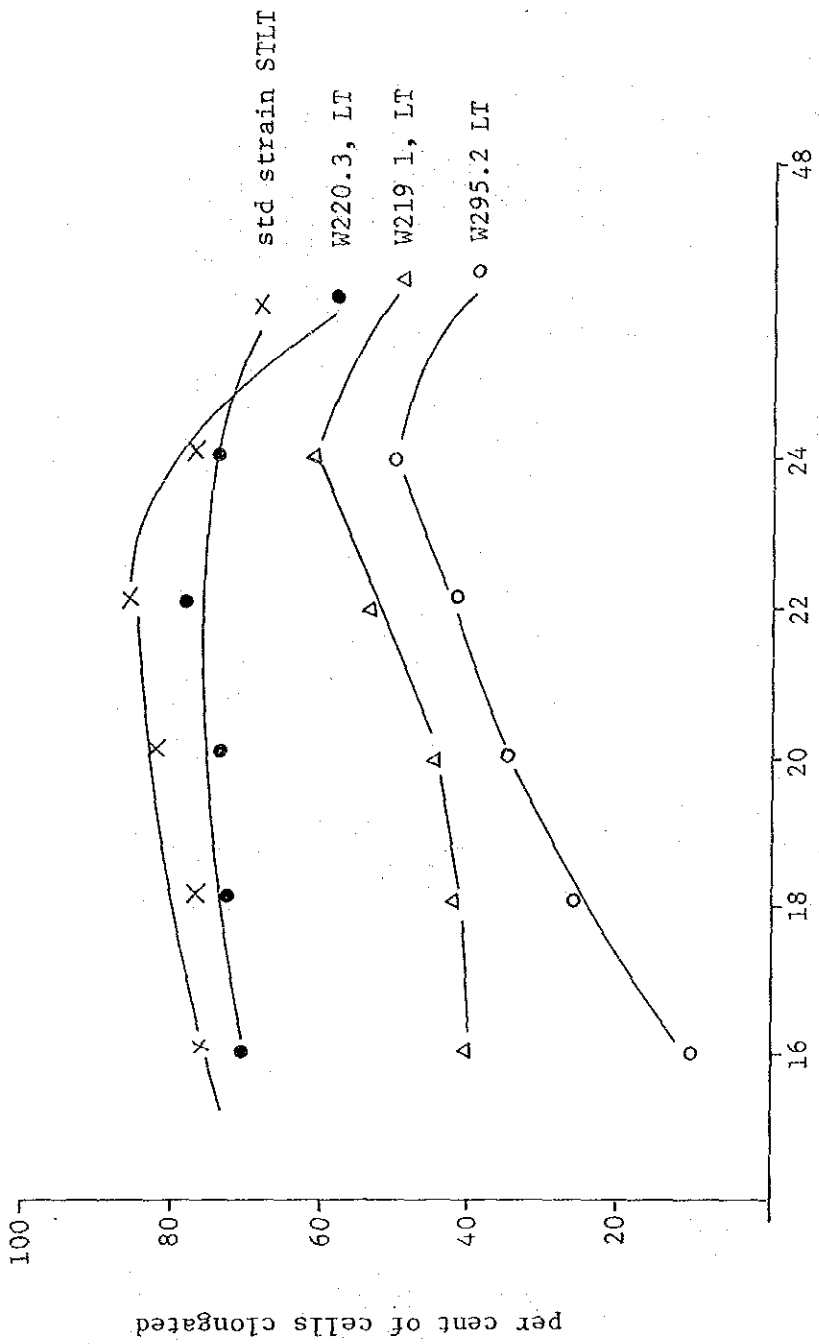


Fig. 24 Effect of toxin in CHO cell

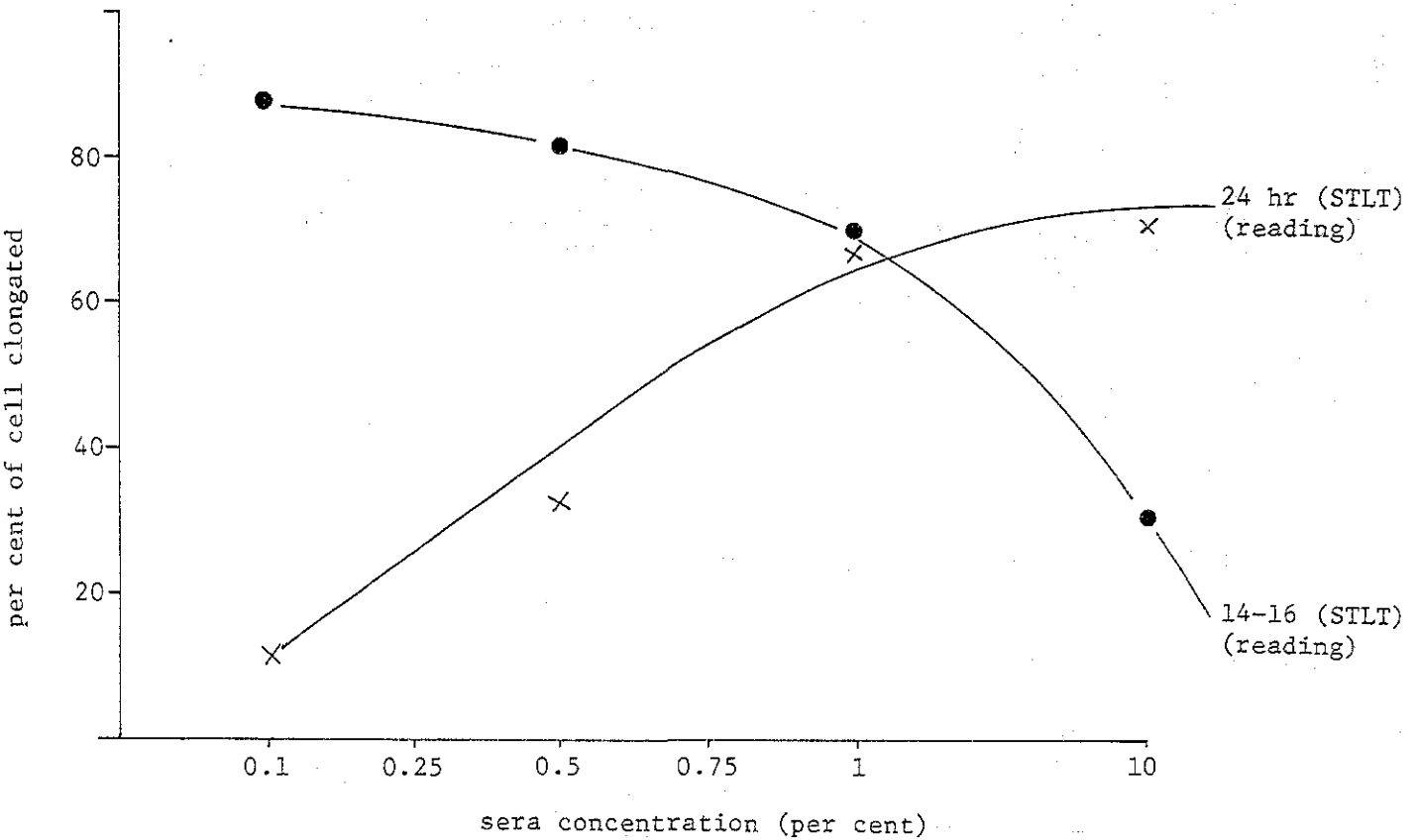


Fig. 25 Effect of sera in CHO cell in relation to time of recording

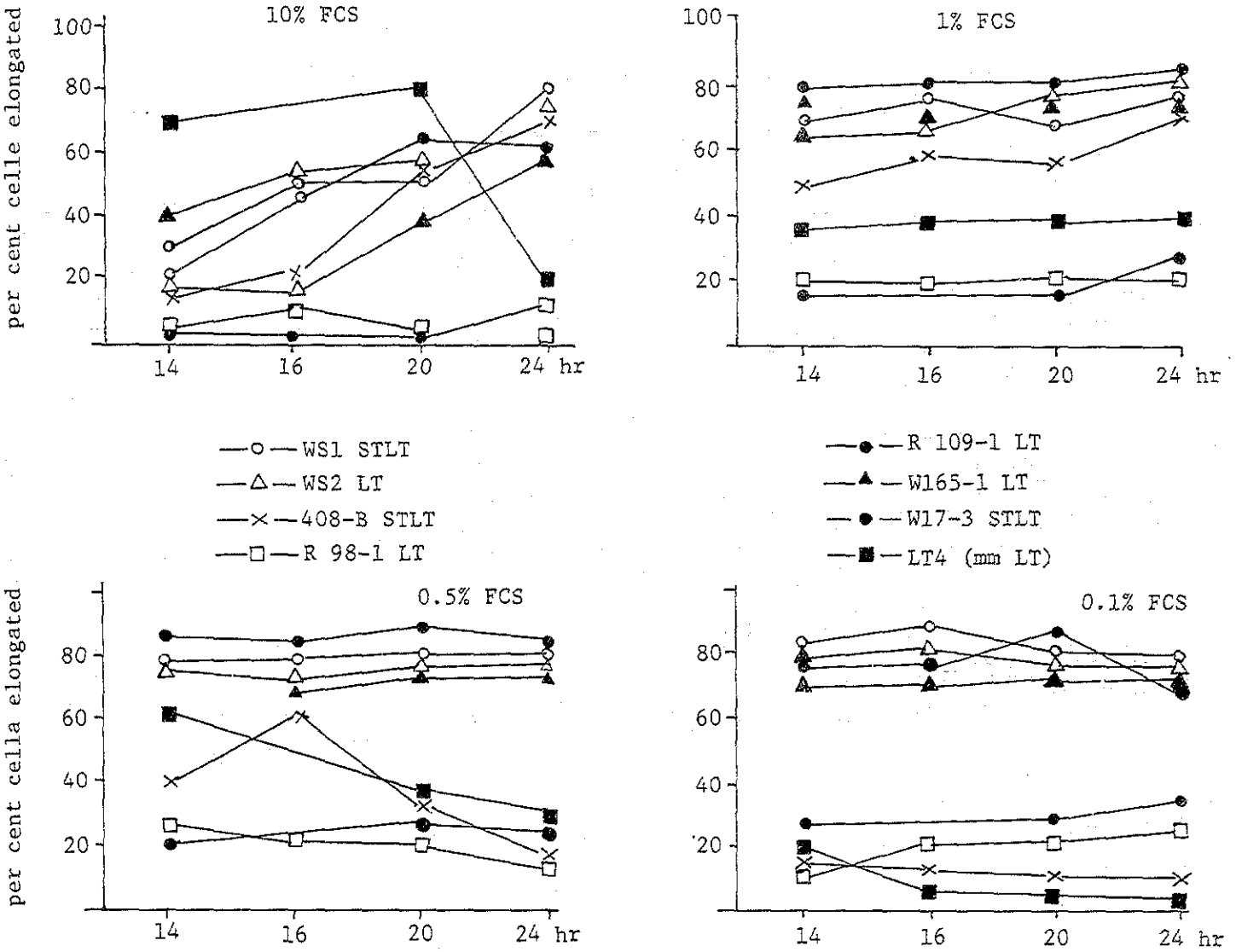


Fig. 26 Effect of sera in CHO assay

2.9 Training Given

(a) Postgraduate students (doctors) from the Institute of Medicine I were given demonstrations as part of the course-work leading to the Diploma in Bacteriology.

(b) Two postgraduate students (doctors) for the Diploma in Bacteriology from the Institute of Medicine I carried out their required research works in the Bacteriology Research Division and were supervised by the senior staff. They completed the following research works.

i. Biochemical and physiological characterization of Shigella isolates collected from N. Okkalapa community based study.

ii. Vibrio parahaemolyticus isolated among diarrhoea patients in Rangoon.

