Proceedings of The 1st Asian Conference on Diarrhoeal Disease

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
DACCA, BANGLADESH

PROCEEDINGS OF THE

FIRST ASIAN CONFERENCE ON DIARRHOEAL DISEASE

February 16-20, 1981
DACCA, BANGLADESH

ORGANIZED BY

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

in technical collaboration with

NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASE, CALCUTTA

Published By:
INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE RESEARCH, BANGLADESH
G.P.O. Box 128, Dacca 2
Bangladesh

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February 1982
Special Publication No. 17
Cover design: Asem Ansari
Manuscript typed by: Ms. Sharon Sargeant

PREFACE

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is an autonomous, international, philanthropic and non-profit centre for research, education and training as well as clinical service. The Centre is derived from the Cholera Research Laboratory (CRL). The activities of the institution are to undertake and promote study, research and dissemination of knowledge in diarrhoeal diseases and directly related subjects of nutrition and fertility with a view to develop improved methods of health care and for the prevention and control of diarrhoeal diseases and improvement of public health programmes with special relevance to developing countries. ICDDR,B issues two types of papers: scientific reports and working papers which demonstrate the type of research activity currently in progress at ICDDR,B. The views expressed in these papers are those of authors and do not necessarily represent views of International Centre for Diarrhoeal Disease Research, Bangladesh. They should not be quoted without the permission of the authors.

FOREWORD

The First Asian Conference on Diarrhoeal Diseases held in Dacca, Bangladesh between February 16-20, 1981 was the culmination of an idea conceived several years ago by a number of research scientists working in this field. Many of the investigators from this part of the world, particularly the subcontinent, became personally acquainted with each other in various meetings and conferences held in the developed countries, although they know each other through their publications. During these informal discussions it was recognised that diarrhoea is not only a major health problem in the Asian countries but the ecological, geographical and cultural characteristics of most of the Asian continent is substantially different from those of the rest of the world, particularly the developed countries where diarrhoea in general has ceased to be a problem. It was also noted that relevant and priority topics for research in the field of diarrhoea are being actively pursued in many countries of Asia.

The Advisory Committee for Medical Research of the South-east Asia Region of WHO in their first meeting held in 1976 officially recognized diarrhoea as a priority health problem of Asia and initiated the formation of an expert committee to identify the research priorities in this field. A resolution was subsequently adopted at the 31st World Health Assembly in 1978 in Geneva which requested WHO to initiate a plan of action for global control of diarrhoeal diseases.

The Conference brought together 24 active workers from many parts of Asia in the field of diarrhoea to Dacca, Bangladesh. The principal sponsors for the Conference, the International Centre for Diarrhoeal Diseases Research, Bangladesh, the National Institute of Cholera and Enteric Diseases, Calcutta under Indian Council of Medical Research can legitimately take some pride in it as they had played a major role in carrying out significant research in the field of diarrhoea. As will be apparent from the proceedings, the papers presented were somewhat variable in quality and interest but it should be emphasized that the Conference was held at a rather short notice. Most of the important fields were covered and the presentations made are of relevance to the Asian countries. Considerable enthusiasm was generated at the Conference and many discussions had to be curtailed due to shortage of time. On the basis of a series of recommendations adopted at the concluding session of the Conference it was decided to hold similar yearly meetings each February and the title of the subsequent conference was changed from the Regional Conference on Diarrhoeal Diseases to Asian Conference on Diarrhoeal Diseases.

Despite our best efforts it was not always possible to obtain corrected version of the discussion from the individual participants. Any omission or possible misrepresentations are regretted. Some presentations were also edited. We also regret any mistakes that may have occurred inadvertantly.

Editors

RECOMMENDATIONS

The participants to the Diarrhoeal Disease Conference met on February 18, 1981 and discussed the follwoing points brought up during the meetings. Since diarrhoea is a major and priority health problem in Asia where cultural habits and geographic ecology are different from those in other continents, future meetings on a regular basis should prove to be very useful.

The following were the concensus of the meeting:

1. Name: In view of the wide geographical representations from Asia it was suggested that the name should be changed from "Regional Conference on Diarrhoeal Diseases" to "Asian Conference on Diarrhoeal Diseases".

2. Interval: It was felt that once-a-year meeting is necessary to keep up with the developments in the field. It should be held from the last Monday of February each year.

3. Venue: The venue should be different each year. The next venue (1982) proposed and accepted was National Institute of Cholera and Enteric Diseases, Calcutta, India. The venue for 1983 will be determined during the Conference in Calcutta in 1982.

4. Organising Committee: It was agreed that until a new Committee with wider geographical representation was constituted, the present Organising Committee should continue to function on an ad hoc basis. All governments in Asia should be contacted and invited to send in nominations and eminent scientists and personalities outside of government should also be contacted for inclusion in the Committee. A more permanent Organising Committee should be formed at the Calcutta meeting.

5. Sponsorship and Funding: In addition to the two current sponsors namely, International Centre for Diarrhoeal Disease Research, Bangladesh, National Institute of Cholera and Enteric Diseases (Indian Council for Medical Research), other potential sponsors should be contacted. World Health Organization, Geneva and WHO-SEARO, New Delhi may be contacted also.

6. Proceedings: Proceedings of the meeting should be published within six months.

7. Suggestions for improving the organization of future meetings:

a. A special day (or half-a-day) should be set aside for holding plenary session on a subject of special interest.

b. An abbreviated text of paper should be pre-circulated.

c. Presentation of findings (free papers) should take no longer than 6-7 minutes. Discussion period should be longer.

d. Specially invited speakers should deliver speeches on important topics. Each year one topic should be discussed in depth. A theme for each conference may be set in advance.

e. Recommendations for research should be discussed and plans formulated for their execution.

f. A permanent forum consisting of about 15 scientists in the field of diarrhoea should be constituted to meet periodically (once in six months) to oversee the progress of research in Asia.

g. An information network should be established to disseminate latest findings on diarrhoeal diseases. Diarrhoea Dialogue published by AHRTAG and Glimpse published by ICDDR,B, Dacca could serve as useful newsletters. A clearing house for diarrhoea literature should be established in each country.

h. Biodata and relevant bibliography of participants may be circulated before the meeting.

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CHAPTER 1

ETIOLOGY AND EPIDEMIOLOGY

CHAPTER 2

PATHOPHYSIOLOGY AND PATHOGENESIS

CHAPTER 3

CLINICAL MEDICINE including NUTRITIONAL CONSIDERATIONS

CHAPTER 4

PREVENTION AND CONTROL

CHAPTER 5

ENVIRONMENTAL HEALTH AND COMMUNITY IMPLEMENTATION

CHAPTER I

ETIOLOGY AND EPIDEMIOLOGY

PATTERN OF *VIBRIO PARAHAEMOLYTICUS* INFECTION IN CALCUTTA

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ABSTRACT

One hundred and three contacts in 23 houses with *V. parahaemolyticus* enteritis cases were examined for five consecutive days to determine the incidences of *V. parahaemolyticus* infections. Among the healthy contacts of *V. parahaemolyticus* positive index cases about 11.6% were detected as carriers in contrast to 3.2% in the control (vibrio negative) houses ($P<0.01$). Majority (91.6%) of carriers among the contacts in the study group were detected with the first 3 days of the occurrence of *V. parahaemolyticus* index diarrhoea cases in the family. In the study group, 16.2% were food handlers as against 2.6% in the control group ($P<0.05$). The possible role of these healthy carriers and other environmental sources in the spread of *V. parahaemolyticus* infection has been discussed in this paper.

INTRODUCTION

Vibrio parahaemolyticus, a halophilic organism has been recognised to cause food borne gastroenteritis in man[1]. These outbreaks were mostly related to consumption of seafish and fish products[2-4]. The organism has been been isolated from coastal sea waters, sea mud and zoo plankton[5,6]. Moreover, there are also reports on isolation of this organism from fresh water fishes[7].

Reports of isolation of *V. parahaemolyticus* from diarrhoeal patients in Calcutta are available since 1968[8,9]. Studies undertaken at the National Institute of Cholera and Enteric Diseases, Calcutta during the last couple of years have indicated that about 10-15% of gastroenteritis patients admitted annually to the local Infectious Diseases Hospital, were due to *V. parahaemolyticus*[10,11]. However, in Calcutta, there is little seasonal variation in incidences[8,10].

A study showed that about one-third of the 60 hospitalized diarrhoea patients due to *V. parahaemolyticus* infection were strict vegetarians with no history of sea food consumption[11]. It has also been observed that about 15-21% of healthy contacts of *V. parahaemolyticus* positive index diarrhoea cases were detected as carriers[10,12]. The present study was conducted to determine the mode of transmission of *V. parahaemolyticus* infection at the community level.

MATERIAL AND METHODS

The study was conducted in the slums of eastern part of Calcutta which is highly endemic for diarrhoeal diseases. A total of 68 houses were studied between April 1977 and December 1979. Of these, *V. parahaemolyticus* were isolated from index cases in 23 houses (study group), whereas in 45 houses no vibrios were isolated from the index case (control group). Information regarding age, sex, socio-economic status and sanitary facilities were collected. As soon as a patient suffering from gastroenteritis was admitted to the local Infectious Diseases Hospital, stool samples were collected before administration of antibiotics and examined for the presence of *V. parahaemolyticus*. The patients family was contacted within 24 hours of hospitalization, stools and finger washing were collected from the contacts for five consecutive days. Samples of leftover cooked food, flies, stored water within the house, nearby pond water and openwell water were also collected for five consecutive days.

All the samples, except water, were collected in alkaline peptone water and processed in the laboratory after enrichment for the presence of *V. parahaemolyticus*, 500 ml. of water samples were collected and examined as described by De and co-workers[7]. All the bacteriological procedures including serotype determination and Kanagawa phenomenon were carried out according to methods described by Sakazaki[13].

Overall sampling coverages of the contacts in the study and control groups of population over the 5 day period were 97.3 and 97.8% respectively.

RESULTS

All the 68 families studied belonged to the lower socioeconomic strata. The general demographic features are presented in Table 1. Age distribution of 316 family contacts are given in Table 2. It may be seen from the above two tables that the study population of the two groups are comparable.

Table 1. Common Demographic Features of the Study and the Control Groups

Features	Study Group	Control Group
Sex Ratio of contacts (M/F)	1:1.3	1:1.1
Monthly family income (%)		
A) RS. ≤ 300	73.9	62.2
B) RS. 301-750	26.1	37.8
Water Sources (%)		
A) Tap (outside)	73.9	64.4
B) Pond (outside)	30.0	35.5
C) Openwell (inside)	43.4	33.3
Latrine facility (%)		
A) Sanitary	47.8	55.6
B) Service	30.4	33.3
C) Open land	21.7	11.1
Number of person per family	4.5	4.7

Table 2. Distribution of the Study and Control Population by Age

Age Group (In Years)	Number of Family Contacts In Study Group	Control Group	Total
< 1	2 (1.9)	6 (2.8)	8 (2.5)
1 - 4	13 (12.6)	27 (12.7)	40 (12.6)
5 - 9	21 (20.4)	34 (16.0)	55 (17.4)
10 - 14	16 (15.5)	36 (16.9)	52 (16.5)
≥ 15	51 (49.5)	110 (51.6)	161 (50.9)
All Ages	103 (100.0)	213 (100.0)	316 (100.0)

N.B. Figures in ( ) indicate percentage

Isolation of *V. parahaemolyticus* from stool and finger washes is shown in Table 3. Examination of the 103 family contacts in the study group revealed that 12 (11.6%) were healthy carriers. As compared to only 7 (3.2%) in the study group had excreted *V. parahaemolyticus*. This difference was statistically significant (P<0.01).

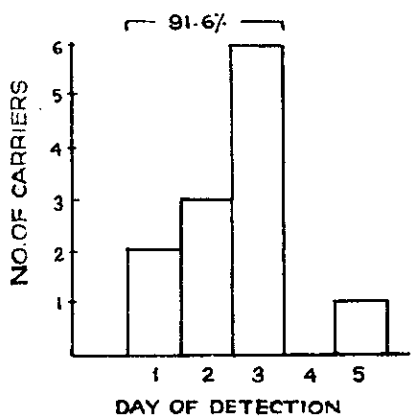
Table 3. *Vibrio parahaemolyticus* Isolated Among Family Contacts of Study and Control Houses

Houses	Number	Persons Sampled Stool +VE	Fingerwash +VE
Study Group	103	12* (11.6)	Nil (-)
Control Group	213	7 (3.2)	2 (0.9)

*Difference was statistically significant (P<0.01)
N.B. Figures in ( ) indicate percentage

There was hardly any significant difference in age distribution of carriers though children below four years of age showed a higher isolation rate in the study group (Table 4). Eight study group families had *V. parahaemolyticus* carriers, 3 families had more than one carrier.

FIG.1 DAY OF DETECTION OF CARRIERS



Carriers among the contacts in the study group, 91.6% of the carriers is the study group were detected within the first 3 days of the hospitalization of *V. parahaemolyticus* associated with gastroenteritis cases (Figure I). Carriers amongst food handlers in the study group was 16.2% as compared to only 2.6% in the control group. This difference in incidences was statistically significant (P< 0.05). Table 5.

Table 4. Age Distribution of *V. parahaemolyticus* in the Study and Control Group

Age Group (In Years)	Study Group		Control Group		Both Groups	
	Number Studied	Number Positive	Number Studied	Number Positive	Number Studied	Number Positive
< 1	2	Nil (-)	6	Nil (-)	8	Nil (-)
1 - 4	13	2 (15.4)	27	Nil (-)	40	2 (5.0)
5 - 9	21	1 (4.7)	34	2 (5.9)	55	3 (5.4)
10 - 14	16	2 (12.5)	37	2 (5.4)	53	4 (7.5)
≥ 15	51	7 (13.7)	110	3 (2.7)	161	10 (6.2)
All Ages	103	12 (11.6)	213	7 (3.2)	316	19 (6.0)

N.B. Figures in ( ) indicate percentages

Table 5. Distribution of Carriers of *V. parahaemolyticus* Amongst Food Handlers (Female ≥ 10 years

Groups	Food Handlers Number Sampled	Number Positive
Study	37	6* (16.2)
Control	77	2 (2.6)

N.B. Figures in ( ) indicate percentages.

*Difference found statistically significant ($P < 0.05$)

There was no conclusive pattern of isolations of *V. parahaemolyticus* from various categories of environmental samples of the study on control group of houses (Table 6).

Table 7 gives the serotypic pattern of *V. parahaemolyticus* strains isolated from the study group. Except 4 houses, there appeared to be no positive correlation between the serotypes of strains isolated from different sources in the same household. Twenty-nine (82.8%) strains isolated from human sources (cases and carriers) were Kanagawa positive. On the otherhand, 9 (75%) of 12 strains isolated from environmental samples, were Kanagawa negative.

DISCUSSION

In Calcutta, *V. parahaemolyticus* has been found in stools, food and water from strictly vegetarian households, with no history of any consumption of fish or other sea food within 7 days of their attack[11]. Moreover, as a rule, meals are sufficiently cooked and usually consumed shortly after their preparation.

The present study clearly demonstrated the higher isolation rate of *V. parahaemolyticus* from stool samples obtained from persons in the study group (11.6%) of families as compared to those in the control group (3.2%). This indicates greates circulation of the organism in houses with confirmed *V. parahaemolyticus* cases. Similar observations have also been made by

Table 6. Isolation of *V. parahaemolyticus* from Various Categories of Samples in Two Groups of Houses

Categories of Samples Examined	Study Group		Control Group	
	No. of Houses Examined	No. found Positive	No. of Houses Examined	No. found Positive
Pond Water	7	4 (57.1)	16	5 (31.2)
Tubewell Water	7	Nil (-)	12	1 (8.3)
Tap Water	17	1 (5.8)	29	Nil (-)
Stored Water	23	2 (8.7)	45	2 (3.7)
Openwell Water	10	3 (30.0)	15	6 (40.0)
Cooked Food	23	Nil (-)	45	1 (2.2)
Flies	23	2 (8.6)	45	6 (13.3)

N.B. Figures in ( ) indicate percentage

Table 7. Serotypes of Different Strains Isolated from Different Categories of Samples in *V. parahaemolyticus* Positive Index Case Houses

House No.	Serotypes of Strains Isolated from Index Cases	Serotypes of Strains Isolated from Carrier			Serotypes of Strains Isolated from other Categories					
		1st Carrier	2nd Carrier	3rd Carrier	Stored Water	Tap Water	Pond Water	Openwell Water	Flies	Cooked Food
2.	UT(-)							UT(-)		
3.	O8:K22(+)	O8:K22(+)								
6.	O2:K3(+)						O2:K28(-)			
10.	O5:K15(+)	UT(+)							UT(-)	
24.	UT(+)	UT(+)								
26.	UT(+)							UT(-)		
29.	UT(-)	O8:K22(+)								
31.	O8:K22(+)					O5:K15(+)		O3:K3O(-)		
46.	O5:K15(+)	O5:K15(+)	O5:K15(+)	O1:K1(-)						
51.	O5:K15(+)				O5:K15(+)		UT(-)			
59.	UT(+)	UT(+)								
68.	O5:K17(+)								UT(-)	
86.	O5:K17(-)	O7:K19(-)	O3:K7(+)							
129.	O2:K3(+)	O2:K28(-)	UT(+)				O2:K3(+)			
131.	UT(+)						UT(-)			
158.	UT(+)				UT(-)					

N.B. Figures in ( ) indicate Kanagawa Phenomenon

workers in this institution in earlier studies[10,12]. However, in Togo, West Africa, only 1.2% of the family contacts were positive for *V. parahaemolyticus*.

It was observed that adult females amongst the study population generally prepared food and were assisted by their daughters (≥ 10 years) in the process. Interestingly, it has been found that carrier rate was higher (statistically significant) amongst the food handlers in the study group as compared to those of control group.

Examination of finger washes in study group did not show any positive isolation which was contrary to the higher finger wash positive rates as observed in contacts of cholera patients during another study[15]. Therefore, the role of contaminated fingers in transmission of *V. parahaemolyticus* infection could not be established during the present study.

About 91.6% of the carriers in the study group were detected within the first 3 days of the onset of illness of the index case. This information might help in controlling the diseases particularly in new areas where the infection is not endemic.

In the study group, 25% of carriers and other categories of samples were found to be positive for the same serotypes as those of their index cases. All these strains were Kanagawa phenomenon positive. During an earlier study also, it was observed that carriers in 37.5% of houses excreted the same serotypes as those in the index cases[11]. But there are reports from diarrhoeal outbreaks that strains of *V. parahaemolyticus* isolated from food or water samples, incriminated as source of infection, were different as regards their serotype and Kanagawa phenomenon test from those isolated from cases[2,3,13]. Moreover, no particular serotype has been strongly associated with Kanagawa phenomenon or with human illness[16].

Though the results obtained in few houses showed positive serotypic correlation between index cases and other categories of samples no such positive indication about vehicle(s) of transmission of *V. parahaemolyticus* was evident in the current study.

During the present study incidence of carriers among food handlers were high in study group which probably suggests that they might have contaminated the cooked food and water sources. However, bacteriological evidence supporting this view was not sufficient in the present study.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the cooperation and help received from

the Superintendent, Infectious Diseases Hospital, Calcutta. The competent assistance of the field and laboratory workers of the Institute is also thankfully acknowledged.

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ROTAVIRUS AND OTHER UNCULTIVABLE ENTERIC VIRUSES IN THE EPIDEMIOLOGY OF ACUTE DIARRHOEAL DISEASE IN SOUTHERN INDIA

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ABSTRACT

The importance of the rotavirus as an aetiological agent in acute diarrhoeal disease in children in southern India has been well established by work during the last five years. At Vellore, an area 80 miles from the sea on the edge of the decan plataeu, rotaviruses accounted for nearly 75% of children with diarrhoea seen in the hospital in the cooler months of the year, November to February during the three years of observation. In the hot dry summer months the prevalence of rotavirus related diarrhoea was much less giving an overall prevalence of rotaviral diarrhoea of 25% in a year. This pattern of rotaviral diarrhoea at Vellore is quite different from that observed at Calicut, 300 miles to the west of Vellore on the west coast of India. The overall prevalence of rotavirus in children hospitalized with diarrhoea at Calicut was 70% and during November to January when acute diarrhoea of childhood assumes epidemic proportions in the community nearly 100% of hospitalized children with diarrhoea were rota positive.

There was striking differences in the seroepidemiology of rotaviruses between these two regions. Using the simian agent SA-11 as the source of antigen in an enzyme linked immunosorbent assay (ELISA) for serum antibodies to rotaviruses, antibodies were detectable in high titres in all sera tested. At Vellore the antibody titres were highest in new born (cord blood) and after the fourth decade of life. There was also a significant fall in the GMT of antibody titres during the hot dry summer (May). At Calicut by age of 5 rotavirus antibody GMT appeared to be falling and in adults the highest GMT was in the 19-20 age group (mothers).

Further work needs to be done to explain these differences in the epidemiology of rotavirus associated diarrhoea. Preliminary data on copro-antibodies (IgA class specific) suggests that the pattern of rotavirus infection in the populations may be different. Electron microscopy, ELISA for detection of antigen and antibody, serotyping of rotavirus isolates and biochemical characterisation of the rotavirus are necessary to understand the problem

further and plan effective control measures.

INTRODUCTION

Direct electronmicroscopic examination of suitably prepared faecal samples is now recognised as an essential method for detecting the uncultivable enteric viruses, which may be causally related to acute diarrhoeal disease[1]. During the later half of the 70's, a large variety of virus particles have been reported from different parts of the world, and it is now well established that several of these could be causing acute diarrhoea both as endemic cases and in epidemics[2].

Since 1974 in Vellore a systematic search for the detection of these uncultivable enteric viruses, has been going on. This paper summarises the current status of these studies at Vellore. Stool samples from patients with acute diarrhoea and appropriate age matched controls were collected. Twenty percent suspension of faecal samples in phosphate buffered saline was clarified by low speed centrifugation, the supernatant was spun down at 90.000g for 30 minutes and the pellet appropriately applied to grids and examined in the electronmicroscope. This methodology was preferred to adding agents like Freon 113 or PEG since these tend to obscure or destroy the details of some of the uncultivable viruses.

The Role of Rotavirus in Acute Diarrhoeal Disease of Childhood

Rotavirus was identified in the stools of children reporting to hospitals with acute diarrhoea in southern India in 1974[3]. A one year hospital based study of children under two years with acute diarrhoea (carried out in 1974 and 1975) rotavirus could be identified in 25% of these children. However, in 66% of the children pathogenic bacteria were isolated which could be causally linked to diarrhoea. These included enterotoxigenic and enteropathogenic strains of E.coli[4]. A subsequent one year survey of cases reporting to the hospital with acute diarrhoeal disease at Vellore confirmed the earlier observations. At Vellore, (which is an area of relatively low rainfall, 90 cm a year, at an elevation of about 700' above mean sea level and 80 miles inland from the coast) rovatirus was detected in hospitalized children with striking seasonal prevalence. Rotavirus was detected during the colder months of the year, August to February and were not during the hot dry summer month of May. The seasonal difference in temperature in the region is - during the winter months temperature ranged from 15° to 35° C and in the hot dry summer from 30° to 45°C.

Calicut, about 300 miles west of Vellore on the west coast of India, has a high rainfall and humidity, as well as more or less uniform temperature

throughout the year. The findings in Calicut were a striking contrast to Vellore[5]. At Calicut, among hospitalized children with acute diarrhoea, rotavirus was detected in 77%. Recognized enteropathogenic bacteria could be cultured only from about 10% of the stools. Rotavirus was detected throughout the year. During November to January, they were present in almost 100% of the children tested The frequency of rotavirus detection was lowest during May which is a relatively hot and dry month.

A seroepidemiological survey was carried out both at Vellore and in Calicut[6]. Enzyme linked Immunosorbent Assay (ELISA) was used for detecting serum antibodies of all three Ig classes, antibody titres in all age groups were found to be very high at Vellore. Antibody titres were significantly higher in adults than children and were found to be highest in those aged over 40, who were mostly grandparents. The antibody titre in children from the higher socioeconomic strata suggested that they were less exposed to rotavirus infections than children from a lower socioeconomic background. The seasonal variation in rotavirus antibody titres suggested that constant infection was a possible factor in the genesis of the very high titres observed.

In contrast to Vellore, in Calicut, antibody titres were found to be highest in children; and children with rotavirus diarrhoea had a lower antibody titre than unaffected children. The highest antibody titres were in the mothers of children with rotavirus diarrhoea while all older age groups had lower geometric mean titres. The geometric mean titres in children without diarrhoea at Calicut and at Vellore were comparable.

The results of these preliminary studies show that in two areas different in the ecology there were differences in rotavirus prevalence in hospitalized children with diarrhoea. To understand these differences further, it is essential to carry out prospective studies in the community.

Epidemics of Diarrhoea possibly Associated with Uncultivable Enteric Viruses

Several epidemics of acute diarrhoea have been studied in this region especially those caused by a variety of bacterial enteric pathogens[7,10]. During 1979, an acute diarrhoeal epidemic in a village 43 km southwest of Vellore was detected and was probably due to an uncultivable enteric virus. The epidemiological and clinical features of this outbreak are described here.

SVV is a predominantly agricultural village with a population of 648. Between 1st of January 1979 and 31st April 1979, 176 cases of acute diarrhoea were detected in this village (Fig. 1). All age groups were affected, with the highest attack rates in preschool children (Table 1). The patients affected were not febrile. They passed watery stools without blood and mucous for a few days without vomiting. Some of the patients complained of increased

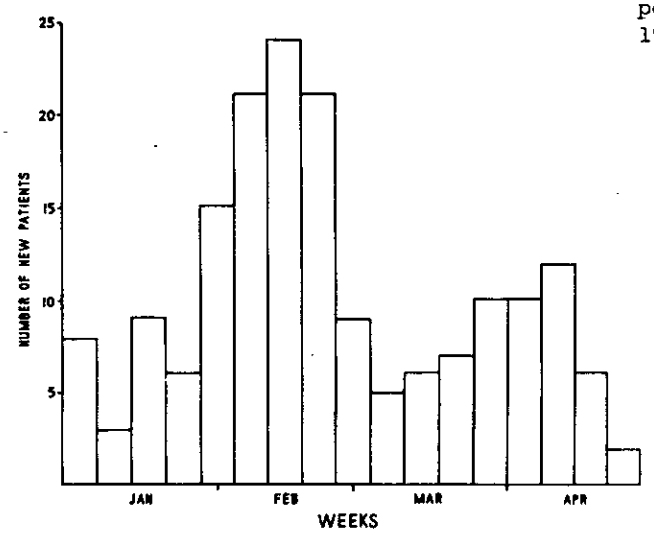

Fig. 1: Epidemic curve of SVV. New cases occurring per week from January 1, 1979 till end of April.

Table 1. Attack Rates

Age Groups	Population	Rate per 100
0 - 4.9	86	51.1
5 - 11.9	120	28.3
12 - 17.9	72	22.2
18 +	370	21.8

borborygmi. The median duration of illness was 4 days and the mean duration was 9 days. Only 23 of the affected individuals had diarrhoea lasting for more than 15 days; among them, there were episodes of remission and relapses but the second attacks could not definitely be identified as a relapse or a reinfection.

Stools for bacteriological and virological studies were obtained from 62 patients (34 during the first seven days of illness) and 23 age matched controls. Using a variety of media pathogenic bacteria were isolated from 4 patients and two controls (3 shigellae and 2 salmonellae). Rotavirus was not detected. Human enteric corona virus was present with equal frequency among the patients and controls (Table 2). A small round virus particle (Fig. 2) was present in 11 out of 34 patients during the first week of diarrhoea and in 1 two-year-old control who developed diarrhoea 2 days after the stool was collected. It was not detected in any patient who had diarrhoea longer than 7 days or any of the other controls (Table 2).

Table 2. Detection of Pathogenic Bacteria and Viruses

	Diarrhoea 1-7 days	Diarrhoea 7+ days	Controls
Bacteria			
Number studied	34	28	23
Pathogens	1	3	2
Viruses			
Number studied	34	10	23
Corona	21	6	16
SRV	11	0	1

Bacterial pathogens included 4 Shigellae and 2 Salmonellae. Corona - Human enteric Corona virus, SRV - Small round virus as shown in figure 2.

The prevalence of this small virus particle only in patients during the early period of the illness, strongly suggests a causal role. Unfortunately it was not possible to obtain blood samples from acute and convalescent cases to confirm the infection by the presence of antibodies. This particle morphologically resembles the calcivirus, known to cause acute diarrhoeal epidemics. The spread of the epidemics over 18 weeks does not suggest a

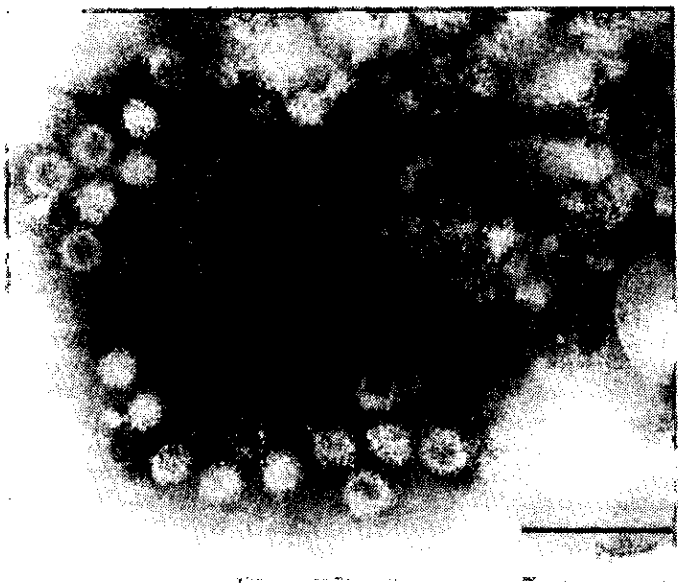

Fig. 2: The 30 nm round particle detected in the stool of early cases from SVV, full and empty particles are seen. Some of them show scalloping resembling Calci viruses. Bar shows 100 nm.

common source epidemic and in fact no common vehicle such as water supply could be identified in the village. The median case to case interval in families with multiple cases was 9 days suggesting a person to person or vector mediated transmission. The epidemic subsided with the onset of the hot dry summer.

DISCUSSION

Viral agents detectable in faecal sample by direct electronmicroscopic examination are now well recognised as the aetiological agents of acute diarrhoeal disease[1,2]. Among these, the rotavirus has now been well established as possibly the single most important cause of this problem[2]. The data presented here establishes that the magnitude of rotavirus infection in the community in southern India is very large judged either by detecting the agent in children with diarrhoea or by seroepidemiological methods and that there are regional differences in epidemiology. However, the role of other uncultivable viruses is not yet clear. Human enteric coronaviruses are widely prevalent in southern India (Table 2) but cannot be yet implicated in the etiology of acute diarrhoeal disease[11]. It has been suggested that they may

be causing the syndrome of neonatal necrotising enterocolities[1,2]. It has not been possible to determine the aetiological roles of the other viral particles that are seen in the stool. Antibody titre level in serum obtained in convalescent cases compared to serum from acute cases strongly suggests an etiological role. In the absence of such proof, the association of a single morphological type of virus particle with diarrhoea of short duration epidemics, and the absence of such particles in controls give presumptive evidence of an aetiological role.

Acute diarrhoeal diseases continue to be a major cause of morbidity and mortality, especially in the rural areas of southern India and in other parts of the developing world. Electronmicroscopy of suitably prepared faecal samples is a powerful new tool in the search for the etiological agents causing acute diarrhoea. It may be argued by some that the cost involved in a proper electronmicroscopic survey for viral aetiological agents is not justified when it is possible to treat the majority of such cases with oral maintenance of hydration. However this is purely a therapeutic approach. The ideal way to combat acute diarrhoeal diseases in the developing world would be to develop vaccines or devise means of interfering with the infection cycle in the community. This can only be achieved if the etiological agent is identified and the mechanism of their transmission in the community known. Direct EM examination of faecal samples in addition to detailed bacteriological studies is an essential part of epidemiological investigations of diarrhoeal diseases, endemic and epidemic in developing countries.

ACKNOWLEDGEMENT

The work of the Wellcome Research Unit is supported by the Wellcome Trust, London, U.K. The technical help of Mr. S.P. Swaminathan, Miss K.M. Roshni and Mr. R. Chandrasekaran is gratefully acknowledged.