(c) One postgraduate student (doctor) for the M. Med. Sc. (Paediatric) degree of the Institutes of Medicine I did the laboratory part of her work in Bacteriology Research Division. The research was on "Neonatal Diarrhoea - major etiological agents and Mortality patterns."

3. ARBOVIRAL DISEASES RESEARCH

3.1 Aims of Research and Development Programme

The Virology Research Division has been doing research on Dengue Haemorrhagic Fever (DHF) and arboviruses since its establishment. Standard serological tests for Dengue viruses and the mosquito-inoculation method of virus isolation had already been established and were being used for research into the epidemiology of DHF.

When the DMR/JICA Project was started in 1980 it was planned that more sophisticated and rapid methods for the identification and diagnosis of Dengue infection should be developed and established for use in advanced epidemiological studies. Research into the biology of Dengue viruses including ultrastructural studies would also be attempted so that a firm foundation for basic research in virology would be established and complement more directly applied research.

Extensive studies into the bacteriology of diarrhoeas under the DMR/JICA project revealed the need for studies of the viral agents causing diarrhoea and therefore plans were also made to undertake these studies in the Virology Research Division.

3.2 Development of advanced and rapid diagnostic methods for the study of dengue and gastroenteritis viruses.

(a) A modification of the mosquito inoculation of dengue virus has been introduced (ie: intracerebral inoculation) during the first two years of DMR/JICA project (1980-82).
During the second two years of the DMR/JICA project (1982-84), this technique was assessed in comparison with the conventional intrathoracic inoculation method for the isolation of dengue viruses from clinical specimens (DHF cases).
Preliminary results of the study suggested that the sensitivity of the intracerebral route is inferior to intrathoracic route for the isolation of dengue viruses from clinical specimens.
The project is continuing to be able to provide a full scientific report.

(b) A rapid diagnostic method for the detection of Rotavirus in stool specimens (Reverse Passive Haemagglutination) was established. The technique has been utilized for the detection of rotavirus in stool specimens in a rural community (see below).

(c) Some of the techniques in relation to Immune Electron Microscopy of viral agents causing diarrhoea has been established and work is continuing to utilize the technology in relation to viral diarrhoeas.

(d) EM techniques to be applied in ultrastructural studies of arboviruses has been made available to one of the trainees in Japan.

(e) Immunological procedures to be utilized in the preparation of biologicals such as (i) isolation of IgM by column chromatography (ii) purification of conjugates by column chromatography and (iii) preparation of therapeutic human γ-globulin were introduced.

3.3 Study of Vector, Amplifier and Human Infection with Japanese Encephalitis Virus in a Rangoon Community

Introduction

Sporadic outbreaks of presumably human Japanese encephalitis virus (JEV) infections have been reported in Burma especially from the Shan State in recent years (1). Unfortunately, most of them have not been confirmed by virological methods except in one area (Soe Thein, 1978, to be published). Serological surveys have also provided evidence of prevalence of JEV in certain parts of Burma (2).

A premonsoon serological survey carried out in two areas of Rangoon (capital city of Burma) in 1968 showed that a small proportion of the sample population had detectable JEV specific neutralizing antibody (Virus Research Centre, Poona, 1968, unpublished data). A survey carried out in a Rangoon community in 1970 indicated a high prevalence of JEV Haemagglutination inhibition

antibodies (JEV HI Ab) in the pigs (91%) but a comparatively low prevalence of the homotypic type of JEV HI Ab was found in 8% of the human (Dr. Mi Mi Khin, personal communication).

The present study was undertaken with a view to (a) quantify the JEV infection in human and pigs in a Rangoon Community and (b) determine prevalence, relative abundance and host preference of Culex mosquitoes in that area. The results of the study will then be compared with data from similar studies in other areas of the world to have an insight into those factors that lead to apparently low JEV human infections in a Dengue endemic area.

Materials and Methods

Study area:

Two areas in Daw-bon township which is a suburb of Rangoon city were the study site. One are had plenty of pigs - ecologically favourable area (EFA) and the other had only a few pigs - ecologically unfavourable area (EUA) (see Table 43).

Study population:

A prospective serological survey of under eight years old children in the study area was carried out to quantify the human JEV infection in the study area. Children were bled twice - premonsoon (May, 1982) and postmonsoon (November, 1982). A similar study was also carried out for under eight month old pigs in the study area (see Table 44).

Virological studies:

Sera were tested for HI Ab against JEV (all specimens) and against Dengue 4 (only some specimens) according to the method of Clarke and Casals (3) and modification to the microtitre technique (4). Dengue 4 haemagglutination antigen (HA Ag) and JEV HA Ag (JaGar 01) were prepared by sucrose acetone extraction of the infected suckling mouse brain (5).

Human sera showing seroconversion to JEV by HI test (37 out of 41 paired sera) were assayed for N Ab against JEV by tissue

culture neutralisation test (6). The cell line utilized was LLC-MK2 which was grown at 37°C with 10 % calf serum in Eagle's minimal essential medium. JEV (JaGar 01 strain) at 100 TCID 50 was utilized in the neutralisation test. Sera were heat inactivated at 56°C for 30 minutes prior to testing. Sera were screened at 1 in 20 dilution to detect the presence of JEV N Ab.

Entomological studies:

The prevalence and relative abundance of Culex mosquitoes in the study area was determined using light traps, cattle bait and human bait.

The host preference of blooded mosquitoes (caught by the light traps) against human, bovine and swine was undertaken using immunoprecipitation (Gel-diffusion) test (7).