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Discussion

Q. *Dr. Ganguly*

Inverse relationship between rotavirus incidence and antibodies was shown in your area? In Calicut a three year spread has shown upto 92 percent incidence what was the status of antibodies? What was the incidence in children below six months in your area when Cord blood titres were high?

A. Children get placental antibody from the mothers. We don't know the exact causes why it is higher in Vellore than Calicut.

Q. *Dr. Swailem (SA)*

When did you bind the maximum seraconversion in rotavirus in your patients?

A. No particular age group was found to have high seraconversion rates.

Q. *Dr. Sanyal*

What antigen do you use for ELISA test for detection of rotavirus infection?

A. Antigen prepared in our own laboratory.

Q. What is your opinion about the role of viruses other than rotavirus (causing) diarrhoea?

A. Yes, it is possible that other virus cause diarrhoea.

Q. *Dr. Nazma Rizvi*

Does colostrum give any immunity to rotavirus?

A. Yes, it does.

ROTAVIRUS DIARRHOEA IN KERALA, INDIA - A FIVE YEAR REPORT

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ABSTRACT

In North Kerala, India very large outbreaks of childhood diarrhoea have been observed during the months November to February, annually from 1971. Attempts to identify a bacterial aetiology have been unsuccessful. The outbreaks were shown to be due to rotavirus infection when in February, 1976, all of ten diarrhoeal stool samples tested were found to contain the virus. Rotavirus has subsequently been found in the majority of diarrhoeal stool samples from hospitalized children tested during the epidemic seasons annually from 1977 to 1980.

Two prospective studies have been carried out to assess the seasonal incidence and role of rotavirus in childhood diarrhoea in Calicut, Kerala. Children below the age of 5 years admitted to hospital with acute diarrhoea were studied. The first study covered the period October 1976 to February 1978.

A second prospective study was conducted during November 1979 to October 1980. Rotavirus infection was tested by ELISA. Of 183 stool samples tested, 102 (56%) were positive for rotavirus. The virus was more frequent in the epidemic season, with a peak of 92% in January 1980. It was much less common in the summer months - 6% and 10% in April and May, 1980.

Very large epidemics of acute diarrhoeal disease affecting infants and children occurred during the months of November to February every year from 1971 onwards in and around Calicut in North Kerala along the southwest coast of India. Though accurate data are not available, the cases are estimated to run into tens of thousands annually. A sample survey in and around Calicut identified some 80,000 cases during the epidemic season in 1973-74. Attempts to identify the aetiological agent were not successful till 1976, when all

the samples tested during the epidemic revealed the presence of rotavirus. Then on, rotavirus has been found in large majority of the faecal samples tested during the epidemics of the five succeeding years. Rotavirus infection in children hospitalized with acute diarrhoea in Calicut has been investigated in two prospective studies, the results are presented here.

The first study was by electron microscopy and was carried out during the period October 1976 to February 1978. Faecal samples were collected with rectal catheters, from children below 5 years admitted with acute diarrhoea, to the Institute of Maternal and Child Health, Medical College, Calicut. During the period 3355 children were admitted with acute diarrhoea, 368 (11%) were studied in detail. After examination for pathogenic bacteria and parasites by routine methods, the faecal samples were stored, frozen and transported on ice, at monthly intervals to the Christian Medical College, Vellore for electron microscopic examination.

Rotavirus was detected all throughout the study period (Oct. 1976 - Feb. 1978) and in 260 (71%) samples. The frequency of detection ranged from a maximum of 100% in November 1976 to a minimum of 27% in May 1977. Rotavirus infection was highest in children aged 6 to 23 months (75% of those tested) and less common in younger infants (35%) or older children (65%). Other viruses, including adenoviruses, astroviruses, caliciviruses, coronaviruses and 23-27 nm small round viruses were detected in 72 (20%) of the samples tested, with almost same frequency in rota positive and negative samples. No seasonal pattern was evident for these viruses, except that adenoviruses were most common in May, when rotavirus infection was least frequent. Pathogenic bacteria could be isolated only from 46 (13%) cases. Parasitic infection, including giardiasis and helminths, was observed in 134 (36%) cases.

The clinical picutre of diarrhoea, including the degree of dehydration, mean duration of the disease, nature of stools and mortality rate, was not noticeably different in rota positive and negative cases. But, vomiting was more common in rota positive patients (92% versus 69%) and fever less common (47% versus 66%). There were only 6 deaths in the series (2%), 3 in rota positive and 3 in rota negative cases.

Stool samples from 31 children without diarrhoea, collected during the epidemic season did not contain rotavirus.

The ELISA method for detection of rotavirus infection was used for the second study. Faecal samples from children admitted to the hospital with acute diarrhoea during November 1979 to October 1980 were tested at the National Institute of Cholera & Enteric Diseases, Calcutta for detection of rotavirus by ELISA. Out of 183 samples tested, 102 (56%) were positive for rotavirus.

The extent and seasonal distribution of rotavirus infection detected by ELISA were similar to the results obtained by electron microscopy. Rotavirus infection was present throughout the year, ranging in frequency from 92% in January to 6% in April.

These studies, spread over 5 years, establish rotavirus as the most common cause of acute diarrhoea in children requiring hospitalization, and also demonstrate a distinct seasonal preponderance. Long term studies in different parts of the world have shown two patterns of seasonal distribution of rotavirus infection. In some, as in the studies in London and Washington, the virus is common in winter and absent or rare during the warmer months. But in Birmingham, Toronto, Melbourne and Guatemala the virus could be detected throughout the year, though its frequency was much more in winter.

In Kerala, Calicut (a coastal city, situated in the tropical monsoon belt - Lat. 10°N; Lon. 75°E.) there is no distinct winter. The temperature remains remarkably steady throughout the year (Max. approx. 30°C; Min. approx. 20°C), with relative humidity around 70 to 90. It has an annual rainfall of over 3000 millimeters a year, a southwest monsoon during June to August and a lesser Northeast monsoon during October-November. The season November to February is the most pleasant with low humidity, cool nights and some dew. This is the period when rotavirus infection is most common, the reasons are not known.

The recognition that epidemic diarrhoea in children is commonly caused by virus infection has an important bearing on the management of such outbreaks. Though antibiotics may not be of any benefit in the treatment of uncomplicated diarrhoeal disease, still it is a common practice in the developing countries. This is probably due to the belief that most diarrhoeal disease in the tropics is caused by bacterial infection. Widespread use of antibiotics during epidemics of diarrhoea has led to the development and dissemination of drug resistant intestinal bacteria. It may not be a coincidence that multiple drug resistance, including resistance to chloramphenicol in typhoid bacilli appeared for the first time in Kerala early in 1972, immediately after the massive use of an oral preparation containing chloramphenicol and streptomycin during a severe epidemic of diarrhoea in children. With the proven efficacy of oral rehydration in rotaviral diarrhoea, this may replace all other measures for the management of diarrhoea once the message percolates down to the community.

Prospective Study of Rotavirus Infection in Calicut by Electron Microscopy

Month	Sample tested	Positive for rota	Percentage
1976			
October	8	5	63
November	12	12	100
December	13	12	92
1977			
January	23	15	65
February	59	50	85
March	36	19	53
April	14	7	50
May	30	8	27
June	14	6	43
July	10	5	50
September	11	6	55
October	15	12	80
November	25	19	76
December	21	20	95
1978			
January	31	25	81
February	46	39	85
TOTAL	368	260	71

Prospective Study of Rotavirus Infection in Calicut by ELISA

Month	Sample tested	Positive for rota	Percentage
1979			
November	7	3	43
December	15	11	73
1980			
January	24	22	92
February	26	19	73
March	29	22	76
April	17	1	6
May	10	1	10
June	10	4	40
July	17	6	35
August	7	1	14
September	17	9	53
October	4	3	75
TOTAL	183	102	56

Discussion

Q. *Dr. Sanyal*

What antiserum do you use for ELISA test for detecting rotavirus?

A. Samples were tested in Calcutta. Reagents were obtained from NIH and WHO.

Q. *Dr. G.H. Rabbani*

What was the endemic level of rotavirus incidence?

A. Normal population were not studied - virus are active throughout the year, but higher in winter.

Q. *Dr. M.U. Khan*

Did you try to isolate ETEC from the stool of patients having rotavirus infection? If so how often was it associated with rotavirus?

A. At that time ETEC and campylobacters were not included in our study.

Q. *Dr. O.P. Ghani*

If we superimpose the graph showing incidence of diarrhoea month by month over the graph showing incidence of rotavirus by ELISA test one finds that incidence of diarrhoea is higher from April to September. But ELISA incidence is low in most partsof India. Great seasonal variation are seen mostly in adults. Children have higher incidence during November - February,

Q. *Dr. Suailem*

Have you seen microscopic features of the stool, of rotavirus diarrhoea e.g. colour, frequency, mucous?

A. Studied - no real significant difference between rota and other stools were seen. In England - stool were seen white flakey. India - brownish and watery, vomiting in 93% cases were also seen.

Q. *Dr. V. Kumar*

Was the normal population also examined?

A. Small percentage were tested.

Q. Do you have information on incidence of multiple pathogens?

A. I do not have the information.

Q. *Dr. Mahalanabis*

What age group did you study?

A. Children below five years.

SHIGELLA DYSENTERIAE TYPE 1 INFECTION IN SOUTHERN INDIA DURING THE 1970's

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ABSTRACT

Four epidemics of diarrhoea in villages of Southern India were studied in detail since 1972. During the same period the frequency of isolation of *Shigella shigae* from patients attending the hospital have also significantly increased. At present this agent of diarrhoea is widely prevalent in Southern India and gives rise to major epidemics especially through contaminated water supplies. Major complications of *Shigella dysenteriae* infection including the haemolytic uraemic syndrome have been documented in hospitalised cases. However, in none of the epidemics that were studied, such major complications were encountered in the field. The possible role of instituting early hydration maintenance methods in the village in the prevention of sequelae will be discussed.

INTRODUCTION

The Shiga bacillus has been considered an unusual enteric pathogen in Southern India [1]. A change in the etiological pattern of acute diarrhoeal disease and dysentery in this region was seen during the 1970's with multi-drug resistant *Shigella dysenteriae 1* becoming a major cause of such episodes of illness [2]. In addition to the etiological change in patients attending hospital with dysentery, six epidemics caused by this organism were studied between 1972 and 1979. This paper records epidemiological data on some of these epidemics and documents that the major vehicle for spread of this pathogen in several instances was the water supply.

The work of the Wellcome Research Unit is supported by The Wellcome Trust, London, United Kingdom.

EPIDEMIOLOGICAL METHODOLOGY

Whenever an unusual number of cases of diarrhoea and dysentery occured, it was reported from several sources: patients attending the hospital, the government public health workers, newspaper reports or by the field team maintained by the Unit for diarrhoeal disease surveillance. Each initial report was followed up by a field visit; if the place of occurrence was farther than 100 km from Vellore, site visits lasting upto a week for spot surveys and collection of bacteriological specimens were done. For village nearer than that entire village was enumerated and detailed clinical and epidemiological data were collected. Patients were treated in field clinics established in the villages, and serious cases transferred to the base hospital. The modifications of the methodology used will be clear as each epidemic is described in detail.

THE EPIDEMICS

1973 Kan

In December 1973 newspapers reported an outbreak of serious diarrhoeal disease affecting a large number of people in a district of Tamil Nadu, 600 km south of Vellore. This was confirmed by the Medical Superintendent of a hospital in that area. A field team visited hospitals in the area and collected clinical data on about 150 patients. The patients had a history of acute dysenteric illness of an average duration of 3-5 days prior to hospitalization. Bloody and mucoid stool with lower abdominal cramps and tenesmus were frequently passed by 87% of the cases. Half of the patients were febrile at the time of admission. They were usually treated with a variety of antibiotics without relief but had a self limited illness lasting about 10-15 days. The disease was more acute and systemic manifestation of toxaemia, decreased urinary output and complicating infections such as pneumonia, were present among the preschool children and those over 50 years.

Shigella dysenteriae type 1 (Table 1) resistant to all the usual antibiotics other than neomycin, kanamycin and gentamycin was isolated from 10 out of 16 patients in the hospital.

New cases continued to appear in this region from September 1973 to February 1974, which died down thereafter. According to conservative estimates based on available records the number of such cases were about 40,000,ore-school children being worst affected.

Table 1. Rate of Isolation of Enteric Bacteria

	Patients			Controls		
	No.	Shi.dys.1	Other	No.	Shi.dys.1	Other
1973 Kan	16	8	-	-	-	-
1976 Vad	23	7	-	7	-	-
1977 Dpm	28	6	3	28	-	2
1977 Kil J*	76	3	9	37*	-	2
1977 Kil N*	27	8	2			

The isolation of *Shigella dysenteriae 1* and other enteropathogenic organisms in the four epidemics. *J July 1977, N November 1977 date on controls for N are pooled for both the periods.

1976 Vad

Vad is a small agricultural village about 40 km. In a population of 395, (between 21.7.76 - 31.8.76) 55 cases of acute dysenteric illness were recorded. After 31.8.76 no new case was recorded (Figure 1). All age groups were affected, but the highest attack rate was among the pre-school children (Table 2). Thirty-eight percent of the families were affected.

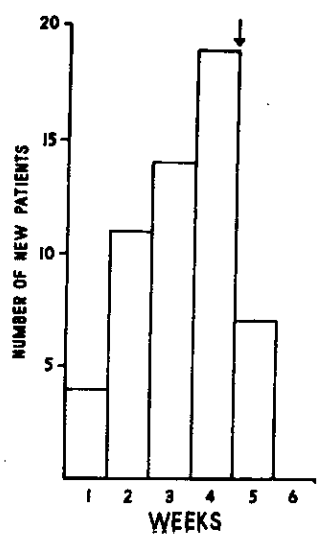

Fig. 1: Epidemic curve of Vad showing number of new cases for week from 21 July 1976. Arrow indicates time of chlorination of all drinking water sources.

Table 2. Attack Rates in Vad and Kil

Age group	Rate per hundred population	
	1976 Vad.	1977 Kil.
0 - 4.9 years	27.1	62.2
5 - 11.9 years	25.8	57.7
12 - 17.9 years	4.8	40.1
18+	10.2	38.2

The only pathogenic organism isolated in 7 out of 23 patients was *Shigella dysenteriae type 1* which was sensitive only to kanamycin, neomycin, polymyxin and septrin (Table 1).

The village was situated in a valley between two low hills, three unprotected shallow wells were its only source of water supply. The water from these wells were unsatisfactory by the presumptive coliform count method although *Shigellae* was not isolated. The village was first visited on 12.8.76 and the water sources were chlorinated on 17.8.76. After which the epidemic rapidly disappeared, no further case occurred after 31.8.76. The chlorination of the wells was maintained till 30.9.76.

Clinically the patients resembled other cases of acute bacillary dysentery seen earlier. None of the patients required hospitalization and all were managed in the field with Kaolin mixture and maintenance of hydration and nutrition. Neomycin syrup was given to three children below five years of age. Before the field team's arrival two children and an elderly man (about 70 years) had died due to dysentery.

1977 Dpm

An unusual number of diarrhoea cases were reported from camp for transients, about 450 km south-east of Vellore. This camp was on the sea shore and transients would arrive there once or twice a week and stayed for about three weeks. During a field visit in May 1977, there were 900 camp residents on that day, sixty one cases of acute diarrhoea were identified by a rapid survey. The clinical picture was not homogenous, only about half of the patients had bloody mucoid diarrhoea with abdominal cramps and fever. The stools passed were watery, like pea soup or mushy.

Piped water was available at the camp, but the cast iron pipes for the water supply and the earthenware pipes for (the water carriage) sewage system,

laid about 80 years earlier were side by side in porous sandy soil and showed considerable corrosion. Water samples from an overhead tank and four distribution taps were found to be faecally contaminated by the presumptive coliform count technique.

Shigella dysenteriae 1 was cultured from the stool of 6 and *Shigella flexneri 1* from 3 out of 28 patients.

1977 - 1978 Kil

In Kil, an agricultural village about 25 km south of Vellore, 462 out of 1028 people developed episodes of acute diarrhoea between April 1977 and February 1978 (Figure 2). The epidemic was in 2 waves with peaks in July and November. The overall attack rate (44.8 per hundred) was high, with the highest attack rate among pre-school children and attack rate decreasing with age (Table 2). This pattern was the same in both waves of the epidemic. Among those who first developed diarrhoea during June to August, 86 again developed a further, usually short, episode during October to December (Fig. 2). Bloody mucoid diarrhoea typical of bacillary dysentery was reported by 42% of those affected in July and 85% of those affected in November. Other patients had watery or mushy stools. About half of those affected had history of fever. The mean duration of diarrhoea (12 days) and median duration (8 days) was similar in both waves of the epidemic. Patients who developed a second episode of diarrhoea in November also had similar duration.

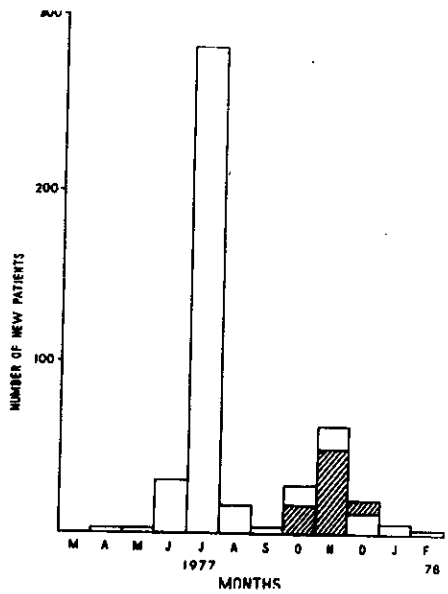

Fig. 2: Epidemic curve for Kil showing number of new cases occurring per month from March 1977. The drinking water was chlorinated at end of July and in November. The shaded bars represent patients who first developed symptoms during June to July who subsequently developed dysentery in October to December.

Stools were obtained from 65 patients in July and 38 in November. In July several organisms were isolated, *Shigella dysenteriae 1*[3], *Shigella flexneri 2*[6] and *Salmonella* group B[2]. In November, *Shigella dysenteriae 1* was isolated from 8 out of 38 stool samples from patients. Two of 37 control stools contained *Shigella flexneri 3* and *Shigella boydii 9*.

The 21 patients that were affected in the epidemic died between June '77 and February '78. Of the 331 affected during June to August 1977, there were 6 deaths, a case fatality rate of 1.8 per hundred. The number of deaths and case fatality rates were much higher during October to February. Five of the 86 patients who developed symptoms again during the period died(case fatality rate 5.8 per hundred). Among those who developed illness for the first time during this period there were 10 deaths (case fatality rate 8.5 per hundred). A field clinic in the village provided symptomatic therapy and the acute cases when willing were taken to the base hospital. The antibiotic resistance pattern of the organism was identified around end of November and thereafter clinically severe patients were given neomycin in appropriate doses. However, the major difficulty in this village was, refusal of the people to accept the necessity of maintaining nutrition and fluid intake, because considerable resistance was offered by several indigenous practitioners in the village.

The village obtained drinking water from a well from which water was pumped to an overhead tank and distributed through a system of street taps. Water supply was intermittent and generally available only in the mornings. Water sampled in July at four of the distribution taps was faecally contaminated. The well from which the water was pumped was chlorinated and chlorination maintained till end of August In November during the second wave of the epidemic water samples again grew abundant faecal coliforms and the well was rechlorinated. No pathogenic bacteria could be detected in the water on either occasion.

DISCUSSION

The four epidemics described here and other reports[2-4] clearly establish that there was an epidemic of *Shigella dysenteriae type 1* in Southern India during the 1970's. The Shiga bacillus was considered a rare pathogen in Southern India accounting for less than 1% of enteric pathogen isolations[1,5]. However, during 1970, a number of children with acute dysenteric illness and major complications were seen in hospitals in this region. Haemolytic uraemic-syndrome[2] was noteworthy among the complications seen.

The high rate of isolation of multidrug resistant strains of *Shigella dysenteriae type 1* in all the four epidemics described here, from only patients (but not controls) suggests that this organism was the major enteric pathogen causing these epidemics. In Vad and Kil during November, the epidemic was exclusively due to this organism as shown by the isolation from nearly 1/3 of the cases sampled, with very few other pathogens isolated. The situation in Dpm and Kil in July is different with a variety of other enteric pathogens also isolated from the population. In these two epidemics the Shiga bacillus was among the several enteric pathogens presumably spread by faecal contamination of the water supply. Since only a small number of hospitalized patients were sampled in Kan, it is difficult to decide the role of other pathogens in this large epidemic. The biotypes and antibiotic sensitivity patterns of all the isolates from these, as well as other epidemics, suggest that it was likely that a single strain was responsible for all these epidemics [6]. No information is available regarding the characteristics of isolates from endemic cases studied in various hospitals during this period.

The relationship of three of these epidemics to sources of water is significant. In Vad and Kil, chlorination of drinking water supply apparently stopped the epidemic with recrudescens in Kil when chlorination was discontinued. At Dpm the water distribution system was old and heavily faecally contaminated, which was relaid shortly after the study described here with a marked reduction in the number of cases of gastroenteritis. The water distribution system in Kil was newly constructed, but, as at Dpm, there was no arrangement for regular chlorination to ensure adequate quality. It is important to note that the mere provision of an overhead tank and a pipe system for distribution of water may only create a vehicle for the occurrence of water borne common source epidemics of diarrhoea. Educating villagers on proper maintenance of water supply is a must which, unfortunately in many rural areas, is not practiced.

The data reported here is reminiscent of the large pandemics due to multidrug resistant Shigella Shiga reported from Central America and Bangladesh [7-10]. These pandemics were associated with a high mortality which at least in Central America was in part ascribed to wrong therapy for Amoebiasis with Emetine, a potentially toxic drug [8]. In the first epidemic due to multidrug resistant shiga bacillus detected in Southern India [3], the mortality was very low (case fatality rate less than 2 per hundred) and it was felt that the maintenance of nutrition and hydration, without the use of antibiotics in all but a few cases, was a successful field approach to this problem. In Vad this technique was very successful with no deaths occurring after the field team started its work. Chlorination of the water source successfully terminated the epidemic. The experience in Kil was different. The village was more than three times the size of Vad and the mortality, especially during that part of the epidemic which could be ascribed to Shiga bacillus was quite high. A possible explanation for

this phenomenon is that the virulence of the organism had increased. However, there was considerable resistance in the village against accepting the necessity for maintenance of nutrient and fluid intake. The traditional regime for all diarrhoea in this area is starvation and fluid deprivation. Any attempts to alter this tradition in this village was hampered also by the non-cooperation of local indigenous practitioners of medicine. Further work needs to be done to devise optimal methods of field therapy of diarrhoeal epidemics.

The data presented in this paper and elsewhere clearly establishes that *Shigella dysenteriae type 1* was a major enteric pathogen in Southern India during most of the 1970's. During 1979 and 1980 the rate of isolation of *Shigella dysenteriae 1* in hospitalized cases at Vellore has decreased and no further epidemics have been detected in this region after 1979. How this pathogen was introduced or why it has not virtually disappeared from this community is not known. This highlights the lack of knowledge on the dynamics of infectious diarrhoea in the community. These questions can only be answered by a carefully planned prospective epidemiological study using all available methods of detecting enteric pathogens.

ACKNOWLEDGEMENTS

The work reported here was supported by the Wellcome Trust, London, U.K. The assistance of Mr. S. Chandrasekaran, Mrs. Savithri and Miss G. Thulasi in field studies is gratefully acknowledged.

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Discussion

Q. *Dr. A.M. Molla*
Was the D-xylose test done on the same cases of diarrhoea?

A. Yes, and the test was not done within a week after diarrhoea.

Q. *Dr. G.H. Rabbani*
What was the method of selection of patients?

A. Willingness to come back to the hospital was used in the principal criterion.

Q. *Dr. G.H. Rabbani*
Did you follow any residual effects of Shigella infection?

A. Yes, we followed for upto 2 years during which no major incidence in these villages were seen.

Q. *Dr. V. Kumar*
One of your slides shows initiation of therapy after an average delay of 4 days, why was this delay?

A. Average duration was 4.4 days before starting the antibiotic therapy as the villagers reported to the hospital late.

DRUG - RESISTANT *SALMONELLAE* IN INDIA

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ABSTRACT

Multiple drug resistant *S. typhi* outbreaks (1972-80) in Calicut, Ernakulum and Chandigarh are described with their phase types and their R-plasmid characters. Spread of phage type O in many cities of South since the end of 1978 was observed. The other serotypes of multi resistant *Salmonellae* carrying R-plasmid like *S. alachua, S. wien, S. anatum, S. oranienberg* and *S. indiana* reported from different cities from time to time are discussed.

Epidemiology of a clone of *S. typhimurium* 66/122/UT which has spread all over the country since 1978 is given in detail.

INTRODUCTION

There has been an unprecedented increase in the isolation of multidrug resistant *salmonellae*, which are almost always resistant to chloramphenicol, all over the world[1]. In India, the first documented outbreak of R-plasmid mediated *salmonellae* was reported in 1972 in Calicut[2]. Since then multiple resistant strains of *salmonellae* responsible for enteric fever, septicemia and gastroenteritis have been reported in increasing numbers from various parts of India. National Salmonella Phage Typing Centre, Lady Hardinge Medical College, New Delhi is continuously monitoring these reports. This communication gives in short the current status of drug resistant *salmonellae* of human origin in India.

MATERIALS AND METHODS

Various serotypes of *Salmonellae* received at the Salmonella Phage Typing Centre from different collaborative centres in India were screened for antibiotic sensitivity by the disc diffusion method of Stokes[3] and strains of *S. typhi*[4] and *S. paratyphi A*[5] and *S. typhimurium*[6] were phage typed.

RESULTS

Prevalence of Resistance among *S. typhi*

The focus of Calicut has persisted since 1972 to date. In December 1974, the prevalent phage type D_1N was replaced by phage type C5 which persisted till the end of 1979. In 1980 phage type O replaced the earlier phage types (Table 1), At Ernakulam 200 km south of Calicut 34 resistant strains (CSSuT phage typhi A-I) were received from 1976 till middle of 1978. No further strains have been received from there since then.

Table 1. Drug Resistant *S. typhi* Foci in India

Place	Year	Phage-type	Number	R-pattern	Inc. group
Calicut	1972-74	D_1N - I	72	CSSuT	H_1
	1974-79	C_5 - II	67	CSSuT	H_1
	1980	O - II	6	CSSuT	H_1
Ernakulam	1976-77	A - I	34	CSSuT	H_1
Chandigarh	1978	A - II	34	CT	H_1

Since October 1978, a multi-resistant phage type O has widely spread all over South India and strains of this clone have been received from Coonoor, Vellore, Madras, Madurai, Belgaum, Bangalore, Manipal, Bombay and Pune (Table 2). It may be noted that phage type O is a phage type of North India[7], where we are still encountering sensitive strains.

A self contained outbreak of illness due to phage type A-II (CSSuT) occurred at Chandigarh in the HMT colony in October - November 1979 involving 33 patients (Table 1 - K.C. Agarwal - personal communication).

Sporadic isolates of multi-resistant *S. typhi* have been received from Trivandrum, Bhilai, Delhi, Nagpur, Kakinada, Amritsar and several other cities.

S. paratyphi A: Two cases occurred in Madras in November 1976 due to phage type 2. One untypable strain was received from Madras in April 1977. No further resistant strains have been encountered.

Table 2. Spread of Multi-Resistant *S. typhi* Phage Type O in South India

Place	Number 1979	Number 1980	R-pattern
Coonoor	26	10	CSSuT (10) CT (25) C (1)
Vellore	14	-	CSSuT (12) CT (1) T (1)
Bangalore	8	17	CSSuT (15) CT (10)
Madras	2	7	CSSuT (3) CT (6)
Madurai	8	38	CSSuT (13) CT (30) C (2)
Belgaum	12	13	T (1)
Manipal	1	-	CT (25)
Sevagram	-	1	CT
Bombay	2	4	CSSuT (1) CT (5)
Pune	2	2	CT (2) CSSuT (1) T (1)
Goa	-	2	CT (2)
Calicut	-	6	CT (6)
Guntur	-	1	CT

Other *Salmonellae* (Table 3).

Salmonella newport: From October 1975 to March 1977, 237 strains of S. newport[8] were isolated from neonates and young children in New Delhi. Isolation of this organism tapered off after January 1977. The interesting feature of this epidemic was that the infection was fatal in about 50% of neonates, there was no mortality in children above one month of age.

S. weltevreden[9]: A small localised outbreak occurred in a Ludhiana Hospital due to *S. weltevreden*. A total of 84 strains were isolated of which only 11 were multi-resistant and were mostly from paediatric ward.

S. alachua[10]: In Calcutta this organism has been isolated in large numbers from C.S.F. of neonates suffering from meningitis, with a high mortality, from 1971 to 1977, sixty five strains were isolated.

S. wien: Seven cases were encountered in Bombay during 1976-78. This strain caused infection in two adults of which one was fatal. It was isolated from five neonates, with fatal results in three. The strain belongs to the same clone causing widespread infections in Europe and Middle East[11].

S. anatum: A small outbreak of diarrhoea due to multiresistant *S. anatum* was reported from P.G.I. Hospital, Chandigarh in April 1977. Seven resistant strains were received at our Centre. At the same time a sensitive strain of *S. anatum* was isolated from the stool of a nurse when a search for carriers was instituted.

S. oranienberg: Very recently in August 1980 there was a localised outbreak in a childrens' hospital in Delhi and 22 strains were isolated within a month. No more isolations have been made since then.

S. indiana: Concurrent to the appearance of *S. oranienberg*, a few strains of multiresistant *S. indiana* were also encountered at the same time and isolations are being made now and then. To date 9 strains have been encountered from several hospitals of Delhi.

It is of interest to note the appearance of *S oranienberg* and *S. indiana* while the outbreak due to multi-resistant *S. typhimurium* is still active (vide infra).

S. typhimurium: Before 1978 only 565 strains of *S. typhimurium* had been reported by the National Salmonella Reference Centre[12,13] over a period of twenty years (1958-1977). Their antibiograms are not available. The multi-resistant strains of *S. typhimurium* (ACKSSuT) first appeared in Bangalore in 1978. These strains belong to clone of 66/122/UT which had also been

Table 3. Drug-Resistance among other *Salmonellae* of Human Origin

Serotype	Year	Place of Isolation	Total No.	Resistance pattern	
S. newport	1975	Delhi	59	ACKSSuT	(56)
				AKSSuT	(2)
				ACSSuT	(1)
	1976	Delhi	138	ACKSSuT	(134)
				ACKST	(2)
				ACSSuT	(2)
	1977	Delhi	17	ACKSSuT	(10)
				ACSSuT	(5)
				AKSSuT	(2)
S. alachua	1971-74*	Calcutta	30	N.D.	
	1975	Calcutta	14	ACKSSuT	(3)
				ND	(11)
	1976	Calcutta	15	AKSSuT	(5)
				ND	(10)
		Bombay	1	ACKSSuTFu	
	1977	Calcutta	6	AKSSuT	(4)
				ACKSSuT	(1)
				ACSSuT	(1)
S. anatum	1977	Chandigarh	7	ACKST	(2)
				AS	(2)
				ACKS	(1)
				KS	(1)
S. wien	1976	Bombay	2	ACKSSuT	(2)
	1977	Bombay	3	ACKSSuT	(3)
	1978	Bombay	2	ACKSSuT	(2)

ND - Not done

* - Chaudhuri, Ghosal & Dutta 1978

encountered in the Philippines and Middle East[4]. This clone spread very rapidly to other cities of India (Table 4). Almost all the strains are untypable by typing phages and the few sensitive strains belong to phage type 66. Lately in some centres a few strains have also acquired gentamicin resistance.

It is of interest to note the extreme communicability of this clone which spread widely in India, while other multi-resistant *salmonellae* remained localised.

DISCUSSION

Acquisition of multidrug resistance in *S. typhi* which almost always includes resistance to chloramphenicol increases the complications of typhoid fever[15] with increase in mortality. The focus in Calicut which has remained active since the first appearance of multi-resistant strains in 1972, has seen a change in phage types twice, though the R-pattern and incompatibility group of the plasmid has remained the same. Other phage types were only confined to the places where they were encountered, till multi-resistant phage type O appeared in Coonoor towards the end of 1978 and spread widely in South Indian cities. The phage type O seems to carry an additional gene on its R-plasmid which contributes to its higher communicability.

Salmonella species other than those causing enteric fever usually cause a mild diarrhoea and very rarely may cause septicaemic complications. Such strains on acquiring a R-plasmid cause severe septicemic illness because the R-plasmids not only have genes for antibiotic resistance, but also genes coding for many other characters such as biochemical properties, enterotoxin production and other genes for virulence, increased communicability and ability to survive in the environment. When such organisms enter the hospital environment through a random patient, they spread rapidly and widely probably due to overcrowding in the hospitals[16,17]. It is also established that such infections occur more in paediatric units than in the other wards and mortality is higher in younger children[18,19].

The clone of *S. typhimurium* type 66/122/UT has spread very widely in India and has also been encountered in Philippines and Saudi Arabia[14]. The epidemiological features resemble that of *S. typhimurium* type 208, which also was distributed over wide geographical areas and food was not to be the primary vehicle of spread. One of the plasmids carried by these strains of *S. typhimurium* 66/122/UT - F_1me is similar to that found in *S. typhimurium* type 208, *S. alachua* and *S. wien*[14].

Table 4. Epidemic of Multi-Registant Salmonella Typhimurium (66/122/UT) in India since 1978

	1978	1979	1980	Total	G Resistance*	Sensitive Strains
NORTH						
Delhi	8	114	55	177	22	6
Rohtak	2	20	4	26	-	3
Chandigarh	-	208	-	208	66	16
Amritsar	-	-	10	10	-	-
Kasuli	-	-	25	25	-	-
Jammu	-	-	2	2	-	-
Varanasi	-	-	2	2	-	-
CENTRAL						
Bombay	14	33	132	179	2	2
Pune	5	6	22	33	-	-
Sevagram	-	6	36	42	-	-
Nagpur	-	-	4	4	-	-
SOUTH						
Bangalore	75	93	42	210	4	6
Belgaum	-	20	2	22	-	-
Manipal	1	33	-	39	3	3
Calicut	8	67	-	75	14	2
Trivandrum	-	18	41	59	-	4
Madras	1	21	126	148	-	4
Vellore**	20	66	146	232	16	10
Madurai	5	7	34	46	-	3
Thanjavur	-	-	4	4	-	-
Guntur	-	-	1	1	-	-

* The usual R-pattern of these strains was ACKSSuT

** Grace Koshi - personal communication

Studies carrying out complete identification and detailed genetic characterisation of plasmids are essential for understanding the epidemiology of multi-resistant strains of *salmonellae*. Especially in *S. typhimurium* such studies are essential because the plasmids and prophages restrict the action of typing phages, thus changing the markers used for epidemiological surveillance[14].

The alarming increase of multi-resistant plasmid mediated Salmonella species in India in the last decade is an ominous sign. Such a sharp increase denotes a very large pool of R-plasmid containing bacteria in the environment. The risk to man is not only by transmission of drug resistant pathogens, but also on that of drug resistant non-pathogenic intestinal bacteria, which must be abundantly communicated from animals to man. The time has come to initiate a surveillance programme for transferable drug resistance to monitor appearance of new drug resistant pathogens and the prevalence of such pathogens.

ACKNOWLEDGEMENTS

We are grateful to the following who have supplied us with the various serotypes of *salmonellae* indicated in this presentation. Prof. C.K.J. Panikar (Calicut), Dr. S.N. Saxena (C.R.I. Kasauli), Prof. K.C. Agarwal (P.G.I. Chandigarh), Dr. A.B. Chaudhury (M.E.D. Calcutta), Prof. Prema Murti (T.N.M.C. Bombay), Prof. D.U. Antani (K.H. Bombay), Dr. D.D. Banker (Sir H.N. Hospital Bombay), Prof. L.P. Deodhar (L.T.M.M.C. Bombay), Dr. Sujata Kuman (Jaslok Hospital, Bombay), Prof. S. Subramanian (B.I.M.S. Madras), Prof. S. Ramajayam (Madurai), Prof. T.D. Chugh & Dr. U. Sabharwal (Rohtak), Prof. Muriel Sukumaran (Trivandrum), Dr. J. Shanmugam (S.C.T.M.C. Trivandrum), Prof. Grace Koshi (Vellore), Prof. Shriniwas (A.I.I.M.S. New Delhi), Dr. R. Gupta (S.J.H. New Delhi), Prof. D.S. Agarwal (M.A.M.C. New Delhi), Lt. Col. D. Raghunath (Delhi Cantt.), Prof. H. Prabhakar (Amritsar).

Our esteemed acknowledgements are due to Prof. E.S. Anderson (Retired Director E.R.L. London) and Dr. Bernard Rowe, Director of WHO Collaborative Centre for Phage Typing and Resistance of Enterobacteria, London for their constant encouragement in this work by confirming most of our results.

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Discussion

Comments

It seems all Shigella dysenteriae has become multi-drug resistant encoded by group B plasmid and such strains are much more virulent than a sensitive strain.

Comments: *Dr. Ganguly*

We do not have clear understanding how the drug-resistant strains spread as no information about total picture is available in the country in spite of free travel in the country,we do not know enough how a drug resistant salmonella restricts itself in one particular area (and also develop a buffer zone).

Q. *Dr. Khan*

I believe your samples are from patients. If so:
(a) Was all of them resistant to multiple antibiotics?
(b) Have you isolated resistant types from environmental sampling? (Water and stool from latrine)

A. Earlier days these were few as the resistance are chromosomal. Now it is plasmid mediated and we get a higher percentage.

A question about efficacy of antibiotics drug resistance determination. Individual case, it might not be useful but in a pattern in epidemic, it should be done though it takes 72 hours.

THE PREVALENCE OF *ASCARIS*, HOOKWORM AND *TRICHURIS* IN PATIENTS ATTENDING A RURAL DIARRHOEA TREATMENT CENTRE IN BANGLADESH

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ABSTRACT

The prevalence of *Ascaris*, Hookworm and *Trichuris* among patients attending a diarrhoea treatment centre in rural Bangladesh was estimated by direct examination of a single stool smear. Between February and August 1977, stool specimens from 2,791 (61%) of the 4,555 patients attending the centre were examined. One or more helminths were detected in 39% (1,094). *Ascaris* was the most common isolate. Four percent of infants under 1 years of age had at least 1 helminth and the rate of isolation increased to 78% by age 4 years. The high prevalence of helminth relation observed in children aged 4 years or more has encouraged us to consider the possible value of treatment and control measures in this high-risk group.

INTRODUCTION

Intestinal helminths are among the most common infections of man and have been associated with widespread morbidity and malnutrition[1,2]. We have surveyed patients attending a diarrhoeal disease hospital in rural Bangladesh to determine the prevalence and the age of acquisition of *Ascaris*, hookworm and *Trichuris*. This information should be helpful in determining the potential value of a program for control or treatment and should identify subgroups of the population deserving priority attention.

Description of the area

The International Centre for Diarrhoeal Disease Research, Bangladesh has operated a treatment centre since 1963 at Matlab Bazar, 45 km southeast of the capital, Dacca. Annually 6,000 - 8,000 patients with diarrhoea are treated in the centre, coming from 228 villages with a total population of 269,000. The average population density in the area was 800 persons per square km.

The area is low-lying and crossed by numerous tidal rivers and canals and has a subtropical climate. During the monsoon (June-September) most of the area is flooded. The average annual rainfall of 215 cm is practically limited to this season. A cool, dry season extends to February and is followed by a hot, dry season, which lasts until the next monsoon.

The inhabitants of this area are mostly rice and jute growing farmers and fishermen. People use unprotected surface water for all purposes. Defecation is indiscriminate on land and water.

METHODS

From February through August 1977, all patients coming to the centre were cultured for enteric pathogens[3]. We examined a single, fresh stool specimen from every patient for all parasites by scanning a saline and an iodine preparation under the microscope. Preservatives, special stains, and ova concentration or counting techniques were not used. A patient was considered to have *Ascaris*, hookworm or *Trichuris* if ova were identified in a 5-minute examination of a saline preparation. Results for *Giardia lamblia* and *Entamoeba histolytica* have been reported previously[3].

RESULTS

During the 7 month period of observation, 4,555 patients were treated at the centre and 2,791 (61%) provided specimens for microscopic examination. The principal reason given for not providing a specimen was failure to remain at the hospital long enough during treatment.

One or more intestinal helminths (*Ascaris*, hookworm, *Trichuris*) were detected in 1,094 (39%) of the 2,791 patients examined. *Ascaris* was found in 85%, hookworm in 44% and *Trichuris* in 36% of the positive cases, either as single or mixed infection. Because *Strongyloides stercoralis* was found in only 1.3% of specimens examined, it was not included in the analysis. Of patients infected with *Ascaris*, 40% also had hookworm, and 35% *Trichuris* infection as well. The prevalence of *Ascaris* (65%), hookworm (52%) and *Trichuris* (36%) was greatest among children 5-14 years of age (Table 1). Adult females were more

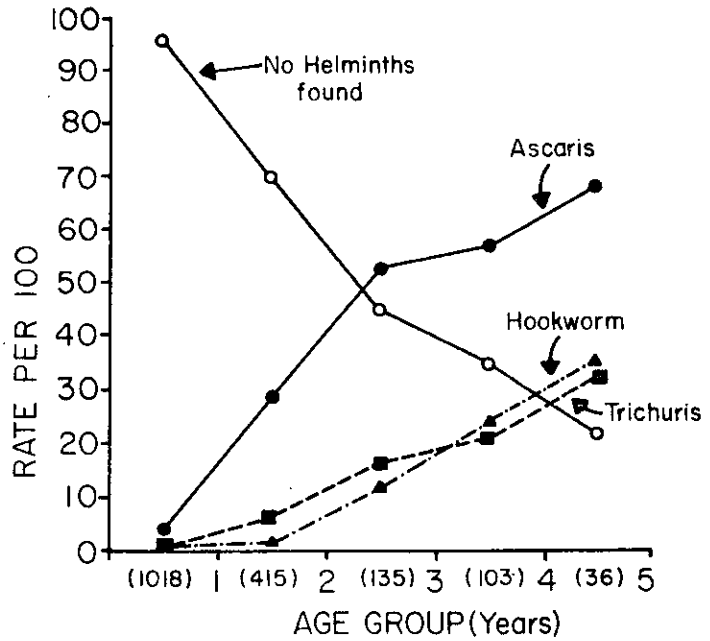
Table 1. Age and Sex-Specific Infection Rates of *Ascaris*, Hookworm, and *Trichuris* in Patients attending the Matlab Treatment Centre Bangladesh, February-August, 1977

Age (Years)	Persons Examined		*Ascaris*		Hookworm		*Trichuris*		Percentage not Infected	
	M	F	M	F	M	F	M	F	M	F
<1	663	355	4	4	1	<1	1	1	96	96
1-4	467	222	40	40	9	9	9	16	57	57
5-9	99	46	63	63	40	33	36	35	28	24
10-14	65	44	66	64	59	43	30	34	19	27
15-19	72	61	51	62	43	28	22	28	33	31
20-29	74	134	46	71	35	33	14	30	34	23
30-49	147	152	39	66	40	33	25	32	42	28
50+	109	81	43	56	43	43	22	35	36	27

likely to have *Ascaris* and *Trichuris* and less likely to have hookworm than adult males, but these differences were not observed in children.

Children were infected with *Ascaris* in the first year of life, whereas hookworm and *Trichuris* infections began during the second year (Fig. 1). By age 4 years, 70% of the children had acquired *Ascaris*, 36% hookworm and 33% *Trichuris* and only 22% were helminth free. The youngest infants passing helminth ova were males, aged 4 months (*Ascaris*) and 5 months (hookworm, *Trichuris*). While the prevalence of hookworm and *Trichuris* rose continuously from age 5 months through 5 years, the curve for *Ascaris* infection was discontinuous and rose less rapidly after age 3 years.

AGE-SPECIFIC INFECTION RATE OF ASCARIS, HOOKWORM AND TRICHURIS IN 1707 CHILDREN UNDER FIVE ATTENDING THE MATLAB TREATMENT CENTRE, (BANGLADESH, FEB-AUG, 1977)



( ) = No. of children examined

DISCUSSION

The high prevalence of *Ascaris*, hookworm and *Trichuris* observed among these patients was not unexpected considering the environmental conditions, the poor sanitation in this area, and the high prevalence of helminth in Bangladesh[4,5]. The bias introduced by surveying only 61% of the patients should not negate the observation of high prevalence. The prevalence might have increased if more sophisticated diagnostic or concentration techniques were used or multiple samples from the same patients were obtained.

In areas with high prevalence of soil-transmitted helminths, individual diagnosis is not practical because of the number of subjects involved and the limitation of resources and personnel. Furthermore, individual treatment of infected people is of transient benefit due to reinfection. Therefore, periodic

mass chemotherapy of targeted subpopulations selected on each criteria as an extreme worm burden might provide a more realistic approach towards treatment and control.

For public health purposes, as an interim measure, people in areas with a high prevalence of intestinal helminths might be screened selectively to identify groups with the greatest worm burden who could then be targeted for special treatment. In our population, this would include aged 4-14 years, who had a prevalence of infection of 73% - 78%.

Control of soil-transmitted helminths will require improved understanding of their ecology and of the human-parasite interaction. Importance of sanitation and hygiene will be needed, but this requires time. Meanwhile, periodic mass treatment of groups with a high prevalence and intensity of helminthic infection may serve as an effective intermediate step. We now need to define levels of prevalence and intensity of infection, the nutritional characteristics of the population and the impact of mass treatment to identify conditions in which it can be most worthwhile.

ACKNOWLEDGEMENT

Thanks are due to Drs. W.B. Greenough III, K.M.S. Aziz and A.R. Samadi of the International Centre for Diarrhoeal Disease Research, Bangladesh, Dacca; Professor G. Huldt of the National Bacteriological Laboratory, Stockholm, Sweden; Professor M. Katz of Babies Hospital, New York City; and Dr. R. Feldman of the Center for Disease Control, Atlanta, Georgia for their helpful comments and suggestions.

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ACUTE GASTRO-ENTERITIS DUE TO SALMONELLA

FOOD POISONING

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ABSTRACT

An outbreak of Salmonella food poisoning was investigated in March 1980. In this outbreak 10 out of 11 members of a family who ate a special kind of food locally named as "Jalar Jao" were attacked with Acute Gastroenterities within 4 to 12 hours of intake. All the 10 patients were hospitalized at ICDDR,B field hospital at Matlab. Rectal Swab was taken from one of the patients and Salmonella Gr. B was isolated. Epidemiological investigation was carried out in the family to find the possible source and mode of transmission of the disease. From the technique of preparation and the history of ingestion of the special food, the short interval between the intake and the onset of the illness, and the culture results, it is suggestive that the special food "Jalar Jao" contaminated by chicken was responsible for the sickness in the family. The Jalar Jao which is traditionally taken by rural people during hot summer months has importance from public health point of view.

INTRODUCTION

Acute gastroenteritis due to food poisoning is one of the common illnesses that affects human being This kind of acute gastroenteritis develops within a few hours (upto 48 hours) after the ingestion of the infested food or drinks. Among the bacterial causes of food poisoning, *Salmonellae* are recognized as one of the most common enteric pathogens in developed nations and many reports of salmonella food poisoning have been published[1-5]. Estimations of its occurrence and significance in the developing countries particularly in Bangladesh have been limited.

In the Matlab Field Hospital of ICDDR,B, 6-8 thousand diarrhoea cases of

various aetiologies are treated annually from a surveillance area consisting of 260,000 population according to 1974 census. Salmonella is a very rare cause of diarrhoea in these patients. During the past 18 years, we have not documented any outbreak of Salmonella food poisoning in the field hospital except one which occurred in March, 1980, involving 10 out of the 11 members of a family who ate a specially prepared food locally named "Jalar Jao".

MATERIALS AND METHODS

The Procedure of Preparation of Jalar Jao

Unhusked paddy is soaked in water and kept for 2-3 days for germination. When the paddy germinate, this is dried in the sun without boiling. This is then husked by indigenous method on the ground. Small portion of the husked rice are ground into powder. Rest of the rice is boiled to which the rice powder is added. When prepared, the food is thick and soft in consistency. After preparation, the mixture is kept in earthen pots covered with banana leaves for 2-3 days then the food is eaten with molasses and coconut.

The Outbreak

10 out of 11 members from a family of a village under Matlab Police Station, Comilla district, were admitted to ICDDR,B Matlab Field Hospital at 7:15 p.m. on March 4, 1980 with history of diarrhoea, vomiting, fever, pain in the abdomen, lethargy and toxemia. From the food history, it was gathered that all the patients ate Jalar Jao in the previous afternoon and the symptoms started within a few hours of ingestion of the food.

Epidemiological Investigation

A team went to the house of the family and collected the epidemiological information. The affected family consists of 11 members which includes the head of the household, his wife, 3 sons, 5 daughters and the wife of his eldest son. Eight other people live in the bari (compound) but do not eat or share food with the index family. The family blamed their illness on Jalar Jao which they ate on March 3, 1980 between 3 p.m. and 4 p.m. The food was prepared on March 2 and was preserved in 3 earthen pots. The family stated that they ate a little of the food, because they were saving it. All of them ate it except one daughter who was in the field and returned late in the afternoon. She did not take this food because she did not like it and also was not feeling well. All the family members except the girl who did not eat the food, became sick between 8 p.m. and 3 a.m. on March 3 and 4, 1980. The sickness continued and all reported to our hospital on March 4, 1980.

Other Possible Source

In the week before illness, the family ate mainly rice with curry of pumpkin and bean. The curry used to be prepared each evening, eaten hot, then the left-over was kept overnight, heated a little and consumed next morning. The girl who did not eat the special food, ate usual rice and curry. None of the family members suffered from diarrhoea in the preceeding week. None from this family visited other places or none from other places visited this family. Rectal swab stool specimen from all the 11 members of the family as well as from two chickens were taken for culture. Environmental samples such as water from handpump tubewell, river ghat and food samples (curry) were also taken for culture. Sample from the special food could not be taken as the food was destroyed before the team went. Rectal swabs from 24 chickens of 5 other families were also taken as control. All the samples taken were cultured in appropriate media using standard procedures.

RESULTS

Clinical Course

The age of the patients varied from 4 to 60 years (mean age 21.1) and the male female ratio was 4:6 (Table 1). The onset was between 8 p.m. on March 3 and 3 a.m. on March 4, 1980 for all the 10 patients and the mean duration between intake of the food and the onset of symptoms was 8.3 hours, the range being 4-12 hours. Diarrhoea and vomiting started simultaneously in 8 out of the 10 patients.

In one case, diarrhoea preceeded vomiting by an hour and reverse happened in another case. Fairly severe abdominal pain was present from the beginning for all the patients. Temperature ranging from 101.8^{F} to 103^{F} was present in all patients. Mild dehydration was seen in 8 patients, moderate dehydration in 2 patients on admission, all were toxic and lethargic. Routine hospital treatment started with oral and/or intravenous rehydration solution as indicated alongwith oral ampicillin. Diarrhoea stopped within 44 to 60 hours of admission, temperature came down to normal and other symptoms like pain in the abdomen passed away, except lethargy and drowsiness. All patients were discharged on the 4th day after recovery. Rectal swab was taken from one of the patients for culture and salmonella java was isolated. Salmonella java was isolated from 3 more members during the follow-up culture (Table 2). One of the two chickens of the affected family was also positive for salmonella java. No salmonella or any other pathogenic bacteria was isolated from water of food samples as well as from the chickens of the control family.

Table 1. Age, Sex, Time of Taking Jalar Jao and Time of Onset

Sl. No.	Patient	Age*	Sex	Jalar Jao Eaten	(Time)	Illness Status Yes	No	Time of Onset**
1.	S.M.	60	M	Yes	3-4 p.m.	"		8 p.m.
2.	M.N.	40	F	"	"	"		10 p.m.
3.	H.M.	22	M	"	"	"		8 p.m.
4.	D.A.	20	M	"	"	"		12 m.n.
5.	D.M.	18	M	"	"	"		12 m.n.
6.	K.N.	18	F	"	"	"		3 a.m.
7.	H.B.	13	F	"	"	"		12 m.n.
8.	R.B.	9	F	"	"	"		12 m.n.
9.	R.K.	7	F	"	"	"		12 m.n.
10.	Ru.K.	4	F	"	"	"		12 m.n.

*$\bar{X}$ = 21.1 years

**$\bar{X}$ = 8.3 hours (Interval between intake and onset)

Table 2. Summary of Culture Results

Source	Number Cultured	Number Salmonella
Affected family members	11	3
Chicken (affected family)	2	1
Tubewell water	1	0
River water	1	0
Canal water	1	0
Food sample (curry)	1	0
Chicken (control family 1)	9	0
Chicken (control family 2)	4	0
Chicken (control family 3)	4	0
Chicken (control family 4)	3	0
Chicken (control family 5)	4	0

DISCUSSION

There are several limitations of this study. One, we could not culture the special food and as such cannot definitely prove that this food was the only vehicle. Second, we did not culture all the patients on admission. Subsequently culture was done after antibiotic therapy. However, from the food history, the technique of preparation of the special food and the epidemiological investigation, it can be inferred that the Jalar Jao was responsible for the outbreak of food poisoning with salmonella in the affected family. Boiled rice with unboiled rice powder, molasses and coconut served as a good medium for bacterial growth. Keeping for more than 24 hours allowed sufficient time for incubation. Incomplete covering with banana leaves allowed scope for contamination from outside - e.g. chicken. Consumption of the food without heating enhanced the scope for infection. From the food history, it is revealed that 10 persons who ate the food, became sick and one person who did not eat the food was not affected. The short interval between taking the food and the onset of illness (4-12 hours, mean 8.3 hours) and 100% attack rate suggested food poisoning with high dose. Chicken from the affected family positive with same group of salmonella (salmonella java) as that of the patients strongly suggests of contamination of the food with salmonella by the chicken, though chickens are usually not carriers of salmonella as chickens of 5 control families were salmonella negative. Salmonella food poisoning in this country is unusual probably because of the fact that food is well cooked and generally eaten hot. But this special food was partially cooked and eaten cold. We believe that this is an interesting finding and is important from public health point of view.

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A COMPARATIVE STUDY OF ROTAVIRUS EPIDEMIOLOGY IN BANGLADESH AND AFRICA

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ABSTRACT

In a study of the epidemiology of rotavirus infection in Bangladesh and Africa, it was found that the frequency with which the virus was isolated varied from country to country and with climate. In Bangladesh, the rate varies from 40-60% during the cold season, whereas in Kenya it is between 29-40%. In South Africa, it is 61% and in Zimbabwe it is 40%. During the dry and warm seasons the rates are 20% and 8% in Mombasa, Kenya and Zaire, respectively. Age distribution of rotavirus infection seems to differ between Bangladesh and Kenya. In Bangladesh, the peak age incidence is between 6-8 months, whereas in Kenya it is between 3-5 months. Infection with rotavirus was found to be independent of the sex of children, and the virus is rarely isolated from asymptomatic children. In Kenya, rotavirus antibodies were found in 20% adult cows, 81% sheep sera and in 2% of sera collected from wild rats. Detection of rotavirus antigen in domestic animals in Bangladesh is going on now, but so far no faecal sample has been found positive.

INTRODUCTION

Although our knowledge that enteropathogenic or toxigenic *E.coli* could cause outbreaks of serious diarrhoea in infants dates back 30 years, three quarters of the cases of childhood acute gastroenteritis could not be classified aetiologically. The scene only changed in 1973 when a new viral contender - "rotavirus" was detected by Bishop and her co-workers in the duodenal epithelium of infants with acute non-bacterial gastroenteritis. Today rotavirus diarrhoea has become as well established as classical entero-bacteria. Rotavirus infections have also been found in calves, antelope, rabbits, piglets, apes, monkeys, lambs, deer, mice and foal.

Infections are common in both developed and developing countries; it has been detected in Bangladesh, India, Nepal, Kenya, S. Africa, Zaire Zimbabwe to mention a few. However, a comparative study of epidemiology of rotavirus in the developing countries has never been undertaken. In this paper an attempt has been made to compare its epidemiology in Bangladesh and some African countries.

DATA COLLECTION

The data presented in this paper were collected from Bangladesh, Kenya, South Africa, Zaire and Zimbabwe. In Kenya studies were carried out in 3 towns, namely Nairobi, Kisumu and Mombasa and also in the Masailand where stools from asymptomatic children were collected.

RESULTS

It has been found that isolation rates of rotavirus vary among countries and according to seasons (Table 1). The highest isolation rate was in S. Africa and the lowest in Zaire. Isolation rates seemed to be constant in Mombasa (Kenya) where temperatures and relative humidity are stable. The pattern of enteropathogens also differed within countries. Rotavirus was most frequently isolated in Nairobi, Kisumu and Kenya. *Shigella* was found to occur more commonly in Mombasa. Similar observations have been reported in Bangladesh by Dr. Black who found enterotoxigenic *E.coli* to be more common in Matlab than rotavirus, while the reverse was true in Dacca.

TABLE 1

ISOLATION RATES OF ROTAVIRUS IN BANGLADESH AND AFRICA

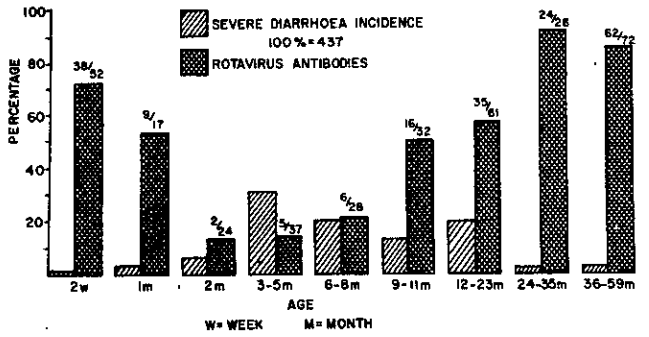
COUNTRY	PERCENT ISOLATED	TECHNIQUE USED	SEASON ISOLATED
BANGLADESH	50 %	ELISA	WET AND WARM
KENYA:			
NAIROBI	40 %	TC	COLD
KISUMU	29 %	"	COLD
MOMBASA	20 %	"	WET AND WARM
MOMBASA	61 %	E.M.	COLD
ZAIRE	8 %	ELISA	DRY AND WARM
ZIMBABWE	40 %	E.M.	COLD

A comparative age distribution of rotavirus infection in Bangladesh and Kenya is shown in Table 2. It can be seen that infection peaked in 6-8 months age-group in Bangladesh, whereas in Kenya it was in the 3-5 months age-group. The 3-5 months age-group (Kenya) coincided with the peak age-incidence of severe diarrhoea, leading to hospitalization. Therefore, in the age-group 0-5 months an inverse relationship appeared between the number of patients with gastroenteritis and the pattern of disappearance of maternal rotavirus antibodies (Figure 1). By 3 years age about 90% of the children in both Bangladesh and African have been found to have acquired rotavirus antibodies. Rotavirus infections in Kenya rotavirus antibodies were found in 20% of the sera of adult cows; 81% of the sera of sheep and in 2% of the sera of wild rats. Detection of rotavirus in animal faeces in Bangladesh has so far yielded negative results.

Table 2. Age Distribution of Rotavirus Infection in Bangladesh and Kenya

	AGE IN MONTHS				
	0 - 2	3 - 5	6 - 8	9 - 11	12 AND OVER
B	% -	% 3/11 (27)	% 14/20 (70)	% 2/5 (40)	% 12/28 (42)
K	10/40 (25)	37/97 (38)	27/82 (33)	13/49 (27)	18/108 (17)

Figure 1. Incidence by Age in Children with Severe Diarrhoea and Percentage of Rotavirus Maternal and Acquired Antibodies. Figures on top of Hystograms indicate number of Seropositive over total Number Tested.



DISCUSSION

The results reported show that isolation rates of rotavirus vary from season to season, country to country and also within areas in the same country. However, seasonal variation of rotavirus infection in both Bangladesh and some African countries is yet to be established. In certain African countries, the virus is isolated more during the cold months, in others, like South Africa, it does not appear to show any significant seasonal variation. Similar observations were also made in Mombasa, Kenya where temperature and relative humidity are rather stable.

The difference of the peak age-incidence observed between children of Bangladesh and Kenya is of particular interest. It indicates that vaccination of children in Bangladesh may be done at a later age than in Kenya; 6-8 months in Bangladesh and 3-5 months in Kenya.

Rotavirus infects domestic animals, but we do not know yet, whether cross-infection occurs between man and animals.

Discussion

Dr. H. Rahman

Q. Do animals serve as resorvoir of rotavirus? Do animals also show diarrhoea?

A. Isolation of rotavirus from a variety of animals in different countries have been reported.

Dr. Nazma Rizvi

Q. Antibody titre was high in 2 week old babies. It went down before it went up again. Do you think there is a transfer of antibodies from mothers milk to the infant? Have you looked for antirota antibodies in mothers milk?

A. Definitely there is transfer from the mother to the infant. But it disappears after 3-5 months.

O.P. Ghai (Comments)

It was interesting that rotavirus antibodies were high in the first few months of life but the incidence of diarrhoea was low till the age of 5-6 months when incidence of diarrhoea was high but rotavirus, antibodies were low. Therefore, rotavirus titre went on rising in subsequent months.

All rotavirus antibodies transmitted transplacentally and protect infants from rotavirus diarrhoea during the first few months.

ETIOLOGIC SPECTRUM OF DIARRHOEAL DISEASES IN CALCUTTA

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ABSTRACT

Faecal specimens from 190 cases of acute gastroenteritis of both sexes and all age groups admitted at the Infectious Diseases Hospital, Calcutta during the year 1979-80 were examined for enteric pathogens. Etiologic agents were detected from 61.6% of the cases; more than one agent was isolated from about 10% of the cases. *V. cholerae* biotype *El Tor*, *V. parahaemolyticus* and *Enteropathogenic E.coli* were found to be the major pathogens. Rotavirus was detected from 26 cases (13.6%). *E.coli* strains not belonging to any of the known enteropathogenic serogroups were tested for their ability of produc producing LT or ST in rabbit illeal loops and infant mouse assay. None of strains were capable of producing ST, though 4.2% of these *E.coli* strains were capable of producing LT in adults rabbit illeal loops. *A. hydrophilia*, *Pl. shigelloides*, Group F vibrios, were detected from fewer number of cases. No strain of *Yersinia enterocolitica* could be detected.

THE ROLE OF CONVALESCENT CARRIERS IN THE TRANSMISSION OF CHOLERA IN TWIN CITIES, OF HYDERABAD AND SECUNDERABAD DURING 1978

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ABSTRACT

Convalescent carrier rate was found to be about 8% on average for 249 index cases. During the earlier part of the year, where antibiotic treatment was not insisted upon, the acute convalescent case rate was about 14%. When 6 tablets of furoxone were given to every patient irrespective of the laboratory diagnosis, from 15th June 1978 onwards the acute convalescent carrier rate has fallen down to 4.3%. The paper deals with the possible beneficial role of antibiotic or chemotherapeutic drugs in reducing the reservoirs of infection of cholera, which may act as sources of infection in maintaining the chain of infection of cholera.

CHAPTER 2

PATHOPHYSIOLOGY & PATHOGENESIS

PATHOGENESIS OF MALABSORPTION IN INFECTION WITH *GIARDIA LAMBLIA*

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ABSTRACT

Although there are evidence to suggest that *Giardia lamblia* can cause malabsorption, its exact pathogenesis is not clear. To investigate this, animal models of giardiasis were used. The following experiments and observations were made:

1. Transport studies using the everted gut sac techniques and the tissue accummulation technique were carried out. There was a significant decrease in the absorption of glucose and amino acids (glycine and alanine), all of which undergo an active, energy dependent absorption. On the other hand, transport of potassium, which diffuses passively across the bowel, was not affected.

2. Transport kinetics were carried out using glucose and glycine as substrates. Results were similar in both. The maximal transport velocity (V max) was considerably reduced in *Giardia* infected animals whereas the affinity constant (Km) of the substrates for their respective receptor sites was not altered.

3. Mucosal enzymes assay showed a significant reduction in the levels of brush border lactase, sucrase and alkaline phosphatase. By contrast, cellular enzymes (LDH, GOT and GPT) were not affected.

4. Histological examination of the small intestinal mucosa did not show any appreciable abnormalities apart from the presence of *Giardia* trophozoites lying in close proximity to the surface epithelium.