Results

Screening of human sera by HI test against JEV revealed a seroconversion rate of 8.3 % (Table 45). However, the HI Ab patterns against JEV and Dengue 4 (Figure 27) of 11 of the 41 seroconvertors indicated that the seroconversions may be due to dengue infections. The absence of JEV N Ab in 37 of the 41 seroconvertors (4 specimens cannot be tested due to insufficient amount of sera for neutralization test) strongly suggested that the seroconversions were due to dengue infections (the study area is laboratory documented dengue endemic area) rather than JEV infections.

Screening of pig sera by HI test against JEV revealed a seroconversion rate of 52.1 % (Table 45). The HI Ab patterns of all 12 of the seroconvertors against JEV and Dengue 4 (Figure 28) strongly suggested that the seroconversions were due to JEV infections.

The known JEV vector mosquito species *Cu. tritaeniorynchus*, *Cu.gelidus*, *Cu.vishnui* and *Cu.fuscocephalus* were prevalent in the study area (Figure 29). The relative abundance of the various species were depicted in Figure 30 and 31.

Host preference determination of the Culex species from the study area revealed that the Culex mosquitoes were zoophilic and the most frequent hosts were bovines (Figure 32).

Discussion

Although JEV infection has been suspected in Rangoon serological studies on suspected hospitalized cases failed to confirm it (Soe Thein, 1982, unpublished data). A cross-sectional serological study which showed a high prevalence of JEV HI Ab in the pigs but a comparatively low prevalence in human in a Rangoon community addresses the question of whether JEV infections in man are modified by previous dengue infections. Thus a prospective serological survey was carried out in a Rangoon community to quantify the JEV infections in human and pigs. The prevalence, relative abundance and host preference of known JEV vector mosquitoes in the area was also determined concurrently to correlate the data educatively.

As dengue infection is highly endemic in Rangoon (8), it was decided to do prospective serological survey of younger children (under 8 years) so as to avoid as much as possible the high titred heterospecific antibody found in sera after sequential infections with group B arboviruses that hinders specific identification of the most recent infection (9). Selection of this age group is justifiable as the peak incidence of human JEV infections was in the five to nine-year old age group (10,11).

As the slaughtering age of pigs was around one year of age, it was necessary to do prospective serosurvey of young pigs (approximately under eight months) so that the pigs that were bled during the premonsoon season will be still alive at the post-monsoon bleeding season.

The HI Ab against Dengue detected in the seroconverted pigs (which are markedly lower than against JEV) is not surprising as broadly reactive serologic patterns identical to those observed in these native domestic mammals have been reported following laboratory infection of pigs, dogs, buffalo and cattle with JEV (12). The reliance place upon the HI test (to identify JEV

infection in the pigs) in this study seems justified as results of a study in India have shown general agreement between the mouse neutralisation and the HI test when antibodies to JEV and West Nile virus were measured in water buffalo and cattle sera (13).

Table 46 shows the comparative data of the present study and previous studies (12, 14 to 20). Tokyo and Taiwan are the areas where there have been no clinicially recognized Dengue infections since just after the second world war. Thailand (Chiangmai) and Burma (Rangoon) are areas where both the Dengue and JEV are endemic.

Comparing the mosquito density as well as JEV infection rate in pigs of the present study and previous studies (where JEV outbreaks have been reported) clearly indicated that the mosquito density as well as JEV infection rate in the pigs (also the pig density if compared to Chiangmai figures) in the present study area was much lower than the previous areas. Moreover it will be noticed that the most frequent hosts of Culex mosquitoes were bovines. This probably of accounts for the fact that human JEV infection was not detected in the study area although JEV infection could be detected in the pigs and the known JEV vector mosquitoes are prevalent in the study area. The present study does not suggest that the high endemicity of Dengue in the study area is modifying JEV infections in man as would be the case if JE infection is absent in spite of very favourable ecological conditions. If the ecological conditions for JEV transmission become more favourable (i.e., increase in the density of mosquitoes as well as density of amplifying hosts-pigs) there is a possibility than human JEV infection may occur in the Rangoon community as have been reported in Thailand.

Summary

A prospective serological study was undertaken in a Rangoon community to quantify JEV infections in human and pigs. The prevalence, relative abundance and host preference of Culex mosquitoes in the area was also determined. JEV infection was

detected in the pigs (52.1%) although concurrent human JEV infection was not detected. The known JEV vector mosquito species were prevalent in the study area. Host preference determination of the Culex species revealed that they were zoophilic and the most frequent hosts were bovines. The absence of concurrent human JEV infection in the presence of JEV infection in the pigs and of vector mosquito species was discussed.

Acknowledgement

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The help rendered by U Aung Myint Thein, Assistant Director, Central Statistical Organization, Rangoon in calculating the projected population figures is also gratefully acknowledged.

Table 43 Demographic data of the study area

Particulars	EFA	EUA
	Set Win NANABAING (SW-NNB)	Aung-Chan-Tha LUMUDAN (AC-LD)
- Total population	3002	3490
- Under 8 years (projected)	690	802
- No. of houses	441	464
- No. of families	586	559
- No. of pigs (under 8 months)*	198	0
(over 8 months)*	82	8
- No. of houses owning pigs	42	3
- No. of bovines	14	0

* approximate age

Table 44 - Data of study population

Season	Particulars	EFA	EUA
		SW - NNB	AC - LD
Pre-monsoon	- Under 8 Children	291 (42)*	384 (47)
	- No. of families	232 (40)	292 (52)
	- No. of pigs (under 8 months)	60 (30)	0
	- No. of houses owning pigs	12 (26)	0
Post-monsoon	- Under 8 Children	218 (32)	276 (34)
	- No. of families	188 (32)	215 (38)
	- No. of pigs (under 8 months)	23 (12)	0
	- No. of houses owning pigs	6 (14)	0

* Figures in parentheses indicate % of existing population that took part in the study.

Table 45 Hit results of human and pig sera aginst JEV

Season	Type of Sera	No. tested	JEV Ab Prevalence	Seroconversion against JEV
Pre-monsoon	Human	675	397 (59)*	-
	Pig	60	10 (16.6)	-
Post-monsoon	Human	494		41 (8.3)
	Pig	23		12 (52.1)

* Figure in parentheses indicate percentages.

Table 46 Comparative data of the present study and previous studies

Area (year)	Human Infection rate %	Pig Infection rate %	Average no.of pigs /household	Mosquito Data							
				Density				Hot reference			
				CT	CV	CG	C	H	B	P	U
Japan (1956-57)	5	98-99	NM	NC	NC	NC	NC	NM	NM	NM	NM
Taiwan (1958)	4	80-100	NM	2000[a] +	0	0	50	NM	NM	NM	NM
				180[b] +	0	0	0	NM	NM	NM	NM
Thailand (1970)	6	93	1.5	900[b]	0	900	50	1-4	78-85	6-10	8-10
Burma (1982)	0	53	0.6	29.3[a]	7	19.8	0.3	1.6	87.4	6.4	5.0
				53.3[b]	19.6	27.6	8.4				

* a = average no. of mosquitoes caught per day by cattle bait for three hours after sunset. (highest no. caught)

b + average no. of mosquitoes caught per night by a light trap (highest no. caught)

Abbreviations

NC = Methods employed not comparable to other studies.

NM = Not mentioned

CT etc. = Cu. tritaeniorhynchus, Cu. vishnui, Cu. gelidus, Cu. fuscocephaius.

H etc. = Human, Bovine, Pig, Unknown.

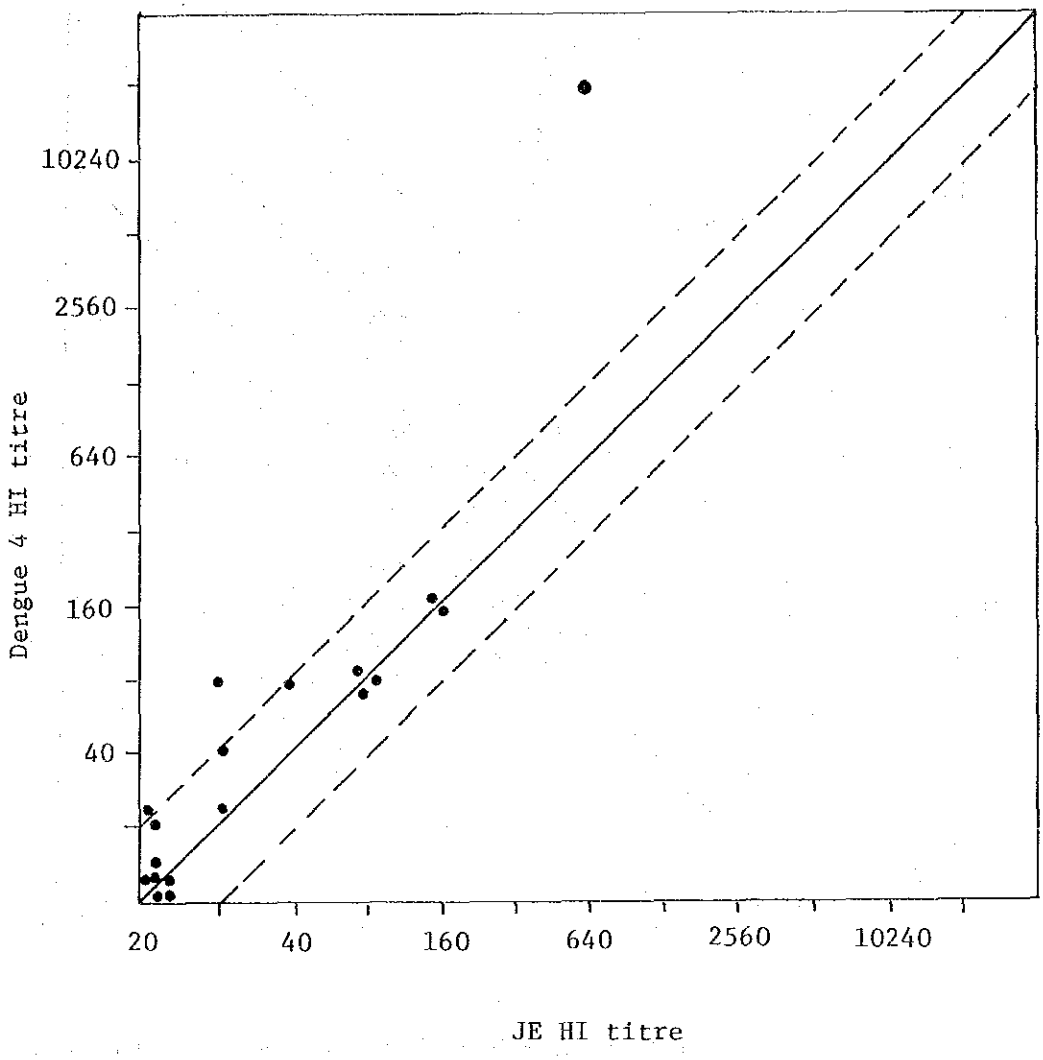


Fig. 27 Dengue 4 and JE HI antibody titre of 11 seroconvertors (human)