It was concluded that infection with *Giardia lamblia* results in damage to the brush border membrane of the enterocyte with a decrease in the number of specific substrate receptor sites.

INTRODUCTION

There is evidence to suggest that *Giardia lamblia* is pathogenic to man. Although the majority of subjects infected with this parasite remain asymptomatic, a small but significant number develop a variety of clinical symptoms including diarrhoea and malabsorption syndrome[1].

The mechanism by which *Giardia lamblia* produces its harmful effect has been the subject of much speculation and a number of theories have been put forward to explain it. Some important ones are discussed briefly: The mechanical barrier theory suggests that large numbers of *Giardia* trophozoites form a covering over the small intestinal mucosa thus creating a barrier to the transport of nutrients[2,3]. Damage to the small intestinal mucosa, as a cause of malabsorption, has been suggested by some workers, on the basis of histological studies[4,5].

Leon-Barua and Lambrares - Cruz have postulated the role of intestinal bacterial flora[6]. They observed that tetracycline suppressed the diarrhoea in patients with *Giardia* infection despite the fact that parasites continued to be excreted in the faeces. Other factors that have been incriminated are: altered bowel motility[7], utilization of dietary nutrients by large numbers of *Giardia* trophozoites[8] and disturbance of exocrine pancreatic functions[9]. More recently, Tandon et al[10] have noted higher levels of deconjugated bile salts in the upper small bowel of patients with *Giardia* infection compared with control subjects and postulate that this may be the responsible factor.

In order to study some of these factors we have used animal models of giardiasis and this paper covers our work in this field over the last 3 years[11,12].

METHODS AND RESULTS

Transport Studies:

Part I: 30 laboratory bred albino rats were used: Group I consisted of 10 normal control animals. Group II (10) rates were infected with *Giardia lamblia*[13], briefly, *Giardia* cysts obtained from human stools were tube fed (0.2 ml of a solution containing 2000-3000 cysts/ml). Group III (10) rats were fed a similar quantity of a Giardia-free stool suspension.

All of 30 rats were killed 11-13 days after the start of the study. Transport studies were carried out using the everted gut sac technique of Wilson and Wiseman[14]. The small intestine was removed and immediately transferred to a Petri dish containing ice cold normal saline. Three

segments, 4 cm each were obtained from the proximal, middle and distal portions of the small intestine. The segments were everted and washed gently in normal saline to remove any adhering matter. One end of segment was closed with a cotton ligature, 0.5 ml of normal saline + a bubble of air was injected with a blunt needle attached to a syringe through the other end. The segments were then suspended in a jar containing 1000 ml normal saline with 100 mg% glucose, 3mg% glycine and 4 mEq/L potassium. The jar was kept in an incubator for 1 hour after which the luminal contents were withdrawn and analysed.

Results: There was a significant fall in the transport of both glucose (Table 1) and glycine (Table 2) in *Giardia* infected animals compared with the control group. On the other hand, the group fed with the *Giardia* free stool suspension showed no such alteration (Table 1 & 2). Potassium absorption was very similar in all the three groups of animals (Table 3).

Table 1. Mean ± SD levels of Glucose Absorption (mg%) in the Three Groups of Experimental Animals.

Group	Proximal Segment	Middle Segment	Distal Segment
I	122.2 ± 11.7	112.2 ± 20	95 ± 12.6
II	47.5 ± 17.6	43.2 ± 17.6	36.6 ± 9.3
III	117.8 ± 12.2	115.4 ± 16	101.7 ± 13.6
I Vs. II	< .001	< .001	< .001
P =			
I Vs. III	N.S.	N.S.	N.S.

Table 2. Mean ± SD Levels of Glycine Absorption (mg%) in the Three Groups of Experimental Animals

Group	Proximal Segment	Middle Segment	Distal Segment
I	7.3 ± 1	5.3 ± 1	4.7 ± 1.5
II	3.7 ± 1	3.7 ± 0.8	1.8 ± 1
III	7 ± 2	6 ± 1.1	7.5 ± 1.8
I Vs. II P =	< .001	< .01	< .001
I Vs. III	N.S.	N.S.	N.S.

Table 3. Mean ± SD Levels of Potassium Transport in the Three Group of Experimental Animals

Group	Proximal Segment	Middle Segment	Distal Segment
I	3.63 ± 0.74	3.49 ± 0.5	3.77 ± 1.46
II	3.53 ± 0.2	3.63 ± 0.22	3.07 ± 0.25
III	4.25 ± 1.7	4.22 ± 0.86	4.2 ± 1.2
I Vs. II P =	N.S.	N.S.	N.S.
I Vs. III	N.S.	N.S.	N.S.

Part II: ~~Four~~ weeks old mice were infected with *Giardia lamblia* by the method described earlier. On this occasion transport studies were carried out using the tissue accumulation technique[14]. The substrates used were ^{14}c labelled D-glucose, L-alanine and glycine. The radioactivity taken up was measured in a liquid scintillation counter.

Results: Similar to the observations with the everted gut sacs, there was a considerable decrease in the transport of glucose, glycine and alanine in the *Giardia* infected group compared with the control group; the differences being statistically significant in respect of all the three substrates.

Transport Kinetics Studies

Kinetics of glucose and glycine transport were examined in the intestinal segments of *Giardia* infected animals by measuring the uptake at different concentrations (2-10 mM) of the substrate. From the data obtained the maximal transport rate (Vmax) and affinity constant (Km) were calculated.

Results: The transport kinetics of glucose in *Giardia* infected animals showed an affinity constant (Km) of 4.37 mM which was similar to that in the normal control animals. On the other hand, the maximal transport rate (Vmax) was considerably reduced in *Giardia* infected animals (158. 7 μ moles/hr/g tissue) compared with the control group (357.1 μ moles/hr/g tissue). Transport kinetics with glycine gave similar results with the Km of *Giardia* infected animals being similar to that of the controls (5.7 mM) while Vmax of infected animals (27.02 μ moles/hr/g) was greatly reduced compared with the control animals (45.5 μ moles/hr/g).

Enzyme Assay

The small intestinal mucosa was analysed for both brush border and cellular enzymes. Brush border enzymes assayed were lactase, sucrase and alkaline phosphatase and the cellular enzyme estimated were lactate dehydrogenase (LDH), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT). Enzyme activity was expressed as units/g protein.

Results: The results are showin Table 4. All the three brush border enzymes tested were significantly reduced in *Giardia* infected animals compared with controls. By contrast, cellular enzymes showed no appreciable differences between the two groups of animals.

Table 4. Enzymes Activity Mean (± S.D.) in Control and Giardia Infected Mice

Experimental group(n)	Enzyme activity (unit/g protein)					
	Alkaline phosphatase	Sucrase	Lactase	LDH	GOT	GPT
Control (8)	45.8±9.4	28.5 ± 8.0	1.9 ± 0.6	187.4 ± 28.2	455.9±11.1	243.2 ± 6.3
Giardia infected (8)	27.1±9.7	14-3 ± 4.1	1.1 ± 0.2	202.9 ± 9.2	460.4±10.3	235.4 ± 5.1
P value	<.01	<.01	<.05	N.S.	N.S.	N.S.

Histological Studies

Full thickness small intestinal specimens were obtained from *Giardia* infected animals and were examined by light microscopy. Numerous *Giardia* trophozoites were seen lying close to the surface epithelium. However, no trophozoites were observed within the mucosa. Furthermore, the small intestinal mucosa was perfectly normal and we could detect no histological abnormalities of the surface epithelium, lamina propria or the crypts.

DISCUSSION

The present series of experiments were carried out to determine the pathogenesis of malabsorption in *Giardia* infection.

Transport studies using both the everted gut sac technique and the tissue accumulation technique showed a significant derangement in the absorption of glucose and amino acids (glycine and alanine). All these substrates undergo an active, energy dependent, carrier based absorption. On the other hand, transport of potassium, which moves passively across the small bowel mucosa and is either absorbed or secreted depending upon its luminal concentration, was not affected. Thus, *Giardia lamblia* interfered only with the active transport mechanisms of the small intestine. Therefore some of the theories mentioned earlier could be safely discarded, for example, a mechanical barrier formed by large number of *Giardia* trophozoites, as a cause of malabsorption, is unlikely since such a barrier would interfere with the transport of all substances, whether these are actively or passively absorbed. Other possibilities such as a motility disorder of the small bowel and disturbance of the exocrine pancreatic functions can be similarly discounted. The possibility that *Giardia* may be consuming the substrates is also unlikely because in the present series of experiments the intestinal segments were first gently shaken to remove any adhering matter before they were employed for the study, and more important, the substrates in the bathing fluid were present in such quantities as to make this hypothesis untenable.

Theoretically, a decrease in the active absorptive process is either due to a reduction in the number of specific receptor sites or a decrease in the affinity of the substrate for the receptor. To determine as to which factor was operating in *Giardia* infection, transport kinetics were carried out. Using glucose and glycine as substrates, it was observed that there was a considerable decrease in the maximal transport velocity (Vmax) in *Giardia* infected animals while the affinity of the substrates for their respective receptors (km) was not altered. These findings suggest a decrease in the number of specific receptor sites on the enterocytes as a cause of deranged absorption.

We next proceeded to determine the site of the absorption defect. For this we took the help of intestinal enzymes. There was a significant reduction in the levels of brush border enzymes whereas cellular enzymes were not affected, thus indicating that in *Giardia* infection, the brush border membrane of the intestinal cell is the primary site of damage.

It is interesting to note that in our study we failed to observe any histological abnormalities of the small intestinal mucosa, as assessed by light microscopy. We should, therefore, like to conclude that *Giardia lamblia* produces its harmful effect by damaging the brush border layer of the enterocyte and that such an abnormality is sufficient to induce absorptive malfunction at least in the experimental animals. It is possible that when the infection is more severe, histological abnormalities of the mucosa such as partial or even subtotal villous atrophy, that have been recorded, become apparent.

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Discussion

Q. *Dr. A.M. Molla*

Active transport of glucose is depressed in giardiasis as shown by you. What is the quantative effect of giardiasis in intact human jejunum?

A. Giardia infection affects the upperbowed, changes can be observed also in the lower part.

IMMUNOLOGICAL AND IMMUNODIAGNOSTIC STUDIES IN EXPERIMENTAL AND HUMAN GIARDIASIS

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ABSTRACT

The basic differences in immunologically mediated susceptibility and protection in experimental and human giardiasis has been worked out along with the mechanism involved in immunopathological events. In Swiss albino mice it was observed that parasitic load negatively correlated with T cell population and function and also correlated negatively with level of antibodies. The susceptibility was related to immuno competence, and infection itself did not lower immunity. Absorptive functions like active transport glucose and alanine and brush border enzymes were affected by parasitic load so also was the level of prostaglandins E and F in the intestinal tissue. It did not depend on the immune competence of the animal. However, the crypt mitosis could have been caused by sensitized lymphocytes. Human giardia positive patients with malabsorption had low total T cells and high crtotoxic and suppressor T cell population compared to giardia positive cases without malabosorption. Antigenic sensitization was only seen in cases with malabsorption. A theory of T cell mediated injury in human situations with severe giardiasis has been postulated. Antibodies in giardiasis with malabsorption were more persistant and had higher G.M. titre compared to control. Although antibodies were found in all the three main classes of immunoglobulins, in cases with malabsorption it was mainly IgA type. Secretory IgA in cases of malabsorption was low. An antibody mediated cytotoxic reaction has been suggested for cases of malabsorption was low. An antibody mediated cytotoxic reaction has been suggested for cases of giardiasis with complications. Lack of minimalsecretory IgA could facilitate entry of antigens and there subsequent contact with immuno competent cells. Variations in antigenic make up of giardia could also be operative along with the host factors and a polyacrylamide analysis has been presented for the support.

INTRODUCTION

Giardia infection is worldwide in distribution[1]. It is widely prevalent in India[2,3,4] and incidence varies from somewhere between 1% to more than 30%. In the United States it is around 4%[6]. It was long considered to he harmless leading to mild transient intestinal complains or diarrhoea with an average of 9.1 day's of incubation period and an average course of four days to six weeks[5] which resolved spontaneously. Recently it has been recognised that it might lead to long standing malabsorption and diarrhoea lasting several months to years[6]. It is acquired readily in endemic regions by travellers from abroad[7,8], transmission in epidemic proportion have occurred through contaminated drinking water[9,10], through Foeco oral route in day care nurseries[11], through sewage[12,13], and in Homosexuals[14].

The G. duodenalis (includes G. lamblia) can infect man, beaver, coyote, cattle, cat and dog and can experimentally infect certain other mammalian species[15] and there can be a man to animal transmission through reservoir as in beavers in colorado[15].

Individuals and experimental animals differ in their susceptibilities to infection[16]. Its particularly severe in immune deficient status like hypogammaglobulinaemia[17]. In immunologically competent mice infection, it is cleared in six weeks[18] and in nuce mice it persists till six months[19]. Normal mice usually show resistance to reinfection[20] butnuce mice show resistance to reinfection only when reconstituted with immunocompetent cells from normal mice. Thymus derived lymphocytes have been shown to cross the epithelium and attach to these trophozoites[21] and low secretory IgA has been shown in giardiasis cases[22,23]. All this shows relationship of susceptibility and resistance of this infection with immune mechanisms of body. Both T cells and B cells have their role however no concrete proof to their role and their dynamics has been available[24]. Normal intestinal biopsy to changes resembling sprue has been described in human giardiasis. Round cell infiltration and hyperplasia of lymphoid nodules[21] and increased intra-epithelial lymphocytes have also been demonstrated[38]. Hence a sensitised lymphocyte mediated injury has been hypothesized. However, there have been lack of convincing proof.

AIMS AND OBJECTIVES

The present attempts to find some of the answers regarding immunologically mediated susceptibility and resistance to this infection and whether a case for immunologically mediated tissue injury exists. Also an attempt has been made to find out immunological differences between an uneventful episode and a sprue like malabsorption state.

MATERIALS AND METHODS

Inbred strains of Swiss albino mice of the same sex, 3-4 weeks old weighing 15-20 gms were used as experimental animals. In case of patients of giardia positive cases with or without malabsorption were studied.

Thymectomy was done according to the method described by Weir[26]. Animals were infected by the method of Robert-Thomson et al[20] and were sacrificed serially on different days after infection starting from 3rd day and trophozoite count was done according to the method of Vasudeva et al[27]. The T & B lymphocytes from the spleen of infected and normal mice were done according to the method of Jondall et al[28], Gravely et al[30] and Gupta and Kapoor[29]. The transport study of ^{14}C glucose and ^{14}C alanine was done according to the method of Anand[31]. The enzyme assay was done according to the method of Dalquist[32]. The prostaglandins were estimated according to the method of Garg and Sharma[33]. The indirect haemagglutination test was performed according to the method described by Mahajan et al[34]. The fluorescent antibody test was performed according to the method of Ridley and Ridley[35]. The immunoglobulins were estimated according to the method of single radial immunodiffusion described by Mancini et al[36]. Polyacrylamide gel electrophoresis was put up by according to the method described by Ganguly et al[37].

RESULTS AND DISCUSSION

Viswesaraya's[25] growth of axenic G. trophozoites in TPS I medium Owens[2] study of scanning beam and cross sectional electronmicroscopy showed tissue invasion with giardian trophozoites. and Robert-Thomson[18,19] demonstrated variation in susceptibility in various animal strains. CH3 A maintained astable infection throughout the study whereas BALBC and DAB_2 showed a resistance and cleared it in six weeks time. The resistance or susceptibility could be transferred to filters. They also stated that BALBC nu/nu showed a persistant infection whereas above mice given lymphoid cells from intact BALB/C showed a drop.

While working in the laboratory with Swiss albino mice it was observed that mice could be infected effectively with 2000 cysts of giardia lamblia isolated from human source and a peak infection was reached on the 9th day, by the 15th day the infection dropped considerably whereas infection was almost eliminated after 28th day. The interesting feature was that although upto 9th or 12th day, the thymectomised irradiated animals showed a sharp increase in trophozoites compared to normal animals. The count in two groups became similar by 15th to 18th day. The severity of infection was related to trophozoite counts obtained. It was reflected in body weight which dropped in thymectomised group significantly by 9th day and then showed gain by 15th day,

When the T & B cells were monitored in the spleen of these mice it was observed that even in thymectomised mice T cells were not completely eliminated and remained at level 20%. Subsequently these cells showed a rising tendency and became near normal by the 12th to 15th week. It is now known that intact BALB/C mice have very few T cells in bone marrow, whereas Swiss albino mice have considerable number of these. Moreover, these mice were given bone marrow transfusion, which might have helped in the picking up of the count of T cells at a late phase. There was no significant dimunition of T cells or B cell count during the course of giardia infection. Rather there was also a concommitant increase in B cell by the 9th day post infection.

Both T and B cell negatively correlated with trophozoite count Transformation studies did not show any depression of lymphocyte function from basal levels against non-specific stimulants like PHA and CONA - Although in thymectomised group it was lower than the normal. Considerable degree of sensitization to giardia, antigen was attained by 9th day which was not existing in preinfection period.

Giardia antibodies in both the groups peaked on 15th and 18th day respectively and in 50% of normal animals it remained at low levels at the end of experiment and in thymectomised group it came down to basal level. Showing that antibody response was not very prolific in these animals and it was transient.

All these experiments demonstrated the arrest of infection in both group depended on both cellular and humoral immunity, perhaps much more in the former. There was no depression of immunity because of the infection itself. However if the T cell count could be lowered in these animals, the load of parasite became high very early and was quantitatively much more till it was arrested around day 15th.

To work out an immunological theory for the functional lesions produced in giardiasis, the above two models were correlated with active transport of ^{14}C glucose and C^{14}. Alanine as also the brush border enzymes. It was observed that active transport of sugar and amino acids were affected in giardiasis but the levels were much lower in case of thymectomised animals and not much recovery was seen till end of experiment. The day to day

variation of the levels was not significant as was true also for day to day variation in trophozoite counts in both the groups. So the magnitude of transport was affected by high trophozoite counts. Brush border Disaccharidases and alkaline phosphatase were all affected. In case of enzymes the peak drop was on 9th to 12th day and it correlated with more faithfully with the parasite load.

Mechanism of diarrhoea in most of the infective conditions have been attributed to cyclic AMP, pathway which in turn can be modulated with tissue prostaglandin E and F. Two of the important causative agents for giardiasis. We shall not go into the details of the mechanism in this particular talk but here also the prostalaandin E and F were higher in thymectomised group. The later one much more than former, which also depended on parasitic load. This function also correlated well with dynamics of the infection, peaking and trailing at the same time.

There have been studies where giardia trophozoite were shown in proximitv to Payer's Patches and sticking to lymphocytes in the gut lumen. Increased intra-epithelial lymphocyte have also been demonstrated in giardiasis[38]. We observed that these lymphocytes proliferated at a much later period and the number increased and stayed increased at a time when the functional aspects were picking up. In thymectomised animals also in our model lymphocytes picked up to a fair number although much lower than normals at days twelve to fifteen

Hence we could postulate that damage to brush border was mainly due to increased load of parasite. However, at a late time both the parasite as well as sensitized lymphocytes which were demonstrated in both the groups could cause additive damage. But this cannot be the only factor as crypt mitosis was coming down when the intra-epithelial lymphocytes were still high.

In human situation we have basically selected two groups. A giardia positive group with at least two malabsorption parameter positive and giardia positive cases without malabsorption. Unlike animals in the group with malabsorption about 45% cases had singificantly low T cell count compared to normals and more than 50% were lower than those without malabsorption. Active T cells which form a percentage of total T cells were also within the normal range but higher than the control group. These are most probably antigen responding cell population. B. cell percentage was normal. Going further in T cell subsets it was observed that the Tu helper cell population were same in both the groups however in most of the cases 10/15 were below the mean normal group whereas Ty cell which contain T cytotoxic cell population or suppressor cell population were high in malabsorption group compared to normals. In T cell functions unlike normal snimals where it was not compromised throughout the study period, in humans in 22% cases stimulation index with PHA was low and another 49% were below

the mean normal. Whereas in the without malabsorption cases only 22% were lower than mean normal. Antigen sensitization was marked in cases with malabsorption compared to the cases without malabsorption where it was within the normal range

Coming to giardia antibodies wer developed IHA test through an improved method of purification of giardia trophozoites through Ficoll density gradient and preparing an antigen for it[39]. It compared well with Ridleys cyst antigens and IFA test system. Although a very low response was obtained as is evident through low G.M. titres. IFA test had an edge over IHA but had a disadvantage that different batches of cysts behaved differently and only precystic stages were suitable. However when giardia positive cases were further broken down into subgroups the geometric mean titre in proven malabsorption cases were much higher. The antibodies in malabsorption cases were maintained in the platu level whereas in cases of acute diarrhoeal diseases it fell sharply within 3-4 weeks. In cases of acute diarrhoeal disease a part of antibody was IgM whereas in cases with malabsorption antibody were present mostly in other classes of immunoglobulin as seen after mercaptoethanol treatment.

In unselected cases of giardiasis the antibodies correlated with all the three classes of immunoglobulins There was no correlation between copro and serum antibodies In most of the cases of malabsorption the secretory IgA was low which picked up only in a fraction of cases after 8 weeks of treatment

From the above studies in human it seems that there is a susceptible group which is immunocompromised with cellular immune parameters, which go into sprue like symptoms when afflicted with giardiasis. Cytotoxic T cells and sensitized T cells which are present in very high proportion can be the cause of tissue damage as reflected in much more severe histological lesions in humans. Subtotal villous atrophy is very rarely found in animals. Antibodies might have an additive effect on pathogenesis as they persist far longer in cases with malabsorption and belong to an immunoglobulin class which participates in antibody mediated cell cytotoxicity. Low secretory IgA which only partially improves during 8 weeks of treatment facilitates higher antigen absorption and penetration and might compound the pathogenic mechanism.

Paradoxically in reports from western literature increased incidence of giardiasis has not been shown in selective T cell defects as well as severe combined immunodeficiency, nor in X linked hypogammaglobulinaemia of childhood outset but has only been seen in variable adult outset primary hypogammaglobulinaemia However, these have been studies in areas where giardia is not endemic and a true picture might emerge from such places.

At last we should not forget the parasite itself. In polyacrylamide analysis of 9 giardia strains although band 6 was the major band but band 4 and 7 were absent from certain strains and two minor bands were present in addition in some other strains. Some more immunochemical studies should be carried to determine the importance of these antigens in protection and pathogenic mechanism of giardiasis.

In conclusion although there are some basic differences in human and animal giardiasis a susceptible population both due to lowered cellular and humoral parameters exists, resistance to infection might be also both due to sensitized T cell and antibodies they operate both at gut level and intraluminaly. Initial damage to brush border membrane might be due to increased parasitic load, however subsequent pathogenic mechanism could be carried out with sensitized cytotoxic T cells and through antibody mediated cell cytoxicity. Variations in susceptibility might also be due to variations in strains with addition and lack of certain important antigens.

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Discussion

Q. *Dr. S. Kabir*

Was it a neonatal thymectomy?

A. It was neonatal thymectomy.

Q. You have looked at the transformation of spleen lymphocytes. Have you looked at stimulation with intestinal lymphocytes?

A. No.

Q. *Dr. Molla*

How would you separate the effect of giardiasis from that of other parasites or protozoas?

A. First select the giardia cases, other parasites from the gut is then eliminated.

EXPERIMENTAL STUDIES ON ENTEROPATHOGENICITY OF THE SO-CALLED NAG VIBRIOS AND THE RELATIONSHIP OF THEIR ENTEROTOXIN WITH THAT OF CHOLERA AND *E.COLI* LT

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ABSTRACT

Vibrio cholerae serotypes other than I have been isolated from sporadic cases and outbreaks of choleric diarrhoea in various parts of the world. A few of them have been shown to produce an enterotoxin immunologically similar to cholera toxin. However, no systematic study has yet been reported about their enteropathogenicity and the immunobiological relationship of their enterotoxin with that of cholera and *E.coli*. The present study was done with all the 59 serotype strains of these vibrios. Live cells and culture filtrates of all the strains caused accumulation of fluid in rabbit illeal loops indicating their enterotoxicity irrespective of serotype. All the culture filtrates caused increased vascular permeability. The enterotoxic substance was found to be heat and pH labile. Chlorpromazine reduced fluid outpouring by 60-70% indicating that the enterotoxin might act through mediation of cAMP. None of the strain could produce keratoconjunctivitis in rabbit's eye indicating lack of invasive capacity. The enterotoxicity of culture filtrates of all the 59 serotype strains could be neutralized by cholera and *E.coli* LT antitoxins both in illeal loops and skin. Culture filtrates of 11 serotype strains gave precipitin bands with cholera antitoxin and reaction of identity with cholera antitoxin.

INTRODUCTION

Vibrio cholerae serotypes other than 1 (the so-called NAG vibrio) have only been recently recognized as one of the important aetiologic agents of diarrhoea. During the last two decades there have been several reports of isolation of these from cases of cholera and diarrhoea outbreaks all over the

world[1,9]. Few strains of these vibrios have been shown to be enterotoxigenic in experimental models[10-17] and some of them have been reported to elaborate an enterotoxin immunobiologically similar to cholera toxin[14,15]. Recently it has also been shown that the cholera toxin and *E.coli* LT are structurally, functionally and immunologically identical[18,19]. Since no systematic study with all the serotype strains of NAG vibrios has been reported so far, the present study was envisaged to see the enteropathogenicity of all the known serotype strains of NAG vibrios in various experimental models and the immunobiological relationship of their enterotoxin with that of *V. cholerae* serotype 1 and *E.coli* LT.

METHODS

Strains

All the serotype strains (2-60) of the so-called NAG vibrios were kindly provided by Dr. R. Sakazaki of National Institute of Health, Tokyo, Japan. The strains were maintained in peptone agar stab cultures at room temperature.

Choleragen

Purified and lypholised choleragen made from *V.cholerae* 569 B was kindly provided by Dr. S. Varallyay of Swiss Serum and Vaccine Institute, Berne, Switzerland.

Antiserum to *E.coli* LT

This was made from *E.coli* strain No. P263 and was kindly supplied by Dr. F. Dorner of Sandox Forschungsinstitut, Wien.

Illeal Loop Test

The test was performed with live cells and culture filtrates of NAG vibrios following the method of De and Chatterjee[20] and Annapurna and Sanyal[21]. Each test was performed in triplicate. Those strains which did not cause accumulation of fluid initially, were passaged in illeal loops until they gave positive reactions[17].

Preparation of Culture Filtrates

Culture filtrates were prepared following the method of Dubey and Sanyal[22] and were preserved at 20°C until use.

Time Course of Fluid Accumulation

One ml quantity of culture filtrates prepared from 4 strains of NAG vibrios alongwith that of *V. cholerae* 569 B in Brain Heart Infusion Broth (BHIB, Difco) were inoculated into illeal loops of each rabbit. The animals were sacrificed after 1, 2, 4, 8 and 18h. The experiment was done in triplicate.

Suckling Mouse Assay

This was done following the method of Dean[23]. The experiment was done in triplicate.

Skin Reactivity of Culture Filtrates

The test was performed following the method of Craig[24]. The results were expressed as mean of the cross diameters of the reaction.

Effect of Temperature on Enterotoxicity of Culture Filtrates

Culture filtrates were held at different temperatures for varying lengths of time as described earlier[21]. Tests for illeal loop and skin reactivities were carried out with these culture filtrates.

Effect of pH on Enterotoxicity of Culture Filtrates

This was done following the method as described earlier[25]. Illeal loop and skin reactivity tests were then performed.

Probable Mode of Action of the Enterotoxin

To determine the probable mode of action of the enterotoxin, the rabbits were pre-treated with inhibitors of various mediators of enterotoxic activity as described below. One rabbit without any drug was included in each experiment to serve as control. Illeal loop tests were performed with culture filtrates both in test and control animals. The animals were sacrificed after 8h and the amount of fluid accumulation per unit length was found out.

Drugs, Dosage and Routes of Administration

1. Prostaglandin (PG) synthesis blockers:

   Diclofenac sodium: 20 mg/kg given intraperitoneally (I.P.) on two consecutive days and on the third day 3h before the experiment.

2. PG receptor blocker:

   SC 19220: 20 mg/kg with 0.1% tween-80 was given I.P. 2h before the experiment.

3. 5-hydroxytryptamine (5-HT) synthesis blocker:

   Parachlorphenylalanine (PCPA): 300 mg/kg was given I.P. on 3 consecutive days, the experiment being done on the fourth day.

4. 5-HT receptor blocker:

   Methysergide: 2mg/kg was given I.P. 3h before the experiment.

5. cAMP inhibitor:

   Chlorpromazine: 5 mg/kg was given intramuscularly (I.M.) 1h before the experiment.

Sereny's Test

The test was performed in rabbit's eye following the method of Sereny[26] as described earlier[27].

Preparation of Antiserum to Choleragen

Albino rabbits weighing about 3.0 kg were immunized with 500 µg choleragen in 0.5 ml phosphate buffer saline (PBS, pH 7.2) mixed with 0.5 ml Freund's incomplete adjuvant intracutaneously in 3 doses at 14 days intervals followed by 2 intravenous injections at 7 days interval. The rabbits were bled 7-10 days after the last injection. The crude serum was used as antitoxin.

Neutralization of Enterotoxicity of Culture Filtrates of NAG Vibrios by Cholera and *E.coli* antitoxins in illeal loop

The experiment was done following the method of Kasai and Burrows[28] as modified by Dubey and Sanyal[25]. The cholera and *E.coli* LT antitoxins and normal rabbit serum (NRS) were inactivated at 56°C for 30 min. Serial 2-fold dilutions of the antitoxins in PBS were mixed with equal volume of culture filtrate of a particular strain. The same culture filtrate was also mixed with equal volume of undiluted NRS. The mixtures were incubated at 37°C for 1h and then injected one in each loop in 1.0 ml volume. The animals were sacrificed after 8h and the accumulation of fluid per unit length was determined. The experiment was done in duplicate.

Neutralization of Skin Reactivities of Culture Filtrates of NAG Vibrios by Cholera and *E.coli* LT antitoxin

The test was performed following the methods of Craig[29], Evans et al[30] and Dubey and Sanyal[22]. The toxin-antitoxin mixtures were prepared as described in neutralization test in illeal loops.

Immunodiffusion Test

This was done following the method of Ouchterlony[31]. The antitoxins were placed in the central wells and the peripheral wells contained cholera toxin, *E.coli* LT and culture filtrates of NAG vibrios. The reactions were allowed to develop in a moist chamber at 4°C upto 48h.

RESULTS

Live Cells

Live cells of all the 59 serotype strains of NAG vibrios caused accumulation of fluid in rabbit illeal loops. However, 11 strains did so only after 1-2 serial passages in rabbit gut. The volume of fluid ranged from 0.5 to 1.6 ml per unit length. In case of *V.cholerae* 569 B, the volume fluid accumulated varied from 0.9 to 1.6 ml per unit length. Strain to strain and loop to loop variations were, however,observed.

Culture Filtrates

Culture filtrates of all the serotype strains gave positive illeal loop reaction. Accumulation of fluid varied from culture filtrate to culture filtrate and loop to loop. Most culture filtrates caused accumulation of fluid comparable to that caused by culture filtrate of *V. cholerae* 569 B.

Time Course of luid Accumulation

Outpouring of fluid in the range of 0.1 - 0.2 ml per cm was started after a lag period of 2h and increased proportionately with time. ull blown reactions were seen at 8h with further increase in volume of fluid at 18h. *V.cholerae* 569 B also behaved similarly (Figure 1).

Suckling Mouse Assay

None of the 13 culture filtrates tested in this model caused distention of intestine and all of them gave gut weight to remaining body weight ratio of less than 0.08.

Skin Reactivity of Culture iltrates

Culture filtrates of all the serotype strains produced increased vascular permeability as judged by bluing reaction. Strain to strain and animal to animal variations were, however, observed. Many of the culture filtrates caused induration and skin necrosis also.

Effect of Temperature and pH on the Enterotoxicity and Skin Reactivity of Culture Filtrates

Enterotoxicity and skin reactivities were considerably reduced on holding the culture filtrates at 56°C for 30 min. These activities were completely lost at 60°C for 20 min. or 65°C for 10 min. (Figure 2). However, the necrotic skin reaction was seen with unheated culture filtrates only. The maximun activity of the culture filtrates was seen in the pH range of 6.0 - 8.0. Enterotoxicity and skin reactivities showed gradual loss at higher and lower pH values (Figure 3).

Effect of Drugs

No inhibition of fluid outpouring was observed in rabbits pre-treated with PGs and 5-HT inhibitors. However, chlorpromazine reduced the volume of

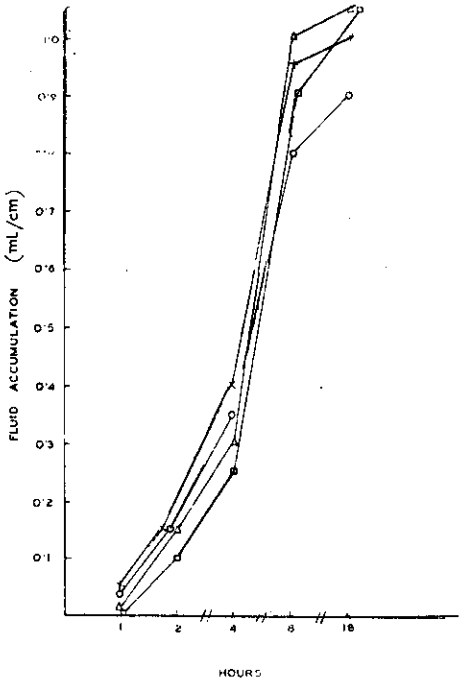
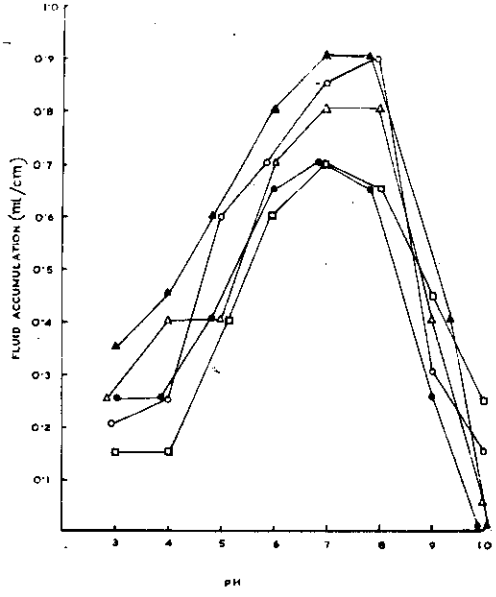


Fig. 1: Time course of fluid accumulation with culture filtrates of NAG vibrios

Fig. 2: Effect of temperature on enterotoxicity of culture filtrates of NAG vibrios



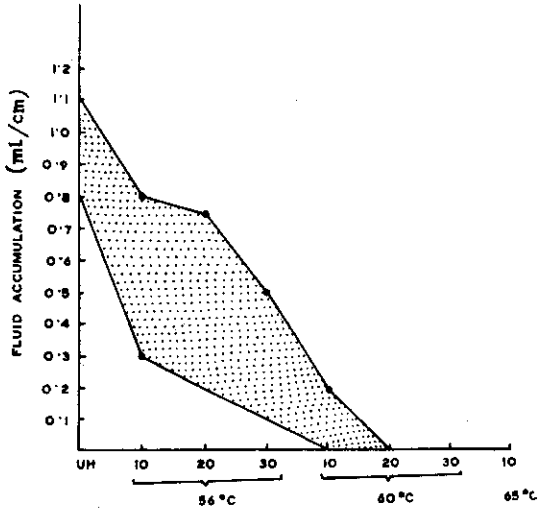


Fig. 3: Effect of pH on exterotoxicity of culture filtrates of NAG vibrios

fluid by 60-70% (Table 1).

Table 1. Effect of Drugs on Illeal Loop Reaction

	Range of fluid accumulation (ml/cm)						
Serotype	Indomethacin	Diclofenac sodium	SC-19220	PCPA	Methy-sergide	Chlorpro mazine	Control
34	0.9-1.1	0.8-1.0	1.1-1.4	0.8-1.0	0.9-1.2	0.2-0.4	0.9-1.
16	0.6-0.8	0.7-0.9	0.6-0.9	0.5-0.8	0.7-0.9	0.1-0.3	0.7-0.
569 B	1.0-1.5	1.0-1.2	0.9-1.2	1.0-1.5	1.0-1.2	0.6-0.8	1.2-1.

Sereny's Test

None of the 10 serotype strains could produce keratoconjunctivities in rabbit's eye. However, *S. flexneri* 543 always induced keratoconjunctivities within 72h.

Neutralization of Enterotoxicity of Culture Filtrates of NAG Vibrios

By cholera antitoxin: Out of the 59 setotype strains, culture filtrates of 35 were completely neutralised and 24 only partially when tested with undiluted cholera antitoxin. There was proportionately less neutralization of enterotoxic activity with serial 2-fold dilutions of the antitoxin (Table 2). None of the culture filtrate could be completely neutralized by antitoxin diluted to 1:16 or more. NRS could not neutralize any of the culture filtrates.

By *E.coli* LT antitoxin: Out of the 18 culture filtrates belonging to different serotype strains, only 12 were completely neutralized and the remaining 6 partially when tested with undiluted antitoxin. There was proportional decrease in neutralization of enterotoxicity with serial dilution of the antitoxin (Table 3). None of the culture filtrate tested could be neutralized completely by the antitoxin diluted to 1:8 or more.

Table 2. Percent Neutralization of Enterotoxicity of Culture Filtrates of NAG Vibrios by Cholera Antitoxin

Neutralization (%)	Dilutions of antitoxin						
	1:1	1:2	1:4	1:8	1:16	1:32	1:64
100	35	32	25	14	-	-	-
76 - 99	24	27	34	25	15	4	-
51 - 75	-	-	-	20	34	11	-
26 - 50	-	-	-	-	10	32	-
0 - 25	-	-	-	-	-	12	59

Figures indicate the number of culture filtrates

Table 3. Percent Neutralization of Enterotoxicity of Culture Filtrates of NAG Vibrios by *E.coli* LT antitoxin

Neutralization (%)	Dilutions of antitoxin						
	1:1	1:2	1:4	1:8	1:16	1:32	1:64
100	12	11	4	-	-	-	-
76 - 99	3	4	9	9	-	-	-
51 - 75	3	0	2	5	10	-	-
26 - 50	-	3	3	4	6	9	-
0 - 25	-	-	-	-	2	9	18

Figures indicate the number of culture filtrates.

Neutralization of Skin Reactivities of Culture Filtrates of NAG Vibrios

By cholera antitoxin: Three parameters of skin reactivity viz. vascular permeability factor, induration factor and dermonecrotic factors were taken into account. With undiluted antitoxin PF activity of 17 culture filtrates out of 25 tested were neutralised completely and the remaining 8, only partially (Table 4) and there was proportional fall in neutralisation of PF following serial dilutions of the antitoxin.

Four of the culture filtrates did not produce induration in the control itself. Out of the remaining 21 culture filtrates 11 were completely neutralised and 10 partially with undiluted antitoxin. With increasing dilutions

of the antitoxin, there was proportional decrease in neutralisation of induration effect (Table 5).

Table 4. Percent Neutralization of PF activity of Culture Filtrates of NAG Vibrios by Cholera Antitoxin

Neutralization (%)	Dilutions of antitoxin				
	1:1	1:2	1:4	1:8	1:16
100	17	11	7	4	-
76 - 99	1	1	1	-	-
51 - 75	6	8	8	5	-
26 - 50	1	4	5	12	4
0 - 25	-	1	4	4	21

Figures indicate the number of culture filtrates

Table 5. Percent Neutralization of Induration Factor Activity of Culture Filtrates of NAG Vibrios by Cholera Antitoxin

Neutralization (%)	Dilutions of antitoxin				
	1:1	1:2	1:4	1:8	1:16
100	11	8	8	5	-
76 - 99	-	-	-	1	-
51 - 75	8	6	1	1	-
26 - 50	2	6	11	13	5
0 - 25	-	1	1	1	16

Figures indicate the number of culture filtrates

No necrosis was observed with 9 controls out of 25 tested. In remaining 16 culture filtrates this factor was completely neutralized with undiluted antitoxin and in 4 even at dilution of 1:16.

By *E.coli* LT antitoxin: Out of the 22 culture filtrates tested, PF activity of 13 was neutralized completely and of 9 only partially with undiluted antitoxin (Table 6). Four of the 22 culture filtrates did not produce induration in control. Out of the remaining 18, complete neutralization was

seen in 8 and the rest were partially neutralized with undiluted antitoxin (Table 7). Proportional decrease in neutralization of both PF and induration activities was observed on serial dilutions of the antitoxin.

Table 6. Percent Neutralization of PF Activity of Culture Filtrates of NAG Vibrios by *E.coli* LT antitoxin

Neutralization (%)	Dilutions of antitoxin				
	1:1	1:2	1:4	1:8	1:16
100	13	6	3	2	-
76 - 99	1	1	0	0	-
51 - 75	6	8	7	3	-
26 - 50	2	6	8	7	3
0 - 25	-	1	4	10	19

Figures indicate the number of culture filtrates

Table 7. Percent Neutralization of Induration Factor Activity of Culture Filtrates of NAG Vibrios by *E.coli* LT Antitoxin

Neutralization (%)	Dilutions of antitoxin				
	1:1	1:2	1:4	1:8	1:16
100	8	6	6	-	-
76 - 99	2	1	-	1	-
51 - 75	3	4	5	5	-
26 - 50	5	6	3	5	-
0 - 25	-	1	4	6	18

Figures indicate the number of culture filtrates

No necrosis was seen with 8 culture filtrates. In the remaining 14, it was completely neutralized and undiluted antitoxin and in one even at 1:16 dilution.

Gel Diffusion Test

Culture filtrates of serotype 2,3,5,9,14,15,16,17,18,19 and 36 gave precipitin bands with cholera antitoxin. All of them showed reaction of identity amongst themselves and with cholera toxin.

Similar studies with *E.coli* LT antitoxin failed to show any precipitin band.

DISCUSSION

Accumulation of fluid in illeal loops indicates that all the strains of NAG vibrios are enterotoxigenic irrespective of serotype. The (individual) variations in volume of fluid outpouring are probably due to difference in quantitative release of toxin which depends on many variable factors[32-36]. It has been observed by some workers[7,14,15] that only some strains of NAG vibrios are enterotoxigenic and are less enterotoxic than *V. cholerae* serotype 1. Our results show that all the NAG vibrios are as enterotoxin as *V. cholerae* serotype 1.

It has been reported by Evans et al[37] that secretory response to heat stable toxin is rapid and short lived, whereas response to heat labile toxin is delayed and more sustained. In the present study the outpouring of fluid started after a lag period of 2h and was sustained upto 18h. This indicates that the enterotoxin of NAG vibrios is heat labile like that of *V. cholerae* serotype 1. The heat labile nature of the enterotoxin factor(s) is further substantiated by the observation of negative suckling mouse assay which is considered to be a specific model for heat labile enterotoxins only[23]. Results of heat treatment also point towards the heat labile nature of the toxin. It has been reported that the complete loss of enterotoxicity takes place at 56°C for 30 min.[14,15], but in our study the enterotoxicity was completely lost by exposing the culture filtrates to 60°C for 20 min. or 65°C for 10 min. The relative heat resistance may either be due to the presence of organic matter in the medium which protects it from the effect of heat or the enterotoxin molecule itself is associated with some other factor(s) as has been observed by many workers[14-16, 38-40].

All the serotype strains of NAG vibrios were found to elaborate vascular permeability factor. Majority of the strains produced induration and a number of strains an additional necrotising and/or haemorrhogic factor(s). The results of this study indicate that vascular permeability factor can be used as an alternate bioassay for enterotoxigenicity of these vibrios.

The mechanism of action of cholera toxin has been shown to be mediated via cAMP[41-43] and chlorpromazine - a cAMP inhibitor has been shown to block the diarrhoeagenic action of cholera and *E.coli* enterotoxins[44-46]. It has been suggested by Lonnroth et al[44] that cAMP levels may be regulated by hormones like PGs and 5-HT. In this study, an attempt was made to elucidate the mode of action of the NAG vibrio enterotoxin. It was observed that PG and 5-HT do not play any role in the causation of diarrhoea by NAG vibrios. However, chlorpromazine reduced the outpouring of fluid by 60-70% indicating that the enterotoxin of NAG vibrios may act through mediation of cAMP.

Negative Sereny's test in this study implies that NAG vibrios lack invasive ability although occasional reports of systemic vibriosis are available[3,8,47] Robins-Brown et al[16] were also unable to produce keratoconjunctivitis by a strain isolated from a case of systemic vibriosis although they could show invasion of the intestinal mucosa. This discrepancy might be due to low invasive potential of the organism.

Enterotoxicity and PF activity of majority of the culture filtrates of NAG vibrios when tested in rabbit illeal loops and skin were completely neutralized by cholera antitoxin indicating similar activities of the two toxins in these biological models. Those culture filtrates which were only partially neutralized might have contained more toxin molecules that could be neutralized by the available titre of the antitoxin. It has also been shown that NAG vibrios elaborate a large amount of LPS in culture medium[48,49] which might have interefered with complete neutralization of enterotoxicity and vascular permeability. Another possibility is that certain enzymes produced by NAG vibrios might have an additive effect and naturally an antitoxin to pure choleragen would be unable to neutralize such a factor.

Complete neutralization of enterotoxic and PF activities of most of the culture filtrates of NAG vibrios by antitoxin to *E.coli* LT indicates that the two toxins are similar in biological activity. Partial neutralization of some culture filtrates might be either due to presence of more toxin molecules or due to presence of other biologically active factors.

Induration and dermonecrotic factors were not constantly associated with PF indicating that they may be unrelated to enterotoxin. Both cholera and *E.coli* LT antitoxins could neutralize the induration factor of NAG vibrios though less efficiently tha PF. This might be due to the presence of certain other factors, such as blanching factor as observed in *E.coli* LT (Finkelstein et al 1976).

Eleven culture filtrates of difference serotypes of NAG vibrios gave precipitin bands in gel diffusion indicating that cholera toxin and enterotoxin elaborated by NAG vibrios are immunologically identical. The remaining culture

filtrates did not give an precipitin band, 6 of them even when concentrated 10 times. However, Finkelstein (personal communication) observed that 100 times concentration of *E.coli* culture filtrate was required to get a precipitin band with cholera antitoxin.

Immunodiffusion studies with *E.coli* LT antitoxin and culture filtrates of NAG vibrios failed to give any positive results, probably because the culture filtrates contained less toxin to give positive precipitin band in gel.

All these results indicate that the observed differences in toxigenicity of NAG vibrios are quantitative rather than qualitative and enterotoxins of *V. cholerae* serotype 1 and serotype other than 1 are similar in their biological activity and at least some of them are immunologically identical as well. It is also indicated that the enterotoxins of NAG vibrios are similar to *E.coli* LT in their biological activity.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the assistance rendered by Snehlata Pandey, Manorama, B. N. Shukla and B. Banerji.

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Discussion

Dr. Rabbani

Q. Have you measured cyclic AMP and adenylate cyclase activity in chlorpromazine treated animals?

A. Yes.

Q. Whether chlopromazine was given prior to or after challenge with *Vibrio cholerae*?

A. It was given prior to the challenge.

Dr. Sharma

Q. What was the significance of passages increasing enterotoxin production by NAG strains. Does it indicate a phenotypic change?

A. The capacity of producing toxin is decreased in all strains when kept in the laboratory. It may be different phenomena rather than phenotypic charge.

Dr. Khan

Q. Did your estimate show rise in vibriocidal titre? Was it for human or animals? If so, what is the result?

A. There was rise both in animals and humans.

Dr. Panikar

Q. What is the source of your NAG strains? Are they all from patients?

A. All from patients who are mostly from India, some were from abroad also.

Dr. Aziz

Q. We could not find any skin permeability factor (PF) activity for NAG vibrios. However, we found skin necrosis at injected sites. Did Dr. Sanyal note any qualitative difference between the bluing caused by NAG vibrios and O1-group of vibrios.

A. We noted induration with *vibrio cholerae* O1 type and necrosis with NAG. Necrosis is not related to enterotoxin. Skin test is not a good assay for measuring NAG enterotoxin.

Dr. Ganguly

Q. Normally common antigens between ETEC and Vibrios has been demonstrated in yield diffusion. In fact only a single band was seen which showed a like of identity. What was the reason?

A. The antisera used was prepared against cholera antigen.

Dr. Deb

Q. You have mentioned that all the NAG strains tested by you were pathogenic. Were these strains isolated from human beings or environment?

A. From human beings.

Q. What percent of NAG strains isolated from environmental sources are toxigenic in your study?

A. Toxin production is different from strain to strain, if given passage then the strains become positive.

MORPHOLOGY OF RECTAL MUCOSA IN HEALTH AND DISEASE IN BANGLADESH

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Institute of Postgraduate Medicine and Research, Dacca and
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ABSTRACT

Rectal mucosal morphology were studied in normal volunteers and in patients with amoebic dysentery, shigellosis, unclassified dysentery and ulcerative colitis in Bangladesh. Essentially no difference was observed in the morphology of normal Bangladeshi volunteers and Europeans. Sigmoidoscopic examinations in 11 cases of amoebic dysentery diagnosed by observance of E. histolytica trophozoites, showed ulcerative colitis - like diffuse inflammation in 5 and patchy ulcers in the rest. Histological appearances in 5 were similar to those of ulcerative colitis while rest had focal collection of mononuclear cells. E. histolytica was observed in the mucosa of 3 cases only. Shigella flexneri (3 cases) and sonnei (1 case) produced sigmoidoscopic appearances similar to ulcerative colitis while histology showed marked neutrophilic infiltration in 2 cases and focal collection of round cells in two. Among 13 unclassified dysenteric cases, sigmoidoscopic and histologic appearances were similar to ulcerative colitis in 8 and patchy inflammation or ulcers in the rest. Sigmoidoscopic and histologic features of 5 ulcerative colitis cases were similar to Europeans. Amoebic dysenteric and even non-specific dysenteric cases responded promptly to tinidazole therapy within one week in majority cases and between 2-3 weeks in the rest. Before confirming ulcerative colitis, antiamoebic therapy should be tried in countries with endemic amoebiasis.

INTRODUCTION

Morphological examination of the rectal mucosa can be helpful in the diagnosis of dysenteric disorders. For proper interpretation of the normal spectra of the rectal mucosal is essential but this has not been widely studied in many geographic areas[1]. This becomes all the more relevant in view

of the variations in the morphology of the jejunal mucosae in the various geographic areas where these have been studied[2,3,4,5].

The purpose of the present study was to delineate the morphology of rectal mucosa of subjects with dysenteric symptoms and normal population.

MATERIALS AND METHODS

Subjects - Only adult subjects were studied.

Normal volunteers: Twenty normal volunteers participated in this study. Informed consents were obtained from them before inclusion in the study. All of the subjects were professional blood donors and were paid a fixed sum for the participation.

Subjects with dysenteric symptoms: Thirty three subjects in this category had complains of diarrhoea with mucous, pus and blood. No consent was needed for inclusion in the study as they were subjected to standard investigations (vide infra) for treatment.

Methods - All the subjects were examined clinically.

Three consecutive faecal samples were examined microscopically from the normal volunteers. None of these samples contained cysts or trophozoites of *Entamoeba histolytica*. Eight had ova of helminths in the faecal samples.

A fresh faecal sample was examined from all the dysenteric subjects. Eleven had trophozoites of *Entamoeba histolytica* in their faeces and they were diagnosed as cases of amoebic dysentery. Faecal samples from all the remaining subjects were cultured for shigella. *Shigella flexneri* was isolated from three and *sonnei* from one. These four subjects were labelled as cases of bacillary dysentery. Five of the remaining subjects were known cases of ulcerative colitis. They presented during an acute episode and they were diagnosed on the basis of clinical presentation, endoscopic, radiologic and histologic appearances. The remaining 13 subjects were labelled as cases of unclassified dysentery.

All the subjects were signoidoscoped without prior preparation and the findings were recorded; a rectal biopsy was taken from the posterior wall about 10 cm. from the anal margin or from the most abnormal looking area. In the case of an ulcer, biopsy of the margin was done. The rectal biopsy specimens were subjected to standard histological examination.

RESULTS

Normal volunteers: Sigmoidoscopic appearances of the rectal mucosa in all the volunteers were similar to those of the normal European population. All had intact mucosa with normal vascular pattern. However patchy erythematon areas were more frequently seen than what is seen in the Europeans.

Histologic appearances (Fig. 1) of the rectal biopsy specimens were similar to the western population. They had usual number of goblet cells, surface epithelium was normal and the lamina propria contained usual types and numbers of round cells.

Amoebic dysentery: Sigmoidoscopic appearances of the rectal mucosa in 5 of the 11 amoebic dysentery cases were similar to those of acute severe ulcerative colitis. The mucosa was diffusely inflammed and covered at places with blood and pus. In the remaining 6 cases the mucosa had patchy ulcers which were covered with dirty grey pus. In these cases the areas intervening the ulcers were either near normal or showed mild inflammation.

Histological appearances in eight of the amoebic dysentery were similar to those of acute ulcerative colitis (Fig. 2). The mucosa was markedly inflammed. The lamina propria was infiltrated by both polymorphs and round cells. These changes were confined to mucosa in almost all places. Crypt abscesses were found less often as compared to a typical case of acute ulcerative colitis. Goblet cells were somewhat depleted but not to the extent of that seen in ulcerative colitis. At places the mucosa was ulcerated. Purulent exudate could be seen at places outside the mucosa. In three instances trophozoites of *Entamoeba histolytica* were seen in the mucosa.

In the remaining three cases the histology revealed focal collections of mononuclear cells with some polymorphs.

Shigella dysentery: Sigmoidoscopic appearances of the rectal mucosa in all three cases with *S. flexneri* and one case with *S. sonnei* were similar to those of acute ulcerative colitis.

Histology showed marked neutrophilic infiltration of the lamina propria in 2 cases (Fig. 3). The remaining two cases showed only focal collections of round cells. None of the biopsies showed crypt abscesses and there was only mild goblet cell depletion.

Ulcerative colitis: The sigmoidoscopic and histologic appearances in all of the five ulcerative colitis cases were similar to what had been described in the Europeans (Fig. 4).

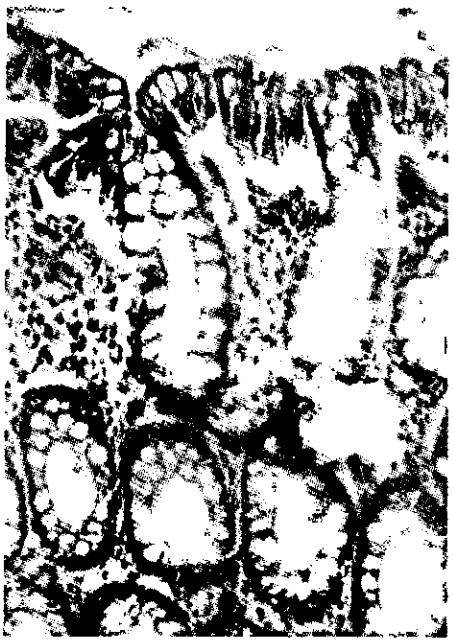
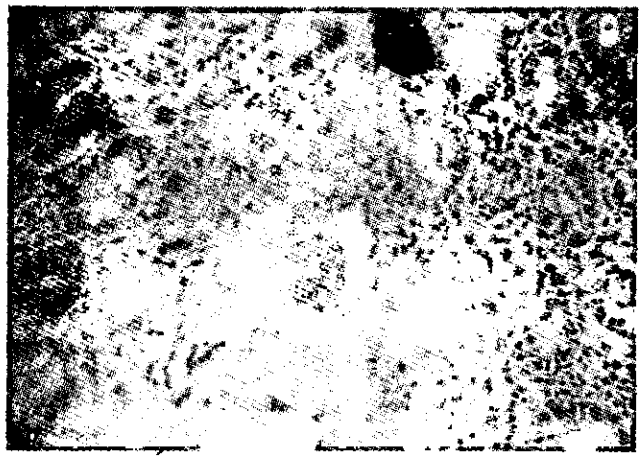


Fig. 1: Normal rectal mucosa (x240)

Fig. 2: Amoebic proctocolitis with one crypt abscess (x240)



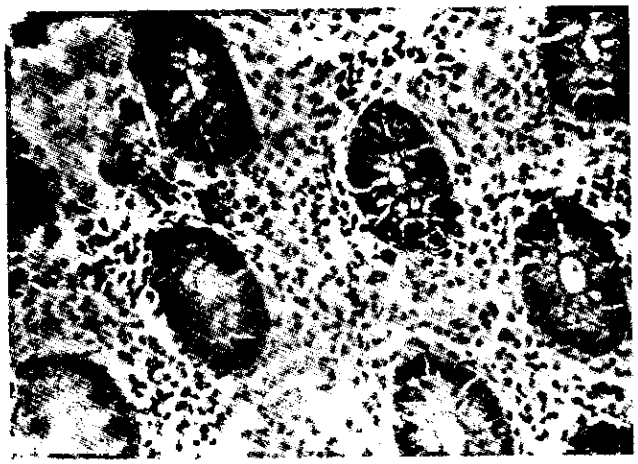


Fig. 3: Shigella dysentery (x240)

Fig. 4: Ulcerative colitis (x65)

Unclassified dysentery: Sigmoidoscopic appearances in eight of the 13 cases were similar to those of acute ulcerative colitis. The remaining five cases showed patchy inflammation or ulcers.

Histological appearances of the eight cases who mimicked ulcerative colitis on signoidoscopy also were similar to ulcerative colitis (Fig. 5). The remaining five cases showed patchy inflammation consisting mainly of round cell infiltration of varying intensity. There were superfician ulcers at places.

DISCUSSION

Contrary to our expectations the morphology of rectal mucosa in normal volunteers from Bangladesh were indistinguishable from the normal Europeans. Considering the differences in the diet and the frequent presence of helminths in the colon in Bangladesh, this was surprising. To confirm the observations, the paraffin blocks were sent to the Department of Pathology, John Radcliffe Hospital, Oxford where these were mixed with similar blocks from normal Europeans and the parapared slides were blindly assessed by one of us (JP). No appreciable difference could be found between the Bangladeshis and the Europeans.

Similar sigmoidoscopic appearances of the rectal mucosa in amoebic or shigella dysentery and ulcerative colitis has been observed by many authors[6,7,1]. In addition we observed that in some cases of amoebic dysentery there were patchy ulcers with comparatively normal mucosa in between. Whether these differences in the sigmoidoscopic appearances depends on the age of evolution of the disease have not been assessed by us. The histology of the rectal mucosa from most of the patients with amoebic dysentery were also seen to be similar to that of patients with ulcerative colitis, however some quantitative differences were observed between these two groups of patients. Prathap and Gilman[6] did not find crypt abscess in patients with amoebic dysentery, while Whitehead[1] observed only some poorly formed crypt abscesses. We observed some well-formed crypt abscesses but these were few and far between.

Contrary to the above observations[7,8,1] some of our patients with amoebic dysentery showed only focal collections of round cells. This was also the experience of Prathap and Gilman[6].

All our patients with amoebic dysentery had vegatative form of *Entamoeba histolytica* in stool. *E. histolytica* was however observed in three out of the 11 rectal biopsy specimens. It was concluded that absence of *E. histolytica* in rectal biopsy specimens does not negate the diagnosis of acute endemic amoebiasis, antiamoebic therapy should be tried before making a diagnosis of

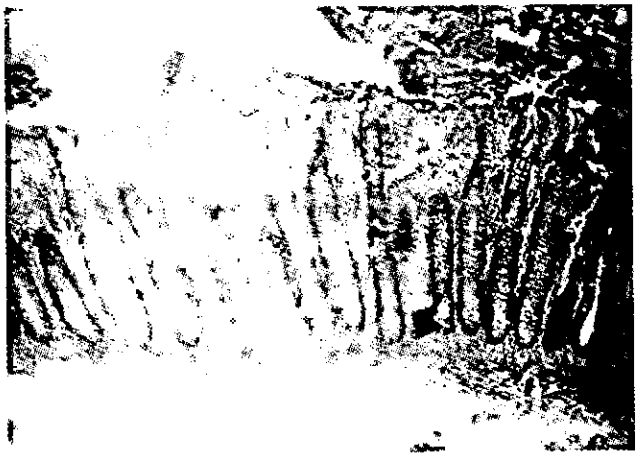


Fig. 5: Unclassified dysentery (x65)

ulcerative colitis. All our patients with unclassified dysentery responded promptly to antiamoebic therapy.

To our surprise we did not find any flask shaped ulcer in any of the amoebic dysentery cases. Whitehead[1] claimed that flash shaped ulcers are typically found in rectal biopsy specimens from patients with amoebic dysentery. Ulcers in the study patients were non-specific in character. When amoeba is not demonstrable, there is no pathogomonic tissue changes under light microscopy in amoebic proctocolitis. We did not examine the tissue samples with recently described methods which have been claimed to be superior for demonstrating amoebia in the tissues[9].

The histological appearances of shigella cases also were non-specific. Neutrophilic infiltration tended to be more marked and goblet cell depletion less marked than what is expected in non-specific inflammatory bowel disease. These differences are quantitative and not of qualitative nature. Histological changes in shigellosis can sometimes be patchy as was the case in amoebic dysentery. Shigellosis can be diagnosed only by growing shigella

from faeces. Shigellosis being a self limiting disease, should not create a proboem for differentiating from ulcerative colitis in long term[10].

Sigmoidoscopic and histologic appearances of the five ulcerative colitis cases of this series were similar to those from western countries. Only five cases could be collected in one year from the only gastroenterology init in Bangladesh. This is a reflection of the rarity of the condition in Bangladesh. Nagaratnam[11] reported 16 cases from Sri Lanka and stated that the condition is not very uncommon; the authors did not mention the time period during which these 16 cases were collected. All our patients with ulcerative colitis received a course of antiamoebic therapy without any appreciable relief.

We could not make a definitive diagnosis in 13 cases Leaving the five cases with ulcerative colitis, these constituted about 50% of the total dysenteric subjects. There are several possibilities: some of these subjects had amoebiasis or shigellosis and had taken some medication before reporting us; although they denied taking any drug. As mentioned earlier we did not use any special stains for demonstrating amoeba in the tissue: We treated them with a course antiamoebic agent (tinidazole) and eight of them showed a prompt response (in 1-2 days). This led us to believe that they had amoebic dysentery. The remaining five subjects recovered by 1-2 weeks and hence they were unlikely to be cases of ulcerative colitis. We did not look for campylobacter for any other infective agents.

Contrary to the observations of Dickinson, Gilmour and Mclelland[12] we did not as a whole find histology of the rectal mucosa of particular diagnostic value except those cases where amoeba could be seen in the biopsy specimens. In our opinion, before making a diagnosis of ulcerative colitis a course of antiamoebic agent should be tried in countries with endemic amoebiasis.

ACKNOWLEDGEMENT

We wish to thank Prof. N. Islam, the Director of the Institute of Postgraduate Medicine and Research for his encouragement while conducting the study.

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Discussion

Comments *Dr. Ganguly*

We had carried out a similar study and reported it in one of our meetings mainly to differentiate between lesions in amoebic colitis and other form of colitis. We observed a large number of monocytic infiltrations. In amoebic colitis we observed eosinophilic infiltration otherwise it was very difficult to differentiate them. The PAS staining should be used to demonstrate amoebiae. In shigellosis mononuclear cell infiltrations have been seen by us.

Q. *Dr. De*

Do the rectal mucosa return to normal following amoebic dysentery?

A. Yes, Rectal mucosa returns to normal.

Q. *Dr. Anand*

In our own study we found some mucosal abnormalities even in normal subjects. What was the criterion of selection of "Normals"? How do you differentiate Amoebic colitis from ulcerative colitis?

A. Absence of amoebic antigens was the criterion used tissue culture method was used to differentiate between amoebic colitis and ulcerative colitis.

Q. *Dr. S. Alam*

Did you find any patient with ulcerative colitis? If it is so, what was the morphological finding of the rectal mucosa?

A. Absence of goblet cells was the findings.

CHARACTERIZATION OF CAMPYLOBACTER SPECIES ISOLATED FROM PATIENTS SUFFERING FROM GASTROENTERITIS

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ABSTRACT

Campylobacter fetus ss. jejuni has been recognized as pathogen for human and animals since early 1950's. As with other intestinal pathogens, infection with Campylobacters does not always produce symptoms; asymptomatic excretors and mild cases are also commonly found.