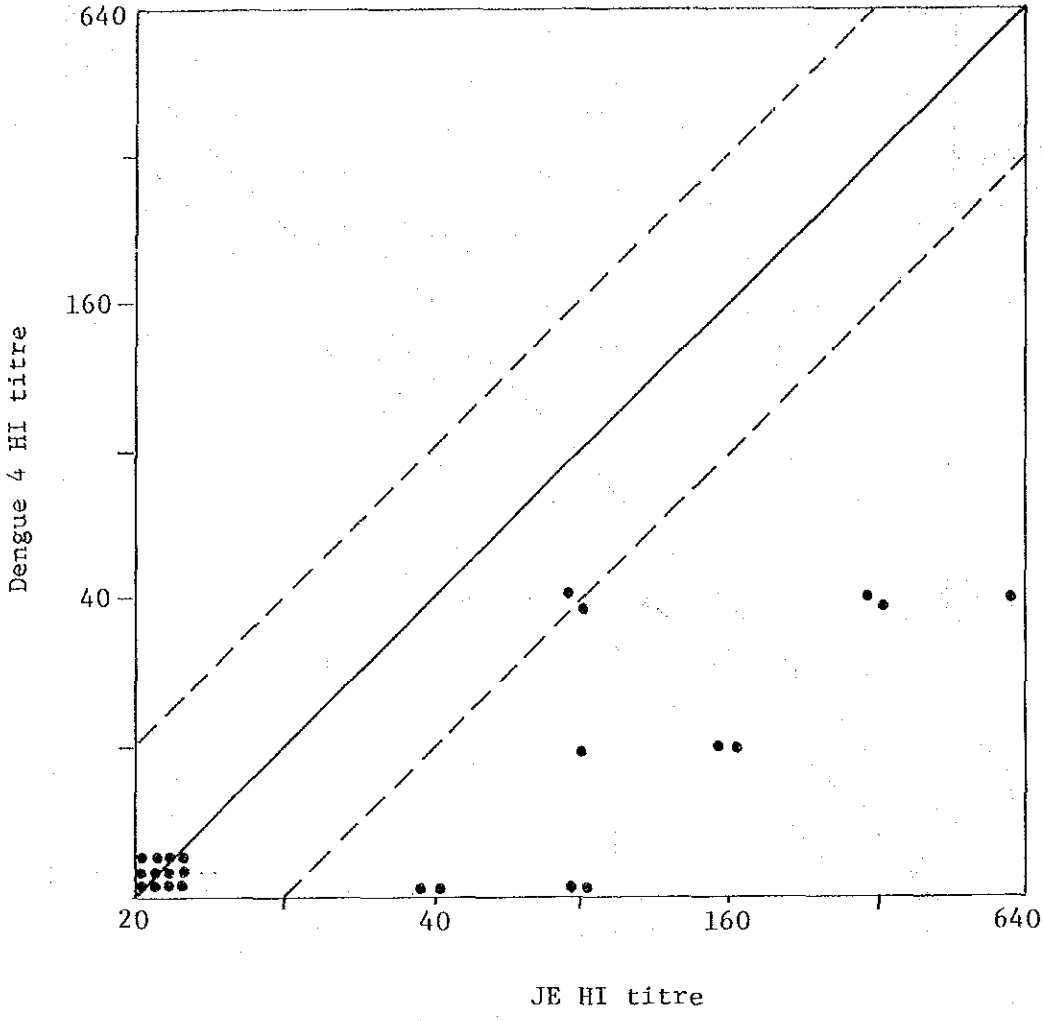
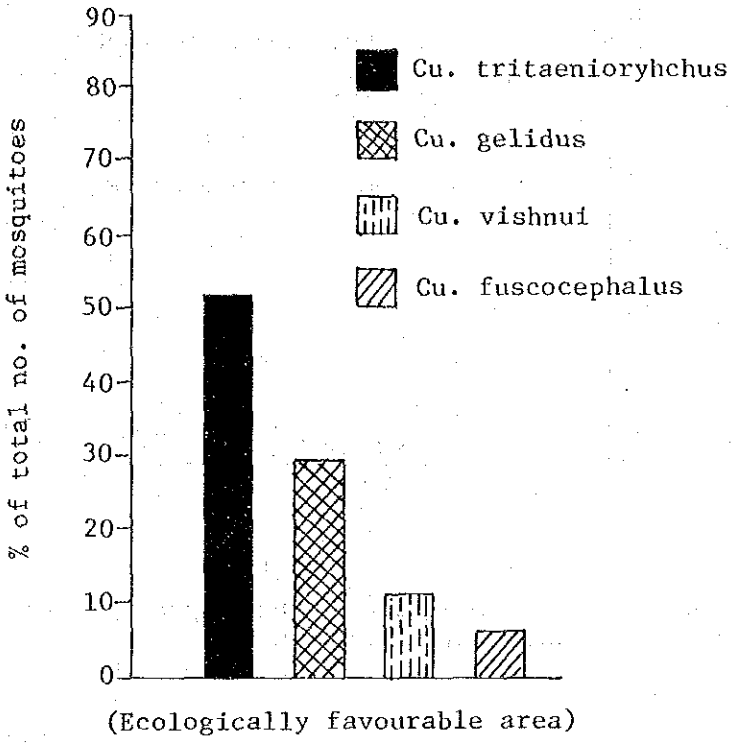


Fig. 28 Dengue 4 and JE HI antibody titre of 12 seroconvertors (pigs)