The isolation of Campylobacter requires specialized media and culture condition. Direct plating onto agar plate containing selective antibiotic has been preferred. Different media have been tested and Brucella agar medium containing 5% sheep blood and 5 antibiotics in different concentrations have been found most suitable. The plates to be incubated at 42°C under conditions of reduced oxygen tension, preferably with added Carbon dioxide. Various characterizing methods such as a) growth at 42°C and 25°C, b) growth in H_2S and d) sensitivity to various antibiotics has been used.

The incidence of Campylobacters were found to be more (10-16%) in age group 0-10 years and less, (2-8%) in the elder age group. The characteristics of Campylobacter isolated from patients have been described in detail.

CHAPTER 3

CLINICAL MEDICINE

INCLUDING

NUTRITIONAL CONSIDERATIONS

LONG-TERM EFFECTS OF ORAL REHYDRATION THERAPY ON THE NUTRITIONAL PROGRESS OF DIARRHOEAL CHILDREN

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S.P. De, D. Sen, M.R. Saha, S.N. Ghosh,
S.K. Niyogi, S. Ghosh, R. Mitra,
S.N. Sikdar, and S.C. Pal

The National Institute of Cholera &
Enteric Diseases
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ABSTRACT

In two slum areas of Calcutta 383 children below 5 years were closely observed (six days-a-week) for three years in an attempt to study the various facets of diarrhoeal diseases. In one area 181 children were provided with prompt oral rehydration therapy as soon as diarrhoea occured. The nutritional improvement in this group was not found to be different from those in the control group after the three-years of surveillance. However, a statistically significant ($P < 0.01$) difference in the weight and height gains between the malnourished children of the above two groups was noticed. Children with malnutrition suffered from higher incidences of diarrhoeas, compared to the nutiritonally normal ones.

INTRODUCTION

Acute diarrhoeal diseases are known to cause high morbidity and associated mortality, specially among the children in the developing nations. Compared to well nourished children, the malnourished children are reported to have higher incidence of diarrhoeas which frequently tends to recur and are often associated with high case-fatality rates[1,3,5,11]. It has been suggested that unless water and electrolyte losses are promptly corrected, diarrhoeal diseases produce malnutrition even in normal children, this has been demonstrated amongst hospitalized diarrhoeal children[7]. However, very few reports are available to show the long-term effect of prompt correction of water and electrolyte losses on the nutritional status of the diarrhoeal children at the community level. The present study was an attempt to determine this objective.

MATERIALS AND METHODS

Two typical slum areas (Corporation Ward Nos. 33 (Area A) and 35 (area B)) in Calcutta were selected for the study, which was carried out during a period of three years between 1977 and 1980. Before the study began, an initial survey was conducted during 1977 to collect and compare the socio-economic status, educational level, sanitational facilities and their utilisation, family size, age, sex and the nutritional status of the population in the two areas.

One hundred and eighty-one children in Area "A" (ORS group) and 202 in area "B" (control group) {total 383} below the age of 5 years were selected for the prospective study. During the entire period of the study all families were under a close six-days-a-week surveillance by teams headed by physicians. Each visit was accompanied by enquiries to detect incidences of diarrhoea among the children. In area "A", mothers or other females (care-takers of children) were provided with packets for half a litre oral rehydration solution (W.H.O. Formula*) and a plastic container to measure 500 ml of water. The preparation, and the method and dose of administration of the ORS was also demonstrated. No oral rehydration therapy was available to the children in area "B" which served as control. In both the areas mothers were asked not to withdraw normal feeding (including fluids) specially breast feeding, during diarrhoea. All mothers were instructed to take their child to the nearby Infectious Disease Hospital in case of any emergency. All the diarrhoeal episodes in both areas were recorded in standard proforma by the physicians. The actual amount of ORS consumed for each episode of diarrhoea was measured and recorded.

Weights and heights of all the children (383) with minimum clothing were measured before the study began and every three months thereafter. Weights were measured to the nearest 100 gms using level actuated balances (SECA, West Germany). Heights were measured to the nearest 1 mm using infantometers having bigger measuring scales.

Stool samples of all the diarrhoea incidences among the study children were bacteriologically examined, following standard procedures, to detect the etiologic agents.

RESULTS

The age distribution of the 383 children are presented in Table 1. There were 123 families in area "A" and 139 in area "B" having a total of 383 children under 5 (1.5 children per family). Percent distributions of the children

* Sod. Chlor. 3.5 gms, Sod. Bicarb. 2.5 gms., Pot. chlor. 1.5 gms., Glucose 20.0 gms., for one litre of solution.

in different age groups were similar in both the groups.

Table 1. Age Distribution of Study Children in Two Areas

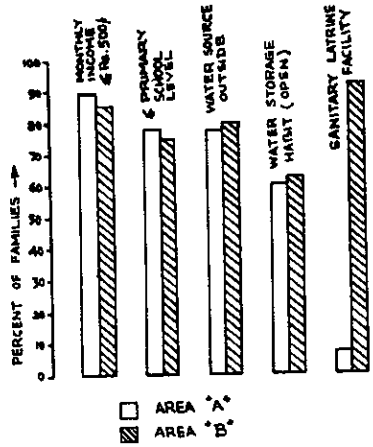
Age Group (Months)	Area "A" No. of Children	%	Area "B" No. of Children	%	Total in Two Areas
< 12	41	22.7	33	16.3	74
12 - 23	43	23.8	33	16.3	76
24 - 35	36	19.9	49	24.3	85
36 - 47	34	18.8	38	18.8	72
48 - 59	27	14.9	49	24.3	76
0 - 59	181	100.0	202	100.0	383
Total No. of Families	123		139		262
No. of Children* Per Family	1.5		1.5		1.5

*Below 5 years

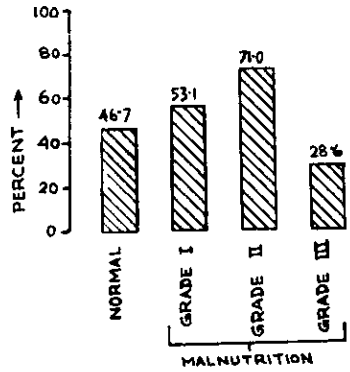
Initial survey revealed no difference in economic status, educational level of mothers, availability of water sources within thehouses and water storage habits among the families of the area "A" and "B" (Fig. 1). The monthly family income of about 85-89% of the families were below Rs. 500/- (approx.$ 65.00 U.S.) per month. About 75-78% mothers' educational levels were upto primary school or below, 76-77% families had no water source within the house. Most of the families (60-62%) stored water without cover.

However, there was a big difference in the sanitary latrine facilities amongst the two areas. Until very recently both the areas had service latrines (manually cleaned). A few months before the initial survey was carried out all the service latrines in area "B" were replaced by the sanitary latrines, but without any water-flushing system. People had to carry water from outside to flush the pans after defaecation. As stated earlier most of

(FIG. 1)
COMPARABILITY OF TWO GROUPS



(FIG. 2)
DIARRHOEAL INCIDENCES IN VARIOUS NUTRITIONAL GROUPS



houses have no water sources. Water was brought from outside for every purpose including water for flushing the latrines. Obviously this was not easy, and latrines remained mostly dirty. Thereby the advantages of sanitary latrines were virtually lost and there was no practical difference in the two areas as far as latrine facilities were concerned. This was further supported by the fact that diarrhoeal episodes per 100 children as well as enteropathogen* detection rates in both A & B groups, during the first six months of surveillance were almost similar (Table 2), inspite of the sanitary latrines in area "B".

Table 2. Diarrhoeal Incidence and Enteropathogen Detection Rates in Two Areas During First Six Months

Area	Diarrhoeal Episodes Per 100 Child/6 Months	Enteropathogen Detection Rate (%)
A	61.9	11.6
B	56.9	10.4

*These included enteropathogenic *E.coli*, *V. cholerae*, *V. parahaemolyticus*, *Shigella*, *Salmonella* and *E. histolytica*

Initial heights and weights of the study children are shown in Table 3. The mean weights and heights of different age groups in two areas were similar. According to the Gomez classification[6] 68.4% of the study children were nutritionally normal, 21.5% were in grade I, 8.2% in grade II and 1.9% in grade III of malnutrition.

Table 3. Initial Heights and Weights of the Study Children

Age Groups (In Months)	Mean Weight (kg)		Mean Height (cm)	
	Area "A"	Area "B"	Area "A"	Area "B"
< 12	5.8	5.9	61.2	61.9
12 - 23	7.6	8.3	72.7	75.2
24 - 35	9.5	9.5	80.7	81.1
36 - 47	11.2	10.8	89.1	88.6
48 - 59	12.4	12.8	93.2	95.8

During the first year of the study there were 421 episodes of diarrhoea, the profile of which are presented in Table 4. One hundred seventy-nine (46.7%) children suffered from diarrhoea during the period, 107 (27.9%) had two or more episodes. Number of episodes per 100 child per year was 110. Average number of loose motions per episode was seven and mean duration of diarrhoea was 35 hours. It was interesting to note that 99.5% of the diarrhoeal episodes were mild in nature and only 0.5% were moderately severe and none was severe.

Average consumption of oral rehydration solution for each diarrhoeal episode in the area "A" was 500 ml.

The malnourished children suffered from high incidence of diarrhoea than the nutritionally normal children as would be evident from Fig. 2, 46.7% of the normal children, 53.1% of the grade I, 71% of the grade II malnourished children had diarrhoeas during the study period. However, the diarrhoea incidence among the grade III malnourished children was found to be 28.6%.

Table 4. Profile of 421 Diarrhoeal Episodes Occuring in Two Areas During the 1st Year of Study

No. of children in study area	: 383
No. suffered from diarrhoea	: 179 (46.7)
Total No. of diarrhoeal episodes	: 421
No. of episodes per 100 children per year	: 110
No. of children with 2 or more diarrhoeal episodes	: 107 (27.9)
Average number of loose stools per episode	: 7
Mean duration of diarrhoea	: 35 hours
Mean volume of oral rehydration solution consumed per episode	: 500 ml
No. of episodes with degrees of severity -	
Mild	: 419 (99.5)
Moderate	: 2 (0.5)
Severe	: Nil (-)

N.B. Figures in ( ) indicate percentage

The nutritional changes of the children after the three-year period of surveillance, are presented in Table 5. The overall weight gain in the "A" (oral fluid) group was 4.6 Kg as against 4.4 Kg in the control group. Similarly overall gain in height in the "A" (oral fluid) group was 21.2 cms and 20.9 cms in the control group. There were no statistically significant differences (in the gains in weights and heights in the two groups).

Table 5. Nutritional Progress in Two Groups after a Three-Year Period of Surveillance

Age Groups (In Months)	Gain in Weight (Kg)		Gain in Height (cm)	
	Oral Fluid Group	Control Group	Oral Fluid Group	Control Group
< 12	5.6	5.1	27.9	29.4
12 - 23	4.5	4.7	21.5	20.4
24 - 35	4.5	4.1	20.1	20.3
36 - 47	4.4	4.1	19.4	17.9
48 - 59	3.9	4.3	17.1	18.0
0 - 59	4.6	4.4	21.2	20.9

Nutritional status of the two groups during different time periods are presented in Table 6. There were no significant differences in weight and height gains during time periods.

Among the nutritionally deficient children of group A & B the overall weight gain after three years was 4.8 Kg in group A (ORS group) as against 4.1 Kg in group B (the control group). This difference was found to be statistically significant ($P < 0.01$). Similar significant difference in height-gain was also observed between the study and the control group.

Table 6. Nutritional Progress in Two Groups During Different Time Periods

Age Groups (In Months)	Gain in Weight (Kg) Oral Fluid Group	Control Group	Gain in Height (Cm) Oral Fluid Group	Control Group
Upto 1 year	1.8	1.6	8.4	7.9
Upto 2 years	3.0	3.0	14.7	14.3
Upto 3 years	4.6	4.4	21.2	20.9

DISCUSSION

Between 1975 and 1977 studies conducted in Philippines[8], Laos[10], Turkey[4] and Iran[2] have shown that during relatively shorter span of studies (1.5 to 7 months) there were significantly higher weight gains in the group of children treated with oral glucose-electrolyte solutions compared to those in the control groups. The current study, however, failed to demonstrate significant or even appreciable difference in weight-gain between children treated promptly with oral rehydration therapy and the control group during the long-term period of surveillance. Even after the completion of one and two year periods, significant differences in weight-gains could not be detected between the two groups. This finding was further supported by a recent study in Philippines (unpublished) which has shown that there were no long-term effects on weight-gain in children who received oral therapy[9]. In the Calcutta study absence of weight-gains in the study group could be attributed to the very mild nature of the diarrhoeal episodes as observed in these children (99.5% of these diarrhoeal episodes were mild in nature and not a single case required hospitalization). On the other hand the studies conducted in Philippines[8], Laon[10], Turkey[4] and Iran[2] dealt with more severely dehydrated children (some of whom had died) who required attendance in the clinics.

Among the malnourished children, on the other hand, the nutritional progress (as shown by weight-gains) in the oral therapy group was found to be significantly higher ($P < 0.01$) than those in the control group. This may possibly be explained by the fact that malnourished children in the control group, due to recurrent incidences of diarrhoea (Fig. 2), suffered from poor nutritional progress. The malnourished diarrhoeal children in the ORS group receiving prompt replenishment of water and electrolyte losses through oral therapy, however, could continue with their normal feeding thus maintained a better progress.

As shown in Fig. 2 the malnourished children in both groups suffered from

higher incidences of diarrhoea which further supported the observations of the earlier workers[1,5,11].

ACKNOWLEDGEMENT

The authors are grateful to Dr. S.G. Srikantia, Director, National Institute of Nutrition, Hyderbad and his scientific staff for their valuable suggestions regarding the anthropometric measurements to be adopted for this particular type of study as well as for providing SECA weighing balance during the early part of this study. Authors are also thankful to the field and laboratory staff of the National Institute of Cholera and Enteric Diseases, Calcutta for their valuable support without which the study would have been difficult.

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Discussion

S.C. Sanyal

Q. Did you look for ETEC? If so by what techniques?

A. Not always when tested sera was used.

Q. What set of antisera for EPEC did you use?

A. 14-18 serotypes - DIFCO (commercial).

Nigar Shahid

Q. Had the usage of the latrines been looked into?

A. People are not using latrines.

R. Islam

Q. 1 Have you observed any difference in the success and failure rate in oral therapy between cholera and non-cholera?

2 Whether tetracycline have been used in the cholera cases?

A. 1 Amongst 50 total cases there were 10 cholera cases.

2 Yes, in all cases tetracycline was used.

SINGLE DOSE AMPICILLIN THERAPY FOR THE TREATMENT OF SEVERE SHIGELLOSIS IN BANGLADESH

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ABSTRACT

A large single dose (SD) of ampicillin (100 mg/kg) was compared to a conventional 5 day course (MD) of ampicillin (100 mg/kg/24 hr) for the treatment of shigellosis in a randomized trial. Adults and children in both groups were of similar age, and had comparable clinical and laboratory findings. In the SD group, clinical relapses were seen in only one of 23 children and in none of 26 adults. None were seen in MD patients, (24 adults, 18 children). Bacteriological relapses were observed in six of 26 SD adults compared to 0 of 24 MD adults, ($p < .05$). Four children, all below 4 years of age, relapsed bacteriologically in the SD group, compared to 1 child in the MD group. Adults and children in both the SD and MD groups had an equal decrease in stool frequency and the number of fecal leukocytes. Fecal *E. coli* from patients given SD had less resistance to ampicillin on the 4th day and 7th day after therapy, than those from the MD patients, but there were no differences 2 weeks after therapy. Invasive *E. coli* was found in 2 of 18 (12%) Shigella-negative dysentery patients, and made up approximately 2% of all clinical bacillary dysentery patients studied. Single dose ampicillin is clinically effective therapy for shigellosis in adults and children over the age of 3.

INTRODUCTION

Tetracycline given as a single large dose has been effective therapy for shigellosis when used in well nourished adults with generally mild disease[1,2]. However, in developing countries, *Shigellae* species are frequently resistant to tetracycline[4,5]. Ampicillin resistance in *Shigellae*, although common in developed countries, is unusual in developing countries such as Bangladesh[4,6].

In a previous study in Bangladesh, we compared high dose (150 mg/kg/24 hr) versus low dose (50 mg/kg/24 hr) ampicillin therapy in adults and children with severe shigellosis. There were no differences in either the clinical or bacterial response between the two regimens[7].

Single dose ampicillin therapy of severe shigellosis, if effective, would provide a single treatment regimen of low cost, and yet give a maximum of dispensing control. This study compared the results of therapy in shigellosis patients treated with either a single large dose of ampicillin or a conventional multidose regimen.

MATERIALS AND METHODS

Patients over 2 years of age, with a history of passing blood and mucous in their stools for less than 1 month and who had not received ampicillin before coming to the hospital were considered potential candidates for the study.

Those patients whose stool on microscopic examination showed mucous and white cells, did not have hematophagour trophozoites of *Entamoeba histolytica* i.e. a bacillary exudate, and who were able to tolerate oral medication were considered eligible for the study. Patients were divided into two groups: adults (more than 10 years) and children (2-10 years). Patients in each group were randomly assigned (by lottery) to one of two treatment groups.

After initial randomization, patients were excluded if: 1) shigella was not isolated, 2) the white count was over 45,000 mm^3), 3) ampicillin-resistant shigellae were isolated, or 4) patients did not complete 7 days of observation in hospital.

Treatment

Patients received one of two treatment schedules. One group received 100 mg/kg of ampicillin (maximum of 4.0 grams) given as a single dose (SD) on the day of admission. The other group received multiple doses (MD) of 100 mg/kg/24 hr of ampicillin given at six hourly intervals for 5 days. All patients were hospitalized for at least 7 days; they were requested to return for examination during the first and second week after hospital discharge.

Patient Evaluation

Patients had an initial history, and physical exam done, and X-rays of the chest and abdomen performed. Proctoscopy was done on admission and weekly thereafter. Patients were questioned daily about the presence of abdominal pain and tenesmus. The frequency, consistency and the presence of blood in the stool were recorded daily. Axillary temperatures were taken every 4 hours.

On the day of admission and on days 4, 7, 14 and 21 after admission patients had a complete blood count, serum specific gravity and microscopic examination of stool, performed. Rectal swabs for bacteriologic culture were taken during proctoscopy and daily for the first 4 hospital days, on the day of discharge, and on each follow-up visit.

Quantitative counts of gram negative aerobic bacterial in stool were done in 68 sequentially selected patients.

Laboratory Studies

Microscopic Exam - A small aliquot (1-2 mg) of stool was emulsified in saline and the number of white cells (pus cells) per high power field were counted. No attempt was made at defining these cells further. Another portion of stool was emulsified in methylene blue. Cells, in which a distinct segmented or band nucleus could be seen were defined as fecal leukocytes and were enumerated per high power field. Diffusely staining cells in which the nucleus was pyknotic or disrupted were not counted.

Bacteriology

Rectal and proctoscopic swabs were streaked onto MacConkey, Shigella-Salmonella (SS) agar and Taurocholate-Tellurite-Gelatin agar. After 18 hours incubation at 37°C lactose negative colonies were identified by routine methods[8,9].

Aerobic quantitative cultures were performed on stool samples by diluting 1 gram of stool in 9cc of phosphate buffered saline (PBS). Tenfold dilution in PBS were made. A 0.1cc aliquot of diluted stool was streaked by glass roller onto MacConkey agar containing either no ampicillin or 200ug of ampicillin. Different bacterial colony types were counted and then picked for identification. *E.coli* were defined as ampicillin sensitive when there was a tenfold or greater difference in the CFU's found on the ampicillin free MacConkey plate compared to the same agar plate containing 200 ug of ampicillin. Conversely, if the difference in the CFU's of *E.coli* on the two agar plates

(0 versus 200 ug of ampicillin) was less than 10 fold, the *E.coli*, population of the stool sample was defined as resistant.

Two individual lactose fermenting colonies (LFC) and a pool of 10 LFC were picked from the agar plate not containing ampicillin and streaked on nutrient agar slants.

One of the individual colonies as well as the pool of 10 LFC were tested for heat labile (LT) and heat stable (ST) enterotoxins by the Chinese Hamster Ovary[10] and infant mice assay[11]. Thirty-one randomly picked shigella patients had their *E.coli* pool tested for guinea pig corneal invasion (Sereny test)[12]. Also, 14 patients from whom shigella was not isolated, had 10 individual LFC colonies tested for invasion.

The minimal inhibitory concentration of 76 shigella isolates to ampicillin was determined by the tube dilution method[13].

Data Analysis

A patient was defined as a clinical failure when, 1) dysentery either did not abate or recurred 7 or more days after the institution of therapy, and 2) stool cultures continued to show the same species of shigellae as isolated on admission.

Bacterial failure was defined as any patient who had shigellae species isolated 3 or more days after the start of therapy.

The characteristics and response to therapy of the 2 patient therapy groups were compared using either the Student t test or the Chi Square test.

RESULTS

There were 132 dysentery patients eligible for randomization. Shigellae was isolated from 108 (82%) of these patients.

Of the 24 shigella-negative dysentery patients there were 2 adults harboring *Vibrio parahaemolyticus*, and one child with Salmonella group E. Another adult was eventually diagnosed as having idiopathic ulcerative colitis. Four additional patients had Heiberg Group III vibrio-like organisms isolated and no other recognizable pathogen. The remaining 16 patients had no potential pathogens recovered by routine bacteriology. Fourteen of these 16 dysentery patients in whom no pathogen was isolated had their LFC's tested by the Sereny test and two patients (14%) had LFC's (both *E.coli*) isolated which invaded the guinea pig cornea (Sereny positive). No LFC's tested from the four patients with Heiberg Group III vibrio-like isolates were Sereny positive.

Ninety-one of the 108 patients with Shigellae were suitable for analysis. Of the 17 excluded patients, 3 had no more dysenteric stools after admission and are not included. Two children (one in each treatment group) were withdrawn because of symptomatic urinary tract infections caused by gram-negative bacteria resistant to ampicillin. Four patients had a *Shigella flexneri* isolated which were resistant to ampicillin (MIC over 160 ug/ml), two other patients had white blood counts on admission of over 45,000 cells per cc^3 and the remaining six left the hospital prior to completion of the study. As seen in Table 1 both adult and children therapy (SD and MD) groups were clinically similar.

Results of Therapy

Side effects from the medication were rare. A portion of the original dose was vomited in two of the children in the single dose group. The amount lost was estimated and given to the child within the next hour. Allergic reactions and rashes were not seen.

Symptomatically, there were no differences in the rapidity of therapeutic response between patients (adult and paediatric) in either therapeutic group (Table 2). Fever lysed within 24 hours in both treatment groups except for one SD and 3 MD children in whom fever disappeared on the following day.

All patients in both treatment groups equally improved proctoscopically after treatment and none worsened proctoscopically. Clearance of blood and mucous from the stool was similar in both groups, six days after therapy 4 children and 8 adults in the single dose group had blood or mucous in their stool compared to 5 children and 6 adults in the multiple dose group.

Only one clinical failure occurred in a 3 year old child given a single dose of ampicillin who relapsed with mild clinical symptoms (3-4 loose stools and pus cells on microscopic examination) one week after discharge.

Four of ten SD children 3 years or less were bacteriological failures while none of the 13 older children (4-10) in the SD failed bacteriologically ($p < 0.02$). In comparison there was only one bacterial failure in the multiple dose group and none in nine children, 3 years or younger, (compared to SD group $p < .05$).

There were no clinical failures in adults treated with either treatment regimen (SD or MD). However, both the number of shigella isolates after therapy (Table 4) and the number of bacteriological failures were significantly increased in the adult SD group compared to the adult MD group (5 versus 0) $p < .02$).

Table 1. Characteristics of Adults and Children in the Ampicillin Single (SD) and Multiple Dose (MD) Treatment Groups[+]

	Treatment group	No. of patients	Mean age (yrs)	Duration of disease (days)	Mean ** WBC in mm^3	Mean hematocrit	On admission day number of patients with fever > $38^{o}C$
Children	SD	23	5 (±3)	6 (±5)	19.1 (± 6.2)	34 (± 4.3)	9
	MD	18	5 (±2)	4 (±2)	15.5 (± 7.5)	34 (± 2.9)	11
Adults	SD	26	33 (±16)	4 (±2)	12.8 (± 4.2)	41 (± 4.0)	15
	MD	24	37 (±16)	4 (±2)	11.9 (± 4.0)	40 (± 3.8)	9

[+] No significant differences were found in any characteristic studied between the SD and MD groups

** On admission

TABLE 2

Number and Type of Shigella Isolates on Admission of the Single Dose and Multiple Dose Groups

Age groups	Treatment[a] group	Shiga	Flexneri	Boydi	Sonnei
Children	SD (23)[b]	13	10	0	0
	MD (18)	8	9	1	0
Adults	SD (26)	8	12	4	2
	MD (24)	13	10	1	0

[a] There were no significant differences in bacterial isolates between the SD and MD groups

[b] ( ) = number of patients examined

TABLE 3. Clinical Response to Therapy in Shigella Patients Treated with Single (SD) and Multiple Dose (MD) Ampicillin Therapy

Age	Treatment[a] group	No.	DAY 0 Mean No. of stools per day	DAY 0 Mean cells per HPF pus cells	DAY 0 Mean cells per HPF leukocytes	DAY 4 Mean No. of stools per day	DAY 4 Mean cells per HPF pus cells	DAY 4 Mean cells per HPF leukocytes	DAY 7 Mean No. of stools per day	DAY 7 Mean cells per HPF pus cells	DAY 7 Mean cells per HPF leukocytes
Children	SD	23	22 (±13)	49 (±35)	6 (±4)	3 (±2.9)	11 (± 9)	1 (±4)	2 (±1)	4 (±7)	1
	MD	18	18 (±14)	40 (±20)	5 (±3)	4.6 (±3.0)	14 (±26)	2 (±2)	2 (±1)	7 (±21)	1 (±4)
Adult	SD	26	25 (±21)	62 (±38)	7 (±7)	3 (±2.0)	11 (±14)	1 (±2)	2 (±1)	2 (±1)	1 (±1)
	MD	24	20 (±15)	59 (±35)	5 (±4)	3 (±2)	13 (±12)	1 (±1)	2 (±1)	1 (±4)	1 (±1)

[a]No significant difference between the two treatment groups was found

TABLE 4

Number of Shigella Isolates and Bacterial Failures
After Single and Multiple Dose Ampicillin Therapy

Age groups		Days after therapy					No. of patients with bacterial failure
		1	2 & 3	7	14	21	
Children	SD	(23)[a] 4	3	1	(15)[a] 1	(11) 1	4 (17%) p < .25
	MD	(18) 2	1	1	(13) 0	(11)	1 (6%)
Adults	SD	(26) 5	5	3	(19) 1	(18) 0	6 (23%) p < .02)
	MD	(24) 5	0	0	(18) 0	(18) 1	0 (0%)

[a] ( ) = the number of patients studies

One asymptomatic adult SD patient had an ampicillin sensitive *Shigella flexneri* initially isolated; 14 days after therapy *Shigella flexneri* was again isolated; but now was ampicillin resistant (MIC > 200 ug/ml to ampicillin).

Of the 76 shigella tested by tube dilution for ampicillin sensitivity, all sensitive strains had an MIC to ampicillin of between 0.625 - 2.5 ug/ml).

Quantitative Studies

There were no significant difference in the CFU's of shigella isolated from 26 patients with shigella dysenteria type 1 compared to 25 patients with *Shigella flexneri* (6.5 ± 0.86 vs 6.7 ± 0.93 log CFU per gm stool).

Shigella counts dropped sharply after either type of ampicillin therapy. Only two of 26 (8%) multiple dose patients and four of 28 (14%) single dose patients still had countable numbers of shigella ($>10^3$ log/ml) present one day post therapy. On days 4 and on day 7 post therapy, only one patient (each) in the SD group and none in the MD group had countable shigella.

In each treatment group (multiple and single dose) there was no difference in the number of adults or children who had ampicillin resistant *E.coli*. These two age groups have therefore been condensed and analysed as one group. As seen in Table 5, there were less SD patients with ampicillin resistant *E.coli* in their stools on day 4 and 7 compared to multiple dose patients. In both treatment groups fecal flora rapidly reverted to normal following therapy.

None of the 60 LFC pools isolated from stools of shigella patients were positive for either ST or LT *E.coli* enterotoxin. In addition, none of the 31 LFC (probable *E.coli*) pools from patients with Shigellosis gave a positive Sereny reaction.

DISCUSSION

Shigellosis patients in both therapy groups (SD and MD) improved clinically at equal rates. This was measured objectively by fever, decrease in stool frequency, the clearance of blood and mucous microscopically and pus cells and fecal leukocytes microscopically from the stool. Since no placebo study was performed it is impossible to compare either of these patient therapy groups (SD or MD) with the natural course of shigella dysentery.

Haltalin used colony counts of Shigella to document the failure of Furazolidone therapy[14]. After one day of therapy with either the SD or MD regimen there was a rapid reduction in shigella counts from a range of (10^5 - 10^8) CFU's to less than 10^3 CFU per gram stool in over 85% of the patients.

Table 5

Ampicillin resistant E. coli in patients treated with multiple or single dose ampicillin therapy

Therapy group		Days after treatment				
		0	1	4	7	14
Single dose	Number of patients[a] with ampicillin resistant fecal E. coli / Total number studied	$9/_{33}$ (27%)	$21/_{31}^{*\ +}$ (68%)	$19/_{34}^{*\ +}$ (56%)	$18/_{35}^{*\ +}$ (51%)	$1/_{20}$ (5%)
	Significance between MD and SD groups	NS	NS	p .001	p .06	NS (p .11)
Multiple dose	Number of patients with ampicillin resistant fecal E. coli / Total number studied	$9/_{28}$ (32%)	$17/_{25}^{*\ o\ +\ *}$ (68%)	$27/_{29}^{o\ +\ *}$ (93%)	$23/_{31}^{*\ +\ o}$ (74%)	$5/_{22}$ (23%)

a) All patients had at least 3 tests performed

*) Significant difference between baseline $p < .05$

+) Significant difference between day 14 and others $p < .05$

o) Significant difference between next day tested $p < .05$

Both children and adults given multiple dose ampicillin therapy responded well; there were no clinical failures and only one bacteriological failure, a 5 year old child.

Bacteriological failure in adults treated with a single dose of ampicillin occurred in 23% of patients, but was not associated with clinical failure. Similarly in the U.S.A. adult volunteers with clinical shigellosis who were treated with a single dose of 3.5 gram of ampicillin had a 44% bacteriological failure rate, but no clinical relapses[2].

In our study no child, age 4-10, who was given a single dose of ampicillin had either a bacterial or clinical relapse. However, 40% of children, 3 years or younger treated with a single dose relapsed bacteriologically and one of these also had associated symptoms of dysentery. (After completion of this study, another 3 year old child with dysentery from whom *Shigella flexneri* (sensitive to ampicillin) was isolated and treated with single dose ampicillin therapy at a village treatment center. The child after initial improvement relapsed with dysentery and again had ampicillin sensitive *Shigella flexneri* isolated.)

Thus, it would appear reasonable to restrict the use of single dose ampicillin therapy to children 4 or more years of age. Isolation of a shigella resistant to ampicillin after SD therapy from a patient whose original isolate was ampicillin sensitive is also disturbing.

Both the isolation of a resistant shigella and the high bacterial failure rate of patients treated with single dose ampicillin therapy make this therapeutic regimen less desirable than a multidose schedule. However, since there is a large reservoir or asymptomatic individuals who are infected with shigellae; therapy is used primarily to shorten clinical disease rather than for eradication of theorganism from patients' stool[15]. Clearly, except in small children, single dose therapy does as well clinically as a multidose regimen and would be especially useful in outpatient clinics. Also, in less developed countries, the advantages of low cost, high patient compliance and decreased personnel requirements of a SD regimen would be more important than the lower bacteriological relapse rate provided by a conventional multi-dose regimen.

Further studies in children using a single dose regimen with other antibiotic agents or adjuvants like probenacid would be desirable.

Several studies have demonstrated that aerobic gram negative flora rapidly becomes resistant after antibiotic therapy[16,17]. The number of patients (both SD and MD) with ampicillin resistant *E.coli* increased significantly after one day of ampicillin therapy. Patients given SD ampicillin therapy had a more rapid return to an ampicillin sensitive fecal *E.coli*

population than did patients in the MD group. This difference was short lived since ampicillin resistance rapidly disappeared (within two weeks) in both treatment groups. In England, patients who had antibiotic resistant strains while on tetracycline therapy rapidly lost these strains after returning home to an antibiotic free encironment[17]. One hundred percent of fecal *E.coli* isolated from Peace Corps Volunteers in Kenya were resistant to doxycycline after 3 weeks of therapy with that agent. However, 2 weeks after therapy was finished tetracycline resistance was found in only 39% of the volunteers fecal *E.coli* isolates[18]. In contrast to tetracycline or its congeners, ampicillin given orally at a low dose caused less than 15% of patients in England to have fecal *E.coli* resistance to ampicillin[17]. In our study, where higher dosages of ampicillin were used, over half the patients receiving SD ampicillin therapy and over 90% of the MD patients had fecal *E. coli* resistant to ampicillin. However, as with tetracycline, most patient's flora rapidly reverted back to being ampicillin sensitive.

This study documents the rarity of invasive *E. coli* as cause of bacillary dysentery in cases presenting to the Cholera Research Hospital. The bulk of clinical dysentery cases, over 80% had shigella isolated from their stool. Only 20 of the 132 (15%) cases had either no agent or a group III non-cholera vibrio (not a known cause of dysentery) isolated from their stool. Of these cases, 18 were tested for the presence of invasive *E.coli* and only two patients were found to have this agent. It would appear that invasive *E.coli*, except in isolated outbreaks[19] is a rare cause of severe dysentery in Bangladesh, as is the case in the U.S. and Canada[20,21]. Less then 2% of all our dysentery cases and less than 12% of those cases without any other isolate, were associated with invasive *E.coli*.

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EFFECTS OF DIARRHOEA ON ABSORPTION OF MACRONUTRIENTS DURING ACUTE STAGE AND AFTER RECOVERY

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ABSTRACT

The effect of diarrhoeal illness caused by rotavirus, enterotoxigenic *E.coli* (ETEC) and shigella on coefficients of absorption of nitrogen, fat, calories and carbohydrates were estimated in a group of children, both in the acute stage and at two and eight weeks after recovery. During the acute stage, absorption of nitrogen was affected in all diarrhoeas being 45%,58% and 41% of the ingested amount for rotavirus, ETEC and shigella respectively. Two weeks after recovery, absorption of nitrogen increased significantly to 69% for rotavirus and 74% for shigella. Absorption of nitrogen for the ETEC group after two weeks was the same as during the acute stage but increased to 73% after eight weeks. Statistically there was no difference in absorption of nitrogen between the three aetiologies eight weeks after recovery. In the acute stage, absorption of fat and calories was comparatively less in rotavirus than in ETEC and shigella. After two weeks, absorption of fat and calories was significantly increased in rotavirus and shigella, and by the eight-week absorption did not vary significantly between the three aetiologies. Absorption of carbohydrate was least affected in any stage of diarrhoea of all aetiologies. In the acute stages, 75% of patients of all aetiologies were xylose malabsorbers compared to only 10% in the recovery period.

INTRODUCTION

Nutritional consequences of diarrhoea are thought to be due to several factors: (a) decreased intake of food resulting from loss of appetite or withholding of food (as practised as a measure to control diarrhoea); (b) loss of major nutrients as well as vitamins and minerals in the faeces due to rapid transit or malabsorption and (c) increased catabolism due to infection.

The main purpose of this presentation is to report our present research on "absorption of macronutrients during and after acute diarrhoeal attacks". To give some information about previous work, first of all, we will summarise the results reported earlier on "loss of enzymatic activity in the gut, secondary to cholera" (1, 2) and then review the work of Chung *et al.* (3, 4) on net absorption of nutrients and the effects of oral feeding and starvation during paediatric diarrhoea.

Two kinds of mucosal enzymes, namely adenosine triphosphatase and disaccharidase in acute cholera and other diarrhoeal disease were studied by Hirschhorn and co-workers (1, 2). The results of ATPase are shown in Table I. The significance of depressed Na-K ATPase in acute diarrhoea is uncertain. Diarrhoea, however, may influence directly the membrane enzyme activity, contributing to the loss of absorptive capacity from the gut. Results on the activity of disaccharidase in acute cholera and other diarrhoeal disease are summarised in Table II. Sucrase, maltase, palatinase activities were low during the acute period. Lactase activity was low during both the acute and convalescent periods compared to healthy North Americans. Enzymatic activities were estimated in serial biopsies on patients. The authors concluded that diarrhoea was probably responsible for the changes in enzymatic activity. However, the significance of the reduction in disaccharidase activities has not been clearly understood. Further research is necessary to correlate these findings with the studies related to nutrient malabsorption in acute diarrhoea.

Net absorption of nutrients in acute diarrhoea was first reported by Chung *et al.* in 1948 (3). The important results are summarised in Tables III and IV. Table III presents the data obtained from a study on four infants who were fed successively three diets; low, medium, and high calorie in the first, second and third 24 hours respectively during the study. Results showed that the absorption of nitrogen and fat was approximately proportional to the amount of intake. Table IV presents the result obtained from a study on two infants treated first with a high calorie diet and then with complete starvation (maintaining a continued I.V. drip). Results showed that during acute diarrhoea, substantial absorption of nitrogen and fat did occur if the food was ingested and no positive absorption occurred during the period of starvation. In a separate report Chung *et al.* (4) studied one hundred and fifteen diarrhoeal patients, fifty-five of whom were starved and sixty fed. They observed that

TABLE I--SODIUM POTASSIUM STIMULATED ADENOSINE TRIPHOSPHATASE OF THE SMALL INTESTINE OF MAN: STUDIES IN CHOLERA AND OTHER DIARRHOEAL DISEASES

By: Norbert Hirschhorn and Irwin H. Rosenberg, 1968

Results and Comments

1) Sodium Potassium stimulated, adenosine Triphosphatase (Na-K ATPase), an enzyme implicated in active sodium transport, was demonstrated in homogenates of human jejunal and ileal mucosa.

2) In cholera, acute non-specific gastro-enteritis and bacterial enteritis there was a significant loss of Na-K ATPase activity during the acute phase of the disease compared to the convalescent period.

3) Loss of Na-K ATPase in cholera and other enteric disease probably contributes to a worsening of, if not the initiation of, the intestinal fluid loss.

TABLE II--DISACCHARIDASE ACTIVITY IN ACUTE CHOLERA AND OTHER DIARRHOEAL DISEASES

By: Norbert Hirschhorn and Ayesha Molla, 1969

Results and Comments

1) Sucrase, Maltase and Palatinase activities were decreased during acute cholera and enteritis.

2) All patients but one had low lactase activity even in convalescence compared to healthy North Americans.

3) Serial biopsy studies suggest that diarrhoea and recovery therefrom were responsible for the changes in enzyme activity.

TABLE III--PERCENTAGE OF ABSORPTION OF NITROGEN AND FAT AT DIFFERENT LEVELS OF CALORIE INTAKE DURING ACUTE DIARRHOEA

No. of Cases	Low (30-45)		Moderate (60-69)		High (100-137)	
	N_2	Fat	N_2	Fat	N_2	Fat
12	40	72	50	67	63	60
26	27	48	40	39	74	59
22	38	-27	-	-	60	30
23	-	-	63	70	60	49

Chung *et al.*, 1948.

TABLE IV--PERCENTAGE OF ABSORPTION OF NITROGEN AND FAT DURING A PERIOD OF HIGH CALORIE INTAKE AND STARVATION IN ACUTE DIARRHOEA

No. of Cases	High (100-106)		Starvation	
	N_2	Fat	N_2	Fat
27	75	68	-0.30	-2.22
28	65	46	-0.15	-0.51

Chung *et al.*, 1948

the duration of diarrhoea was comparatively longer in the starved group though the statistical difference between the starved and the fed groups was not significant. The observations are listed in Table V.

TABLE V--MEAN OBSERVATIONS ON FIFTY-FIVE DIARRHOEAL PATIENTS TREATED BY STARVATION AND SIXTY BY FEEDING

Observations	Starvation	Feeding
Age (Months)	7.9	7.3
Initial Wt. (Kg)	5.97	5.56
Wt. gain at discharge	Lesser gain	Greater gain
Duration of diarrhoea (days)	7.8	6.1

Chung *et al.*, 1948.

Since the work of Chung *et al.*, no attempt has been made to demonstrate the importance of continuing feeding during acute diarrhoea. Cholera, rotavirus, shigella and ETEC are the major causes of diarrhoeal illness among the under-five population in most developing countries (5, 6). Rotavirus continues to be one of the important causative organisms responsible for childhood diarrhoea even in developed countries (7). The aim of the present study was to determine the effect of diarrhoea caused by rotavirus, shigella and ETEC on net absorption of fat, calories, nitrogen and carbohydrates during acute diarrhoea and two weeks after discharge. The absorption study was repeated eight weeks after discharge to see if there was any difference in the absorption of nutrients between the two stages of recoveries. The absorption data obtained from the above study was analysed to determine if the pattern varies between the three aetiologies. However, before we go into the details of our study we would like to point out an important difference between the study of Chung *et al.* and the present research (Table VI). In this study specific aetiologic agents were identified and their relation to nutrient absorption studied, whereas previous work dealt with non-specific diarrhoeas only.

TABLE VI--DIFFERENCES BETWEEN THE STUDY OF CHUNG *ET AL.*, AND THE PRESENT RESEARCH

Chung *et al.*	Molla *et al.*
1. Subjects - Infant, Age 12 - 70 days	Subjects - Children Age 1-4 years
2. Sample size - 6	Sample size - 29
3. Causative organisms - not isolated	Causative organisms - isolated from the stool
4. A liquid formula diet commonly employed for the age group was used	Familiar semi-solid and solid diet was used
5. Three different calorie formulae fed - either low, medium or full	No restriction was applied
6. Study period - 24 hours duration, with each formula diet	Study period - 72 hours duration, with the same food

METHODS AND PATIENTS

Twenty-nine male children below five years of age with a history of acute watery diarrhoea and moderate to severe dehydration were selected at random for the study, those with any obvious complications being excluded. Informed consent was obtained from their parents or guardians. There were 29 patients altogether, 13 of whom had rotavirus infection diagnosed by the enzyme-linked immunosorbent assay, ELISA (8). Ten had *E.coli* : colonies were tested for toxigenicity by infant mouse assay for heat stable toxin (ST) and adrenal cell assay (9) for heat labile toxin (LT). The other six had shigella isolated by standard methods (10). The patients were rehydrated by intravenous route with the Dacca solution* and maintained with the same for 18 to 24 hours.

* Composition: Na^+ 133 mmol/l; K^+ = 13 mmol/l; Cl = 99 mmol/l and HCO^-3 (as acetate) - 48 mmol/l.

After an overnight fast, the patients were fed 5 grams of d-xylose in 100 ml. of water. One hour after ingestion one millitre of venous blood was drawn to estimate the xylose. Charcoal tablets were fed as markers, the time taken by the charcoal to make its appearance in the stool being taken as the transit time. Four meals were offered daily ad libitum to each patient at regular intervals during the study period. Two types of diet were used: a semi-solid cooked meal containing rice, chicken, lentils, and oil was offered to the patients at lunch and supper. Another cooked meal, consisting of a mixture of rice, egg, milk, butter and sugar was given at breakfast time. Banana, breast-milk, cow's milk and bread were given to the patients at specified intervals during the period of the study. Each item of food was weighed before offering it to the patients and any left over was weighed again to quantify the actual intake. Test weighing was done before and after each breast feeding to measure the amount of milk consumed. Samples from all types of food consumed were saved for analysis of fat, nitrogen and calories. Breastmilk was collected from the mothers, and analysed for calorie, fat and nitrogen. Collection of stools was started after the appearance of the first marker and was continued till the appearance of the second marker fed at 72 hours. The stools collected between markers were pooled and homogenized in a blender. An aliquot from this stool was saved for analyses of fat, nitrogen and calories. Any vomitus collected from the patients during the study was analysed and subtracted. No antibiotics were administered during the study. Patients went home after diarrhoea had stopped. All of the 29 patients were studied two weeks after discharge. Eighteen of them were studied eight weeks after discharge (8 rota, 6 ETEC, 4 shigella). These two recovery stages will be termed respectively R_1 and R_2 in the text.

Serum xylose estimation was done by the Roe and Rice (11) method. Fat was estimated by the Van de Kamer procedure (12). Total nitrogen content was measured according to microkjeldahl procedure (13). Calorie estimation from all samples was performed by using an adiabatic bomb caloriemeter, benzoic acid acting as the standard.

Co-efficients of absorption of nutrients at both the acute stage and at recovery were calculated using the following expression: (Intake - Output) x 100/Intake.

RESULTS

Some physical and clinical features of the study patients are presented in Table VII. Rotavirus patients were comparatively younger than those with ETEC and shigella. Body weight increased between acute and recovery stages 1 and 2 in all groups. All of the patients were anemic.

TABLE VII--CHARACTERISTICS OF THE STUDY PATIENTS (MEAN ± 1SD)

Particulars	Rotavirus	ETEC	Shigella
No.	13	10	6
Age (months)	23 ± 14.2	36.3 ± 10.6	34.1 ± 8.8
Admission body wt (kg)	8.7 ± 1.8	9.2 ± 2	9.4 ± 1.7
Body wt at recovery 1	9.1 ± 1.9	9.8 ± 2	9.9 ± 2.2
Body wt at recovery 2*	10.2 ± 2	10.0 ± 1.8	10.9 ± 2.4
Serum HCT on admission	31.2 ± 3.8	32.6 ± 2.5	33.3 ± 4.9
1st 24 hr stool volume (litres)	0.70 ± 0.31	0.68 ± 0.55	0.93 ± 0.57

* The number of patients available for the study at stage 2 was 8, 6 and 4 for rotavirus, ETEC and shigella respectively.

The co-efficient of absorption of nitrogen was compared between the three aetiologies of diarrhoea (Table VIII). During the acute stage, absorption of nitrogen was significantly less (P<.01) in rotavirus than in ETEC. At recovery 1, absorption of nitrogen did not differ significantly between the three aetiologies. However, even eight weeks after recovery, absorption of nitrogen was comparatively less in the rotavirus group than in the others. Co-efficients of absorption of fat, calories and carbohydrates are shown in Tables IX, X and XI respectively. During the acute stage, absorption of fat and calories was

TABLE VIII--CO-EFFICIENT OF ABSORPTION (±1SD) OF NITROGEN DURING ACUTE (A) DIARRHOEA AND AFTER RECOVERIES (R_1 and R_2)

Aetiology	Co-efficient of Absorption		
	A	R_1	R_2
Rotavirus (13)	43.3 ± 22.3^a	68.5 ± 13.0^b	59.9 ± 28.2^c
ETEC (10)	58.3 ± 13.9^d	54.0 ± 33.3^e	72.8 ± 9.3^f
Shigella (6)	41.3 ± 45.6^g	73.6 ± 4.8^h	72.3 ± 9.7^i

Student's T Test. a vs d : s

Figures in parentheses indicate number of patients.

significantly less (P<.01) in rotavirus patients compared to ETEC patients. There was no statistical difference in the absorption of either fat or calories between rotavirus and shigella patients during the acute diarrhoea. In all aetiologies, absorption of carbohydrate was least affected, even during acute diarrhoea (Table XII). In ETEC, absorption of all nutrients was comparatively better in the acute stage compared to recovery stage 1. However, in recovery stage 2, absorption of nutrients in ETEC increased compared to stage 1.

Serum xylose levels were measured during the acute stage and after recovery (Table XII). Patients absorbing less than 20 mg% of xylose were defined as xylose malabsorbers. On an average 75% patients were found to be xylose malabsorbers at the acute stage, which was reduced to 10% at recovery 1. An attempt was made to see the relationship between xylose absorption and the

TABLE IX--CO-EFFICIENT OF ABSORPTION (±1SD) OF FAT DURING ACUTE (A) DIARRHOEA AND AFTER RECOVERIES (R_1 AND R_2)

Aetiology	Co-efficient of Absorption		
	A	R_1	R_2
Rotavirus (13)	42.3 ± 27.6[a]	79.0 ± 20.0[b]	81.3 ± 10.1[c] (8)
ETEC (10)	78 ± 14.2[d]	80.4 ± 15.3[e]	88.4 ± 8.3[f] (6)
Shigella (6)	61.8 ± 34.8[g]	88.8 ± 3.6[h]	93.8 ± 2.1[i] (4)

Student's T Test a vs d : s f vs i : s
c vs i : s

Figures in parentheses indicate number of patients.

TABLE X--CO-EFFICIENT OF ABSORPTION (±1SD) OF CALORIES DURING ACUTE (A) DIARRHOEA AND AFTER RECOVERIES (R_1 AND R_2)

Aetiology	Co-efficient of Absorption		
	A	R_1	R_2
Rotavirus (13)	55.4 ± 23.7[a]	91.1 ± 4.6[b]	81.2 ± 10.1[c] (8)
ETEC (10)	86.7 ± 7.7[d]	82.2 ± 10.9[e]	88.7 ± 6.5[f] (6)
Shigella (6)	68.1 ± 32.0[g]	79.7 ± 13.9[h]	90.4 ± 2.5[i] (4)

Student's T Test. a vs d : s b vs e : s c vs i : s

Figures in parentheses indicate number of patients.

TABLE XI--CO-EFFICIENT OF ABSORPTION (±1SD) OF CARBOHYDRATE DURING ACUTE (A) DIARRHOEA AND AFTER RECOVERIES (R_1 AND R_2)

Aetiology	Co-efficient of Absorption		
	A	R_1	R_2
Rotavirus (13)	73.8 ± 26.0[a]	88.8 ± 5.8[b]	84.1 ± 11.6[c]
ETEC (10)	91.7 ± 5.2[d]	86.8 ± 9.8[e]	91.0 ± 6.3[f]
Shigella (6)	77.0 ± 31.7[g]	78.3 ±20.8[h]	91.6 ± 3.7[i]

Student's T Test. a vs d : s

Figures in parentheses indicate number of patients.

TABLE XII--MEAN ONE-HOUR SERUM XYLOSE LEVEL (±1SD) DURING ACUTE (A) AND RECOVERY STAGES (R_1 AND R_2) OF DIARRHOEA DUE TO DIFFERENT AETIOLOGY

Aetiology	Serum Xylose Level (mg%)		
	A	R_1	R_2
Rotavirus (13)	13.9 ± 6	26.3 ± 7	33.7 ± 10
ETEC (10)	15.2 ± 6	26.7 ± 8	24.3 ± 5
Shigella (6)	38.6 ± 38	28.6 ± 5	32.1 ± 14

Figures in parentheses indicate number of patients.

absorption of nutrients during acute and recovery stages (R_1 and R_2) of diarrhoea. Figure 1 shows that in rotavirus infection when xylose absorption was poor carbohydrate absorption was as high as 74% even during acute diarrhoea. However, results were not similar for the shigella group. Results in Figure 2 indicate that in shigella, though xylose absorption remained unaffected (39 mg%) in acute diarrhoea, carbohydrate absorption (77%) was not comparatively higher than in rotavirus group (74%).

Stool weight was taken after every 24 hour period during both the acute and recovery stages. Table XIII shows the mean percent change of stool weight in the first and second 24 hour period during acute diarrhoea. A general tendency for stool weight to decrease gradually was noticed between the first and second 24 hour periods at the acute stage of diarrhoea.

TABLE XIII--MEAN PERCENT CHANGE IN STOOL WEIGHT IN THE FIRST AND SECOND 24 HOUR PERIOD DURING ACUTE DIARRHOEA

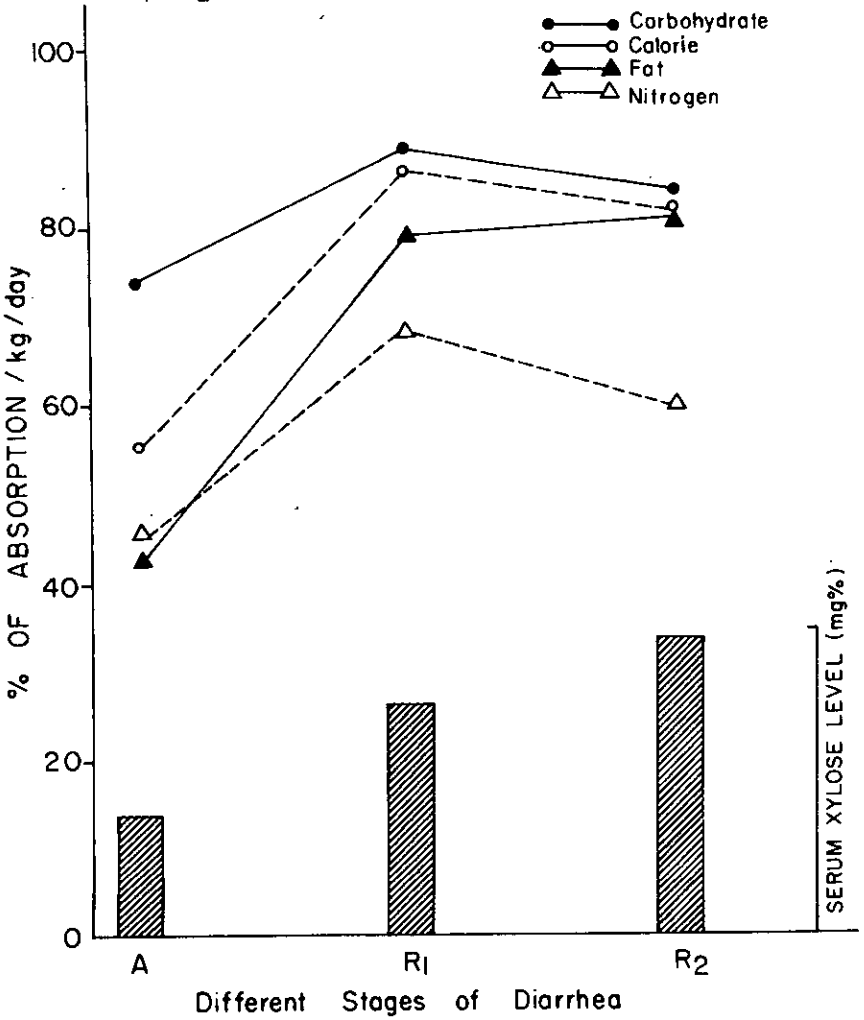
		% Change in Stool Wt.	
Aetiology	Number	1st 24 hour	2nd 24 hour
Rotavirus	13	- 19	- 26
ETEC	10	- 56	- 33
Shigella	6	- 47	- 69

DISCUSSION

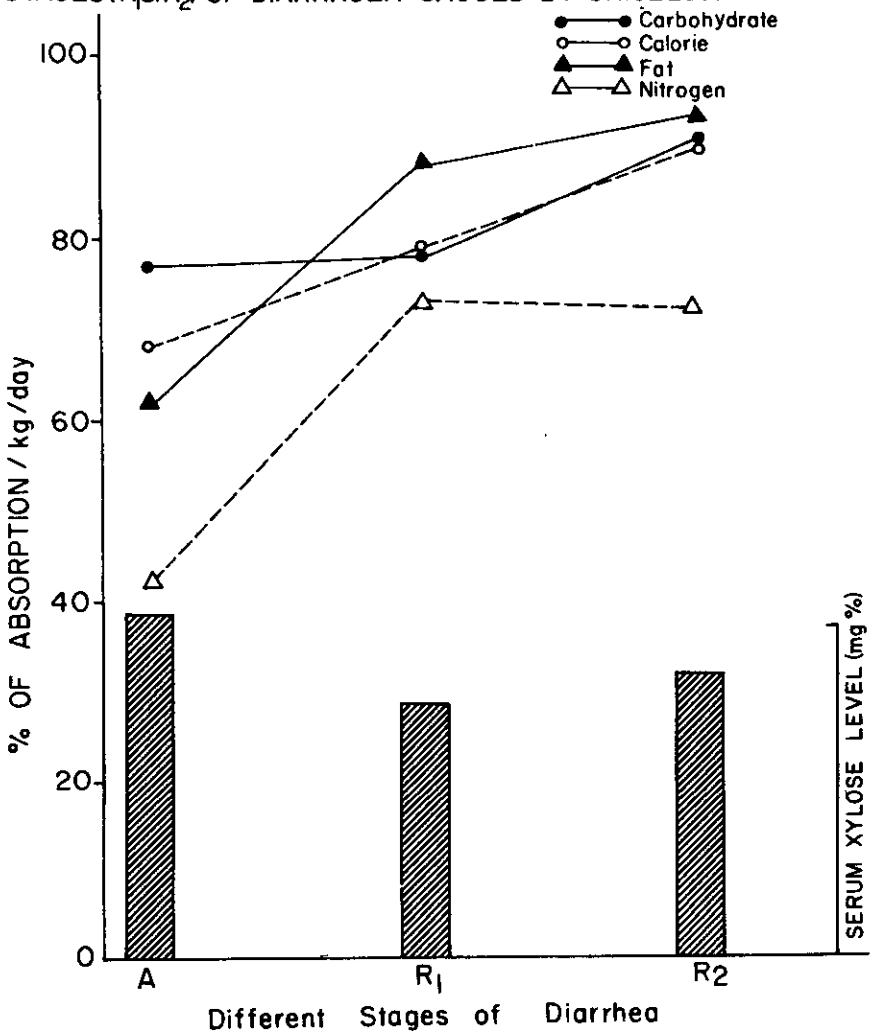
The results of the present research indicate several differences between the aetiologies with regard to absorption of nutrients during acute and recovery stages of diarrhoea.

In the acute stage of rotavirus, absorption of all the nutrients was significantly lower compared to absorption in ETEC diarrhoea. Absorption of nitrogen in rotavirus did not improve even eight weeks after recovery, suggesting the presence of a prolonged malabsorption period. This observation may be explained by the fact that in rotavirus infection villous epithelial cells are specifically affected (14) and the inflammation may continue longer. Previously, it was observed that rotavirus stool contains a large amount of reducing substance

RELATION BETWEEN XYLOSE ABSORPTION AND PERCENTAGE OF NUTRIENT ABSORPTION DURING ACUTE (A) AND RECOVERY STAGES (R_1 & R_2) OF **DIARRHOEA CAUSED BY ROTAVIRUS**



RELATION BETWEEN XYLOSE ABSORPTION AND PERCENTAGE OF NUTRIENT ABSORPTION DURING ACUTE (A) AND RECOVERY STAGES(R_1&R_2) OF DIARRHOEA CAUSED BY SHIGELLA



compared to the stool in cholera (15, 16). However, in this study we observed that as much as 74% of the ingested carbohydrate was absorbed in the acute stage compared to 92% absorption in ETEC and 77% in shigella.

During the acute stage of ETEC, absorption of all nutrients was comparatively better than in other aetiologies but failed to show improvement two weeks after recovery. After eight weeks, an increase in absorption of nutrients was clearly evident. The mechanism of mucosal damage by the ST toxin is not yet clearly known. In the present group of ETEC patients, we had four with pure ST, two with pure LT and four with a mixture of ST and LT. The combined presence of ST and LT toxins on the epithelial cells may have affected the absorption of nutrients two weeks after recovery but improved thereafter.

In shigella, during the acute stage, absorption of nitrogen was minimum (41%) compared to the absorption of other nutrients which was in the range of 62-77%. Shigella is a disease of the lower bowel and absorption of nitrogen was probably due to direct loss of protein from the gut rather than failure of intestinal absorption. Absorption of all nutrients was significantly higher two weeks after recovery.

Similarly, the serum xylose level in acute stage of rotavirus and ETEC diarrhoea was below 20 mg% but in shigella it was 37 mg%. Despite the lower xylose level, absorption of carbohydrate was 74% in rotavirus and 92% in ETEC. In shigella during the acute stage it was 77%, though the serum xylose level was much higher. This discrepancy between xylose absorption and carbohydrate absorption may indicate that the absorption status of xylose is not a valid reflection of the absorption of a natural carbohydrate and the other nutrients.

Early feeding of these diarrhoeal patients did not seem to increase the stool volume, which is evident from Table XIII. However, Chung *et al.* (3, 4) showed that in some of their patients feeding during diarrhoea was associated with increase in stool volume though absorption of nutrients was not affected.

Finally, the following conclusions can be derived from this study: Despite reduced absorption of nutrients, feeding of children should be encouraged because substantial absorption does take place in acute diarrhoeal infection. This may help in the prevention of further malnutrition. This observation has profound implications, especially in developing countries where diarrhoea is endemic.

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HOME ADMINISTERED ORAL REHYDRATION THERAPY:

A MEASURE OF SAFETY AND EFFICACY

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ABSTRACT

Serum electrolytes were measured from persons with diarrhoea treated at home with prepackaged or locally available sugar and salt oral rehydration therapy (ORT) solutions and from persons with diarrhoea treated according to local customs. No detrimental effect was found in persons treated with ORT at home. The rates of hyponatremia and hypokalemia were significantly lower in persons who took estimated appropriate volumes of ORT than in persons who took less than appropriate volumes or in persons treated according to local customs. No significant difference was found between the groups in development of hypernatremia. These results indicate that ORT can be safely and efficaciously used at home under the conditions of our study.

INTRODUCTION

Oral sugar-electrolyte therapy (ORT) solutions have been shown to be effective in treating diarrhoeal dehydration associated with various enteric pathogens[1-7]. Because ORT solutions are produced from inexpensive ingredients, it can be widely distributed and are easily administered. ORT reduces the use of intravenous (IV) fluids, they offer a practical outpatient method for decreasing the severe dehydration and mortality from diarrhoeal disease[8,9].

Several important questions remain to be answered about the use of ORT. Whether ORT administered at home, and made from locally available sugar-salt mixtures will cause the development of electrolyte abnormalities. This paper reports on the serum electrolytes of persons (mostly children) with diarrhoea who were treated with ORT at home and from persons who were admitted to a treatment centre. The 3 study groups included: persons receiving ORT made from packets made according to the WHO formula (packet group), persons

receiving ORT made from salt (lobon) and molasses (gur) available in the home (lobon-gur group), and persons who received only hospital treatment - comparison group.

METHODS

From January 1979 to Debember 1980, an oral therapy field trial was carried out by the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B - formerly the Cholera Research Laboratory) in its rural field station in Matlab Thana, Bangladesh.

Three cluster of villages with approximately 40,000 persons in each cluster, and with almost equal access to the ICDDR,B's treatment centre, comprised 2 study and 1 comparison group. A woman was selected from each bari (consisting of 1 to 20 related families) in each treatment area village to store and prepare ORT for the members of her 'bari'. In the packet area these women were given 1 litre bottles for measuring water and plastic packets containing necessary amount of glucose and electrolytes to make ORT according to the WHO formula. In the lobon-gur area, the women received 1 litre bottles, supplies of locally available molasses (gur) and salt (lobon), and a 2-ended plastic spoons designed to measure a mixture similar in composition to the packet. One female village worker (FVW) with special training on treatment of diarrhoeal disease and use of ORT resided in each treatment area village (approximately 1,000 population). They visited every house once in two months and provided repeated instructions and encouragement on the preparation and use of ORT. During these visits they also supervised the preparation of the ORT by the women. FVW's with only demographic surveillance duties resided in the comparison villages.

Indepth Follow-ups

Information on the incidence and home treatment of diarrhoea was collected by field assistants (FAs) who visited each home in the 2 treatment areas every 2 months and recorded cases and the mode of treatment of diarrhoea which had occured. If the FA arrived within 48 hours of the onset of diarrhoea, he completed the first portion of a health history and physical examination questionnaire, obtained a rectal swab stool specimen, and an oral therapy fluid sample from each person with diarrhoea who received ORT. Two days later he obtained a finger-prick blood specimen and completed the second portion of the questionnaire. Six days after the initial visit, the final portion of the questionnaire was completed. We selected ¼ seer per day for children less than 2 years old and 1 seer per day for persons older than 2 years as conservative volume intake minimums.

Home and Treatment Centre Therapy

Information were obtained from patients admitted to the treatment centre from the control and the study areas. Rectal swab stool specimens were obtained for culture. Blood samples were drawn by finger-prick method from all study area patients on week days between 8:30 a.m. to 5 p.m. A conservative estimate of the appropriate amount of fluid needed was selected as 75% or more of the calculated daily requirement based on body weight and dehydration status.