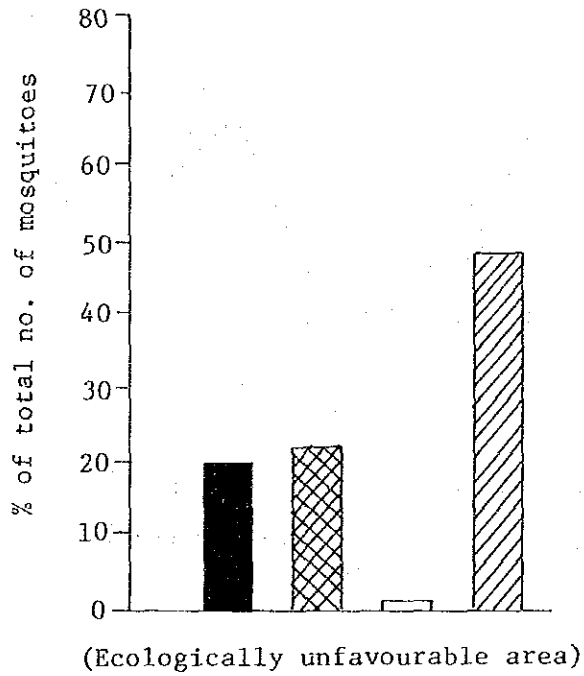
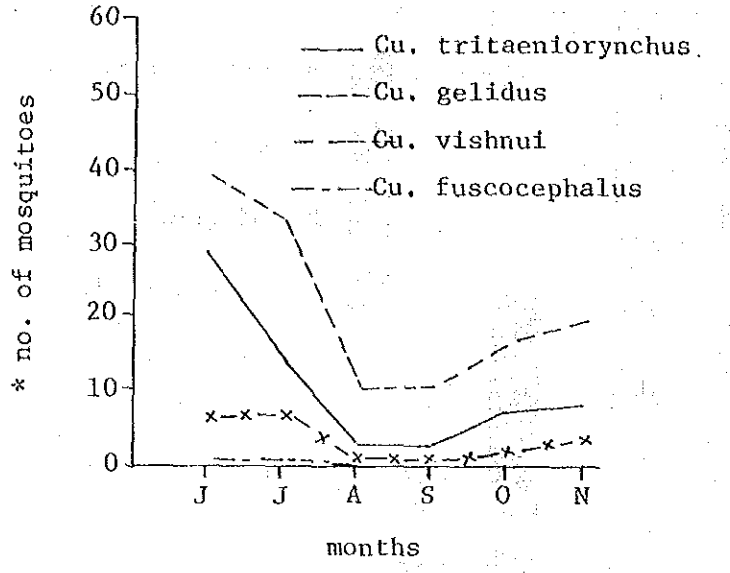
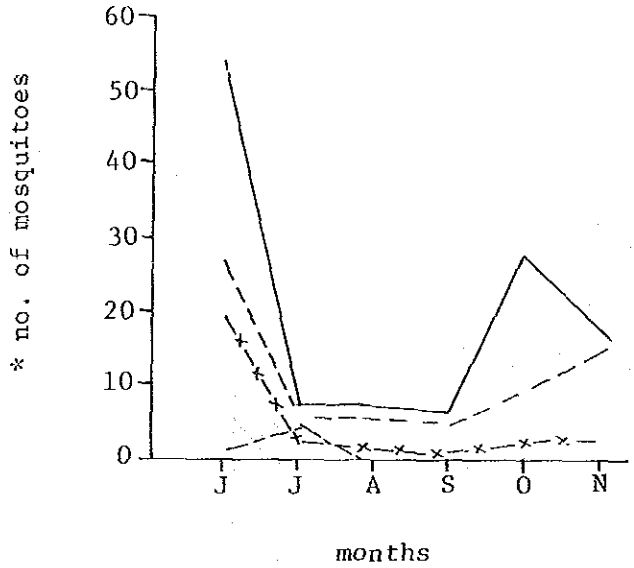


Fig. 29 Prevalence of Culex species in the study area. (Light trap data)

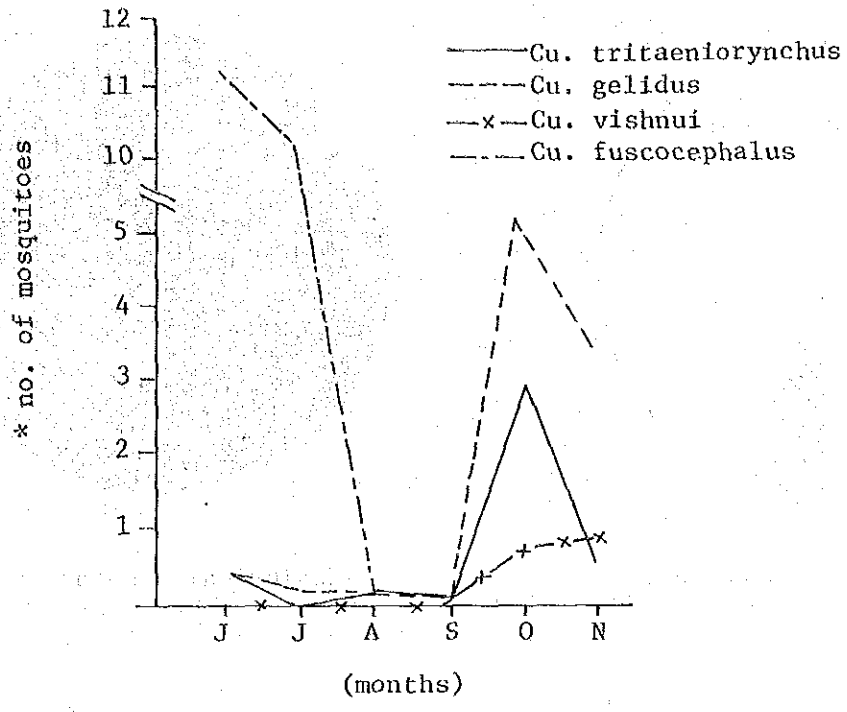


* average no. of mosquitoes caught per day by cattle bait for three hours after sunset.

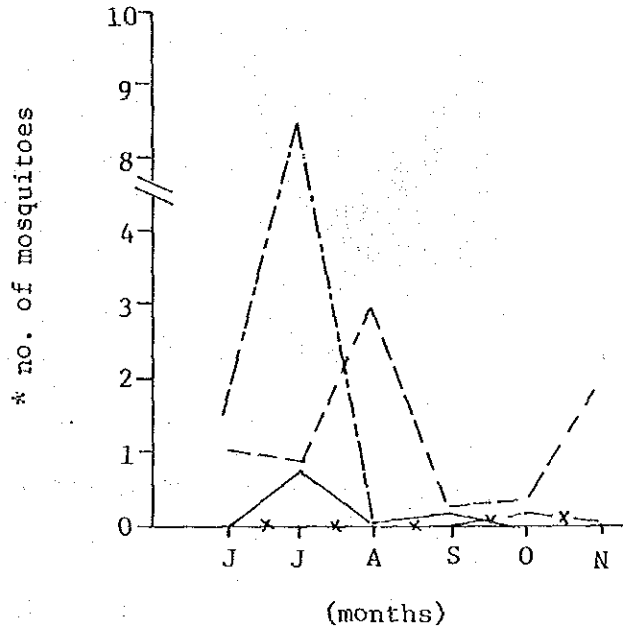


* average no. of mosquitoes caught per night by a light trap.

Fig. 30 Relative abundance of Culex species in the study area (Ecologically favourable area)

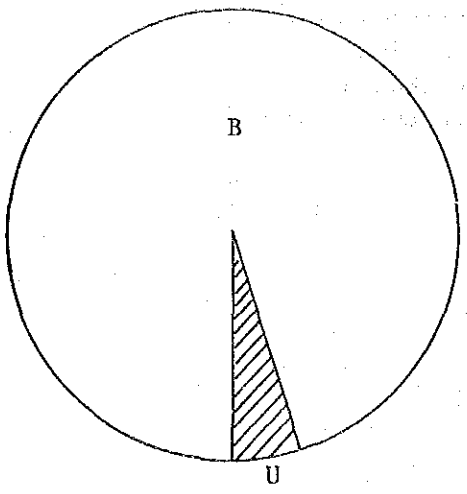


* average no. of mosquitoes caught per day by human bait for three hours after sunset.

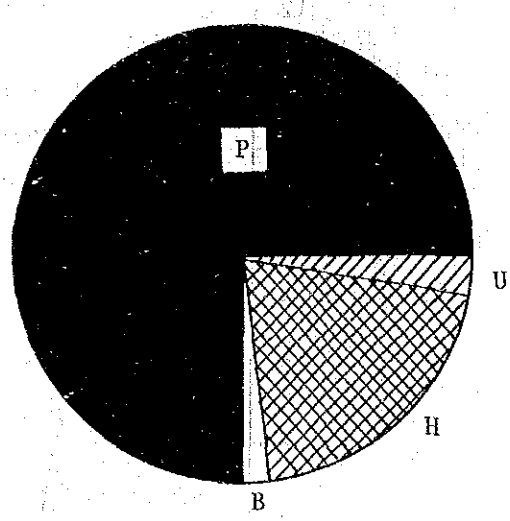


* average no. of mosquitoes caught per night by a light trap.

Fig. 31 Relative abundance of Culex species in the study area (Ecologically unfavourable area)

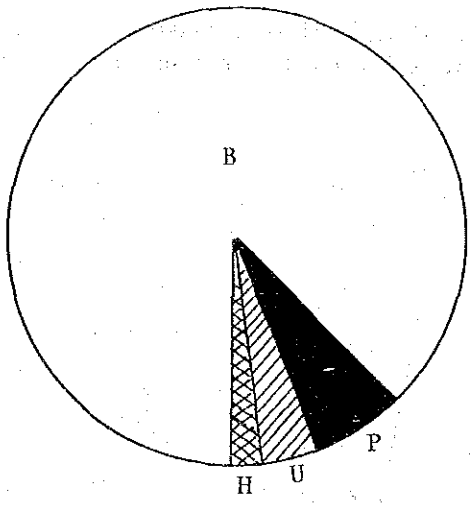


(Ecologically favourable area)



(Ecologically unfavourable area)

B = Bovines
P = Pigs
H = Human
U = Unidentified



(Both areas combined)

Fig. 32 Host preference of Culex species in the study area.

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3-4 Rotavirus Infection in Children in an Urban and Rural Community

Introduction

Acute diarrhoea disease continue to be an important cause of morbidity and mortality in children, particularly in developing countries (1). Recent evidence has indicated that viruses are responsible for the majority of diarrhoeal episodes in infants and young children in both developed and developing countries and may account to a considerable extent for malnutrition due to associated malabsorption (2).

In Burma, although studies have been carried out to evaluate the bacterial aetiology of diarrhoeal diseases, studies pertaining to evaluation of viral aetiology of diarrhoeal diseases is still in infancy. The present study is undertaken to determine the relative importance of rotavirus as an enteric pathogen in the causation of acute diarrhoea in children under 5 years residing in an urban and rural community.

Materials and Methods

Urban Survey (North Okkalapa)

Study area and Population

North Okkalapa Township, a satellite town, is situated 20 km north of Rangoon City..Out of 17 wards in the study township, 12 wards were included in the study and only two adjoining electroral units in each ward were randomly selected for the collection of stool samples. Stool specimens for the identification of rotavirus were collected from children under five years (who had diarrhoea as well as from non-diarrhoea controls) inhibiting in the sampled 24 electroral units during January (dry season) and in the sampled 21 electroal units during July (wet season). There were 407 and 1545 under five years children in the study area during the respective study periods. There were a total of 451 stool samples (166 from diarrhoea cases and 285 from non-diarrhoea controls) and 323 stool specimen (216 from diarrhoea cases and 107 from non-diarrhoea controls) collected during the dry and wet seasons respectively. However, virological studies to detect rotavirus was undertaken in subsampled samples from the wet season stool specimens only due to limited laboratory facilities.

Virologic Studies:

Rotazyme (Rotavirus Diagnostic Kit) available from Abbott Laboratories, Diagnostics Division, North Chicago, IL 60064, U.S.A. was utilized to detect the presence of Rotavirus in the stool specimens. The Rotazyme system utilizes, the "sandwich principle" (solid phase enzyme immunoassay technique) to measure rotavirus antigen level in feces. Plastic beads coated with guinea pig antibody are supplied in the kit. An aliquot of patient feces suspended in a diluent buffer is incubated with the bead. During the incubation rotavirus antigens, if present in the specimen, bind to the antibody on the bead. After removal of the unreacted specimen, the bead is reacted with virus antibody conjugated to horseradish peroxidase. This results in the formation on the bead of an antibody-antigen-antibody-enzyme complex. The unbound material is removed and a substrate is added to the bead for measurement of the