Laboratory

All blood specimens were centrifuged and the serum separated on the same day. The samples were analyzed for sodium and potassium on a flame photometer. Rectal swab stool specimens were cultured for salmonella, shigella, *E.coli*, *Vibrio cholerae*, and rotavirus using standard procedure.

Electrolyte Analysis

Values indicating electrolyte abnormality were selected using guidelines from Nelson's Textbook of Pediatrics[10]. The definitions of abnormal values include: Hypernatremia, $Na^+ \geq 150$ mEq/l, hyponatremia, $Na^+ \leq 130$ mEq/l, and hypokalemia, $K^+ < 3.0$ mEq/l. Serum specimens were excluded from analysis if they contained evidence of hemolysis.

RESULTS

Completed questionnaires and serum electrolyte results were available from 72 persons from the packet area and 66 persons from the lobon-gur area. The rate of electrolyte abnormalities for persons from the 2 study areas by age group was 2% (1/60) for children less than 10 years old, 5% (2/44) for persons 10 to 39 years old, and 15% (5/33) for persons over 40 years old. There was no relation between rate and type of abnormality and age group. No electrolyte abnormalities were found in children less than 2 years old. Only 2 out of the 72 persons from the packet area and one out of the 66 persons from the lobon-gur area had 5% or greater dehydration, and none of these persons developed electrolyte abnormalities. None of the persons surveyed were treated at the treatment centre.

Five persons treated with packet ORT developed electrolyte abnormalities: 3 had hypernatremia (4%) and 2 (3%) had hyponatremia; there were no cases of hypokalemia (Table 1). No person from the packet area who took greater than 1 seer of ORS per day had an electrolyte abnormality.

Table 1. Electrolyte abnormalities in persons treated with ORT at home by volume of ORT taken

A. Hypernatremia ([Na] $\geq$150 mEq/1)

	Appropriate Volume*	< Appropriate Volume*
Packet	0/7	3/65 (4%)
Labon-gur	0/2	0/64

B. Hyponatremia ([Na] $\leq$ 130 mEq/1)

	Appropriate Volume*	< Appropriate Volume
Packet	0/7	2/65 (3%)
Labon-gur	0/2	2/64 (3%)

C. Hypokalemia ([K] 3.0 mEq/1)

	Appropriate Volume*	< Appropriate Volume
Packet	0/7	0/65
Labon-gur	0/2	1/64 (1%)

*Appropriate volume:

For children <2 years: 1/2 seer ORT/day (about 500 cc/day)
For persons $\geq$2 years: 1 seer ORT/dat (about 1 litre/day)

Electrolyte abnormalities were found in 3 persons treated with lobon-gur; none had hypernatremia, 2 persons (3%) had hyponatremia, and 1 person (1%) had hypokalemia (Table 1). Only 2 persons took more than 1 seer of ORT per day and neither person had an electrolyte abnormality. The median volume of ORT taken by persons in either treatment area was 1/3 seer per day for the first 3 days of therapy.

There was no association of electrolyte abnormalities and etiologic agents recovered from the stools. No statistically significant association was found in seasonality and the occurence of electrolyte abnormalities.

Home and Treatment Centre Therapy

Serum electrolytes were evaluated from 434 persons admitted to the treatment centre from the 3 study areas (Table 2). Approximately 85% of these persons had mild (less than 5%) dehydration compared to 70% of all persons admitted from the study areas (unpublished data). Children less than 10 years old accounted for about 75% of the persons with mild rehydration from the 3 study areas and about 33% of the persons with greater dehydration. There was no statistical difference in the rate of electrolyte abnormalities in persons who had less than 5% dehydration and persons who had 5% or greater dehydration. Approximately 80% of the electrolyte abnormalities occured in children less than 10 years old and this age group comprised about 70% of the population sampled. Forty-five percent of the abnormalities occurred in children less than 2 years old, and this group totalled 45% of the population studied. There was no significant difference in the rate of electrolyte abnormalities according to age group.

Table 2. Severity of Dehydration of Persons Admitted to the Treatment Centre from the Study Areas

	Percent Dehydration				
	< 5%		≥ 5%		
Study Area	Persons	(%)	Persons	(%)	Total
Packet	108	(83)	22	(17)	130
Lobon-gur	117	(84)	23	(16)	140
Comparison	140	(85)	24	(15)	164
					434

None of the persons who received an appropriate volume of packet ORT (defined as equal to or greater than 75% of the calculated required daily total) had an electrolyte abnormality (Table 3). There was no statistical difference in the rate of hypernatremia found in persons who took appropriate, less than appropriate, or no packet ORT and persons from the lobon-gur or comparison group. Neither hyponatremia nor hypokalemia was found in persons taking an appropriate volume of packet ORT, but the numbers were too small to show a statistical association with volume taken. Only 22% of persons from the packet area took an appropriate volume of fluid.

Similar results were found in persons from the lobon-gur area. No significant difference was found in the rates of hypernatremia in persons taking appropriate, less than appropriate, or no lobon-gur ORT. Neither hyponatremia nor hypokalemia was found in the 23% of persons taking an appropriate volume of lobon-gur, but the number was too small to show a statistically significant difference between persons taking appropriate and less than appropriate volumes. However, when persons taking an appropriate volume of packet and lobon-gur ORT were compared to persons from the treatment areas taking less than appropriate or no ORT, there was a statistically significant protective effect (P=0.046, Fisher's 2-tailed exact test). When the group taking an appropriate volume of either fluid was compared with the comparison group, the difference in rates of hypokalemia and hyponatremia was also significantly different (p=0.04).

No association was found between the occurrence of electrolyte abnormalities and infection by a particular etiologic agent and no seasonal pattern of electrolyte abnormalities was found.

DISCUSSION

Several limitations of the study must be kept in mind when trying to use the results to answer questions concerning the safety and efficacy of the home preparation and administration of ORT. The number of persons sampled was too small to show statistical differences in electrolyte abnormalities within the different study groups. Larger numbers may have confirmed the trends indicated by the fact that no one taking an estimated appropriate volume of either packet or lobon-gur developed hyponatremia or hypokalemia. Only when persons taking estimated appropriate volumes of both packets and lobon-gur ORT are considered as one group were the numbers large enough to show a statistical difference indicating a protective effect against the development of hyponatremia and hypokalemia. These findings support the contention for persons who replace their fluid and electrolyte losses should do better than persons who take little or nothing by mouth during diarrhoea. Although the numbers were small, if a major detrimental effect of taking ORT existed, our study could have been expected to have shown such a relation.

Table 3: Electrolyte abnormalities related to volume and nature of fluid taken.

A. Hypernatremia ([Na] $\geq$ 150 mEq/l)

Electrolyte abnormalities in persons taking:

Study Area	Appropriate Volume*	<Appropriate Volume*	None
Packet	0/28 (-)	2/60 (3%)	2/42 (5%)
Labon-gur	3/32 (9%)	1/63 (2%)	0/45 (-)
Comparison			5/164 (3%)

B. Hyponatremia ([Na] $\leq$ 130 mEq/l)

Study Area	Appropriate Volume*	<Appropriate Volume*	None
Packet	0/28 (-)	3/60 (5%)	1/42 (2%)
Labon-gur	0/32 (-)	0/63 (-)	1/45 (2%)
Comparison			5/164 (3%)

C. Hypokalemia ([K]<3.0 mEq/l)

Study Area	Appropriate Volume*	<Appropriate Volume*	None
Packet	0/28 (-)	2/60 (3%)	4/42 (10%)
Labon-gur	0/32 (-)	5/63 (8%)	1/45 (2%)
Comparison			7/164 (4%)

*Volume taken was at least 75% of the calculated required daily total.

Since the great majority of dehydrations reported were mild, the possible effect of ORT intake on electrolyte abnormalities associated with severe dehydration was difficult to assess. However, we found no difference in the rate of electrolyte abnormalities in persons with mild and more severe dehydration, and the evaluation of persons treated at the treatment centre allowed us to see severe cases of dehydration.

The unexpected discovery that potassium and bicarbonate were present in the lobon-gur mixture meant that we could not evaluate the efficacy of a local sodium chloride and sugar mixture with the premixed packet containing sodium, potassium, chloride, bicarbonate, and sugar. The results do indicate that the presence of potassium is necessary in ORT mixtures.

Care must be taken in extrapolating the results of this study of home preparation and administration of ORT to larger populations because of the intensive supervision, training, and encouragement given by the field staff. However, the results do indicate that villagers can be taught to prepare ORT at home.

Despite the limitations mentioned, we feel that the study does show that ORT can be done and administered safely by villagers receiving close supervision and that ORT administered at home may have a beneficial effect in preventing the development of hyponatremia and hypokalemia.

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HAND PACKAGED ORS FOR DIARRHOEA

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ABSTRACT

The feasibility of a simple method for the preparation of Oral Rehydration Solution (ORS) packets suitable for a cottage industry was tried out. Contents of the packet contained ingredients to make one litre of ORS according to formula approved by WHO excepting that glucose was replaced by common sugar (sucrose). Salts and sugar measured for 200 packets were mixed thoroughly in a bowl. A standardized scoop was used to measure the salt-mixture for one litre of ORS and packed in polythelene bags. Trained housewives prepared the packets. A printed leaflet containing the composition and instructions for making the solution and administration of ORS was wrapped around each packet. Every step of the preparation of the packets was controlled.

For quality control, 3 to 5 packets were tested for weight and electrolyte contents from each lot of 200 ORS packets. The results were found to be satisfactory. The shelf life of the ORS packets in different seasons and in various conditions of temperature and humidity was also tested. Some physical changes were observed; but composition remained almost constant upto 8 months of storage. A simple method for quality control was developed by estimating chloride by simple titrimetric analysis. A good correlation was found between Na^+ and Cl^- ions in ORS.

INTRODUCTION

The efficacy of oral rehydration solution (ORS) in the treatment of diarrhoeal diseases has been accepted worldwide[1-2]. But when ORS is packaged centrally there is a tremendous amount of logistics involved in distributing this throughout the country. This problem is especially faced by rural areas of developing countries, and this is where ORS is most needed because of predominance of diarrhoea. The logistics and supply problems may result in scarcity and thereby unnaturally pushing the prices up.

Another alternative is to use either the pinch and scoop method or the double-ended spoon and make ORS at home. But field trials have shown that even though rural women with no education can make ORS satisfactorily, repeated training by a trained person is essential. This is not practical for all the areas where diarrhoea in endemic. To overcome this disadvantage a simple method, suitable as a cottage industry for the preparation of safe oral-rehydration salt packets was developed. All ingredients were tested in the Biochemistry laboratory of ICDDR,B; the ORS formula recommended by WHO was followed except 20 gm glucose was substituted by 40 gm sucrose. Ingredients for preparing 200 packets, each for 1 litre ORS, were measured in a balance and mixed thoroughly in a bowl. For testing the homogeneity of the mixture of salts and sugar, 3 samples each containing 47.5 gms. of the mixture (which is required for making 1 litre ORS) were taken from bowl and assayed separately. When the results were found to be within the satisfactory range the salt-sugar mixture was then packed. It was found that 30 minutes of continuous mixing by hand was needed for making a homogenous mixture. Sometimes sodium bicarbonate had to be ground by hand and sieved for proper mixing. A standardized plastic scoop measuring 47.5 gms. was used to measure mixture for making one litre packets. The scoop was made in this laboratory by cutting a 50 ml plastic measuring cylinder to hold exactly 47.5 gms. of the mixture. The measured mixture was then sealed in a polythelene bag by an electric sealer or a candle. Housewives were instructed to prepare packets and mastered the technique very well within three months. A leaflet describing the composition and instructions on making the solution and for administering it was wrapped around the packets.

From each lot of 200 p-ckets 3 to 5 bags were tested for quality by measuring the gross weight and electrolytes contents. Electrolytes were estimated in the Biochemistry laboratory of ICDDR,B using IL analyser. A total of 3359 samples were analyzed and the results were tabulated. The mean net weight of the packets tested was 48.2 gms. with S.D. ± 0.35. The optimum weight of the mixture for 1 litre ORS is 47·5 gm. The mean weight of polythelene bags was 1.4 gm.

The shelf life of the ORS packets was also tested. Sixty packets prepared in May 1980 were kept exposed to the atmosphere for eight months and analysed. It was found that the glucose, HCO_3^-, pH and moisture content varied during the period of storage. Physical changes noticed were: colour, changed from white to brown and formed soft lump. But the lump did not effect solubility of the mixture. CO_2 came down from 29.6 to 23.7 mmol/l, glucose from 107 to 100 mmol/l, pH increased from 8.4 to 9.1 and 1.3% moisture was absorbed by those packets during the eight months of storage. The bicarbonate content of the salt mixture decreased gradually during storage, packets that are usually stored from 3-5 months were safe and contained optimum concentration[7] of bicarbonate to treat diarrhoea. In the

rainy season those packets were also analysed after one month and 3 months. Except moisture content all other components remained stable. The mean moisture content was 5% and varied from 2.6 - 10.5% during 3 months of storage. During the rainy season in Bangladesh humidity varies from 98 - 100% and temperature from 28° - 30°C. When the rainy season was over, water absorbed, diffused out of the packets because the polythelene bags were semipermeable to water.

The concentration of Na^+ ion in the ORS is considered to be the most critical item for its safety and efficacy (14-15). Estimation of sodium requires the use of a flame photometer which is expensive and requires a high level technical competence for operation and maintenance and is not suitable for quality control in rural areas. We evaluated an indirect method for the estimation of sodium in ORS solution by titrating chloride against silver nitrate in presence of an indicator[16].

The modified Mohr method was used to titrate chloride in oral solution. The chloride in neutral or weak alkaline solution containing chromates is titrated with silver nitrate. Silver chloride precipitates and at the end point red silver chromate is formed. Oral therapy solution is weekly alkaline, therefore adjustment of alkalinity or neutrality of the solution was not necessary. This method is ideal to titrate chloride in oral therapy solution because the interfering substances iodine, bromide, phosphate, sulfide and cyanide are not likely to be present in ORS in important amounts.

Reagents:

(a) Standard $AgNO_3$ solution (0.01 N)' 1.699 gm $AgNO_3$ was dissolved in 1000 ml. volumetric flask and the volume was adjusted up to the mark.

(b) Standard Nacl solution (0.01 N)' 0.5845 gm of reagent grade Nacl was dissolved in 1000 ml. volumetric flask and volume was made up to the mark. This solution is used to standardize silver nitrate solution.

(c) Potassium chromate indicator solution: 50 gm of K_2CrO_4 was dissolved in about 700 ml of distilled water and to this solution silver nitrate solution was added to produce a slight red precipitation. After keeping undisturbed overnight the solution was filtered and diluted to one litre.

Procedure:

To 1 ml of oral therapy solution in an 100 ml Erleenmeyer flask about 20 ml of water was added. The solution was warmed slightly on a heater or a spirit lamp and then 0.5 ml of K_2CrO_4 solution was added to it. The titration was carried out against standard $AgNO_3$ solution from burate until a colour change from pure yellow to pinkish yellow, coloured precipitation appeared. The indicator blank should be

determined by titrating water in the same way. One standard shold be run in each lot of analysis.

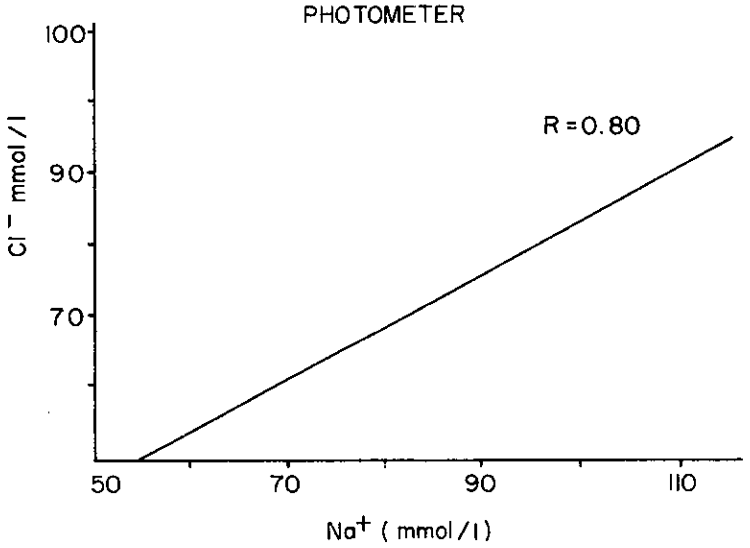
calculation $X = V \times S \times 1000 = Cl^-$ in mmol/l of ORS.

Where, X = Chloride concentration in ORS in mmol/l.
V = Vol. of $AgNO_3$ used to titrate the solution.
S = the actual strength in normality of $AgNO_3$.

The chloride values obtained by the automatic chloride counter and by the manual titrimetric method correlate strongly. The correlation coefficient was found to be 0.98. The correlation coefficient between chloride values measured by the titrimetric method and Na values measured by flame photometry was found to be 0.8 (Figure 1).

Fig. 1

CORRELATION BETWEEN CHLORIDE CONC. ESTIMATED BY TITRIMETRIC METHOD AND SODIUM CONC. ESTIMATED BY FLAME PHOTOMETER



It may be concluded that if centrally tested ingredients are supplied, cottage industry for making ORS packets may be set up and housewives can be trained to make safe oral rehydration packets. The simple titrimetric method not requiring sophisticated instruments and adaptable to simple field use has been found to provide an accurate estimation of chloride and therefore can

be used to estimate sodium for ensuring the safety of oral rehydration salt packets. The estimation of chloride requires glass ware and reagents which are easily available in Bangladesh. Centrally prepared and standardized reagents may be supplied. To simplify the preparation of ORS packets sodium-bicarbonate was mixed with other ingredients. Results show that even when the packets are exposed to normal conditions of temperature and humidity for upto eight months, those packets could be safely used for rehydration. However, as a safety measure, it is recommended that packets should be used by three months of preparation. Another advantage of mixing ingredients for 200 packets at a time was that the large quantity was easier to measure and reduced the margin of error incurred by using a rough balance.

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THE SUCCESSFUL USE OF (RICE-POWDER) ELECTROLYTE SOLUTION AS ORAL THERAPY IN DIARRHOEA DUE TO *V.CHOLERAE* AND *E.COLI*

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ABSTRACT

A cereal-based electrolyte solution, containing 30 g of rice powder per litre and electrolytes as recommended by WHO, was administered to 124 patients with acute diarrhoea due to *V.cholerae* and *E.coli* in place of the previously standardized sucrose-electrolyte solution. The effect of this cereal-based therapy was assessed by the rate of purging, change in body weight, serum specific gracity, urine output and post hydrolysis surgar content in the stool. The average rate of success in the rice-powder group was 80% for cholera patients and 88% for *E.coli* patients, no different from those receiving the sucrose-electrolyte solution. Failure was due to rates of purging that exceeded the patient's ability to drink enough replacement solution. This study suggests that a cereal-based, rice-powder electrolyte solution is efficient and safe to use as a rehydrating oral fluid in acute diarrhoea.

INTRODUCTION

Darrow in 1949[1], Chatterjee in 1953[2], Harrison in 1954[3] and Meneghello in 1960[4] recommended the use of a salt and glucose solution as oral therapy in the treatment of diarrhoea. These workers considered this salt-sugar solution as a nutritional medium. It was discovered later that sodium and glucose-transport are coupled in the small intestine, so that glucose accelerates the absorption of the solute and water[5,6,7,8]. Following this, several practical regimens were devised to treat cholera and other non-cholera diarrhoeas, in adults as well as children [9,10,11,12,13]. Glucose, a monosaccharide which effectively promotes the absorption of sodium and concomitantly water, is relatively expensive and is not produced in some countries where the incidence of diarrhoea is highest. A successful alternative to glucose in oral rehydration solutions [14,15,16] has been sucrose. However, research continues on oral rehydration therapy to simplify, reduce cost, and provide nutrition during diarrhoea. Studies have shown direct or indirect nutritional benefit from oral therapy [17,18] in diarrhoea but this aspect needs further study. The use of chicken soup with glucose or a cereal starch has been suggested [19].

We have utilized a cereal, rice-powder, to replace sucrose or glucose in a standard oral rehydration solution in a case-control study with an objective to see if this cereal base electrolyte solution is effective as rehydration media in diarrhoea.

PATIENTS AND METHODS

Adults and children with less than 24 hour history of diarrhoea and moderate to severe dehydration were selected from the patients presenting themselves at the treatment centre of the International Centre for Diarrhoeal Disease Research, Dacca, Bangladesh. Informed consent was obtained from the patients, or from their parents or guardians. 63 patients without signs of systemic disease or any other complications were treated with rice powder electrolyte solution. 61 patients matched for age, sex, aetiology and severity of dehydration who were receiving sucrose electrolyte solution as a standard therapy at the same time and in the same facility were used as control. Weight and height were taken and a thorough clinical examination was made. Blood for CBC, Hct, sp.gr. and electrolytes was drawn on admission, at 24 hours, and before discharge. Microscopic examination and culture of the stool were carried out. *E.coli* isolates from each admission stool specimen were tested for heat labile enterotoxin (LT) using CHO cell culture assay[20] and for heat stable enterotoxin (ST) using the infant mouse assay[21].

Composition and Preparation of the Solution

The electrolyte content of both solutions was that recommended by WHO (Sodium 90 mMol, Chloride 80 mMol, Potassium 20 mMol and bicarbonate 30 mMol per litre). Sucrose (40g) was replaced by 30g of rice-powder. In vitro hydrolysis converts 80-86% of the rice-powder into glucose. Thus 30g of rice-powder were taken so that at least 20g of glucose would be liberated in the intestinal lumen. The electrolytes and rice-powder were packaged separately. To prepare the solution, the rice-powder was first dissolved in several hundred mls of ordinary water and boiled for 1-2 minutes in order to make a uniform solution. The solution was then cooked, the electrolytes were dissolved, and water was added to make one litre.

Plain water was allowed on demand. The study was designed to be carried out under minimum supervision by the nursing staff and the physicians, as was true for patients receiving the sucrose-electrolyte solution. The oral fluid was administered mainly by attendants or by the patients themselves. Strict intake and output records were maintained at 8-hour intervals. The effect of therapy was assessed by the rate of purging, change in body weight, serum specific gravity, urine output and post hydrolysis sugar content in the stool.

Recourse to intravenous fluid after initiation of oral therapy was considered as failure. This was left to the judgment of the physicians and nurses. Intravenous fluid was administered when stool output could not be matched by oral fluid or when the patient went back into a state of severe dehydration. Pre and post hydrolysis glucose content was determined from the stool every 24 hours and the difference was considered as the amount of starch passed unhydrolysed.

RESULTS

The results of 63 patients in the rice powder group and 61 patients in the sucrose electrolytes group of matched serum specific gravity were analysed. The majority of patients had cholera. Among the cholera patients 38 were adults and 35 were below 10 years of age. All the remaining patients were adults with *ETEC* diarrhoea. For practical reasons very young children with rotavirus diarrhoea were excluded. Comparative information on study and control patients is presented in Table 1. The mean age, body weight, and serum specific gravity on admission were comparable. Many patients stopped purging after 24 hours of study. Results after completing the first 24 hours of treatment were analysed to assess the effect of therapy on the patients. Stool output, intake of oral fluid and urine output after the first 24 hours of treatment were comparable in adults with cholera in both groups (Table 2). About 80% of the patients gained weight after the first 24 hours of treatment. Serum specific gravity returned to normal levels and the rate of success was the same in both groups.

The results in cholera patients below 10 years are presented in Table 3. After 24 hours of treatment the patients in the sucrose group consumed more oral solution ($P<0.05$). The stool output was high, urine output was low and post hydrolysis glucose content in the stool was less in the sucrose electrolyte group compared to the rice powder group, but statistically these differences were not significant. The gain in body weight and the rate of success in both groups were similar. The results for the *E.coli* patients are presented in Table 4. The mean age, body weight and serum specific gravity were very comparable. Most patients of both groups showed gain in body weight within 24 hours of therapy and the success rate was identical.

The ability of sucrose-electrolyte solution to correct and maintain electrolyte balance has been adequately shown before [16,23]. Rice-powder electrolyte solution was able to correct electrolyte abnormality including both moderate and severe degrees of acidosis. Except for a few patients who developed complications unrelated to oral therapy, failure was mostly caused by an inability to match the output due to heavy purging or vomiting through either of the oral solutions. A comparison between success and failure among

Table 1. Comparison of Study Patients Treated with Rice-Powder and Sucrose-Electrolyte Solution on Admission (Mean ± SEM)

Particulars	Rice-Powder group (63*)	Sucrose group (61*)
Age (yrs)	27.8 ± 2.6	22.7 ± 1.7
Male	38	43
Female	25	18
Bacteriol. diag.		
V.cholerae	37	36
ETEC	26	25
Ser. sp. gr.	1.031 ± 0.0007	1.032 ± 0.0004
Weight (Kg)	32.8 ± 1.8	30.6 ± 1.9

*Number of patients studied

Table 2. Effect of Treatment by Two Kinds of Oral Solutions on Cholera Patients Above 10 Years (Mean ± SEM)

Parameters	Rice-Powder group (17*)	Sucrose group (21*)
Stool Output (ml/kg/24 hrs)	123.4 ± 36.4	100 ± 19
Intake of Oral Fluid (ml/kg/24 hrs)	194 ± 26	223 ± 24
Urine Output (ml/kg/24 hrs)	42 ± 14	67 ± 13
% of Patients Who Gained Weight	86	82
Mean of ser. sp. gr.	1.025 ± .0005	1.025 ± .0002
Post Hydrolysis Sugar Content in the Stool (mMols/L)	10.31± 2.91	9.34 ± 2.55
% of Success	82	81

* Number of patients studied.

Table 3. Effect of Treatment by Two Kinds of Oral Solutions on Cholera Patients Below 10 Years (Mean ± SEM)

Parameters	Rice-powder group (15*)	Sucrose group (19*)
Stool output (mls/kg/24 hrs)	130 ± 30	184 ± 23
Intake of oral fluid (mls/kg/24 hrs)	189 ± 32**	326 ± 31**
Urine output (mls/kg/24 hrs)	48 ± 15	20 ± 4
Serum sp. gr. after 24 hrs of treatment	1.026 ± 0.0009	1.026 ± 0.0011
% patient weight gained	87	89
% body weight gained	5.5 ± 0.07	6.9 ± 0.047
Post hydrolysis sugar content (mMol/l)	13.7 ± 3.2	7 ± 0.70
% success	80	85

*Number of patients studied
** P 0.05

the cholera patients less than 10 years of age taking rice-powder electrolyte solution is presented in Table 5. Those who were more dehydrated and who purged more heavily had a significantly higher failure rate ($P < 0.001$) than those with a milder form of the disease. This has been shown previously in cholera patients treated with standard ORS[31].

DISCUSSION

The results of this preliminary study, conducted in a hospital but with minimum supervision, show that a cereal-based electrolyte solution made of rice-powder was as effective as a standard sucrose-electrolyte solution in correcting dehydration and maintaining hydration in diarrhoea caused by *E.coli* and *V. cholerae*. The rate of success was more than 80% in all age groups which is comparable to that in other studies[10,16,22].

Table 4. Comparison of the *E.coli* Patients Treated with Two Kinds of Oral Solutions (Mean ± SEM)

Parameters	Rice-powder group (27*)	Sucrose group (25*)
Age (yrs)	37.8 ± 3.4	32.4 ± 4
Weight (kg)	37.9 ± 1.7	39 ± 0.9
Ser. sp. gr.	1.032 ± 0.0006	1.033 ± 0.0009
Stool output (mls/kg/24 hrs)	126.1 ± 13.5	195 ± 24
Change in serum sp. gr. (after 24 hrs)	1.025 ± 0.0005	1.025 ± 0.0003
% patients who gained weight	100	96
% body weight gained	7.6 ± 0.7	9 ± 0.6
% success	89	88

*Number of patients studied

Table 5. Comparison between the Success and Failure Groups Below 10 Years Taking Rice-Powder Electrolyte Solution (Mean ± SEM)

Particulars	Success group	Failure group
Percent	80	20
Ser. sp. gr.	1.0295 ± 0.0006	1.0318 ± 0.0012
Stool output (mls/kg/24 hrs)	75.67 ± 6.4*	176 ± 15.6*
Oral fluid taken (ml/kg/24 hrs)	210 ± 6.0*	87 ± 10.4*
I.V. fluid received (ml/kg/24 hrs)	52.2 ± 1.9*	80 ± 4.0*
Urine output (ml/kg/24 hrs)	24 ± 3.5*	14 ± 1.0*

*P 0.001

Failure must be viewed as a function of the rate of fluid loss rather than the type of solution used (Table 5). Patients losing fluid at a rate higher than their ability to drink fell behind in hydration and required intravenous fluid therapy. Oral therapy with glucose or sucrose may increase the stool output upto 20%[8,23] during acute diarrhoea. In this study no significant increase in stool output was observed in any of the study groups. In children with cholera taking rice-electrolyte solution there was a tendency to pass less stool and more urine but this was not statistically significant.

In adults as well as in children starch is rapidly hydrolysed by salivary and pancreatic amylase to glucose, maltose, maltotriose and branched dextrins[24]. These carbohydrates are further hydrolysed to glucose by the maltases of the brush border of the enterocytes[25]. Most of the active disaccharidases are fully developed at birth[26] and even a one month old infant can digest and absorb a large amount of rice starch[27].

Rice is a unique starch containing a mixture of two different polyglucoses, amylose and amylopectin. Amylose has a linear structure and is composed of glucose units linked by 1,4 glucosidic bonds and in amylopectic the majority of the glucose units are also connected by 1,4 glucosidic bonds. It has 7-10% protein, and very little electrolyte[28]. Acid hydrolysis converts 80-86% of the rice-powder into glucose. The protein content of rice has important amino acids, such as glycine (30-36 mg) and lysine (30-34 mg) per 100 gm of rice and that of leucine and isoleucine are 30-40 mg[28]. Glycine has already been shown to promote transportation of sodium from the intestinal lumen[29]. Intraluminal digestion of rice-powder liberates the monosaccharide glucose slowly without causing an osmolar load, which makes it possible to give a higher quantity of rice-powder without it losing its effectiveness or causing an osmolar drag of fluid from the vascular space to the gut lumen. The efficiency of the specific intestinal enzymes in hydrolysing rice-powder remains at a satisfactory level in diarrhoea due to *V. cholerae* and *E.coli*. This has been shown by the similar amountsof post hydrolysis sugar in the 24 hour stool in both groups. Studies in this Centre have demonstrated that carbohydrate absorption is least affected furing diarrhoea due to *V. cholerae* and *E.coli* when a rice meal is taken; and even with invasive organisms such as rotavirus and shigella absorption remains excellent[30]. Moreover, severely purging cholera patients can eat a full meal of rice without any change in the character of the watery stool. Soaked rice in some form, with added salt or sugar, has been a traditional dietary therapy during diarrhoea in Bangladesh and many other developing countries for centuries, but without attention to the proper concentration of salts and water. Rice is cheaper and more readily available than glucose or sucrose. It is a familiar component of treatment for diarrhoea and thus may be more acceptable. Since there is no osmotic penalty the only limitation of rice is the thickness of the gruel used and the only measurement of concern is that enough be used. Above this limit there should be no hazard and a potential benefit of increased caloric density.

Hence, effective use of rice-powder, or any other appropriate cereal for a specific geographic area, in the oral therapy of diarrhoea may provide a unique opportunity for rehydration and nutritional rehabilitation for patients during the hazard of nutritional compromise during and after illness.

ACKNOWLEDGEMENT

We thank the research technicians of the Department of Biochemistry and Microbiology of the International Centre for Diarrhoeal Disease Research, Bangladesh for their skillful technical help for this study. Thanks are due to the physicians and nursing staff of the clinical research unit of ICDDR,B for their active support and participants in this study. The skilled secretarial help of Mrs. S. Ahmed is also gratefully acknowledged.

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ORAL GLUCOSE-ELECTROLYTE SOLUTION IN THE TREATMENT OF CHOLERA AND OTHER DIARRHOEAS - A TRIAL ON SEVERELY DEHYDRATED PATIENTS

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ABSTRACT

Efficacy of oral rehydration therapy in the treatment of mild and moderately dehydrated cholera patients has been well established. However, the utility of oral rehydration in the management of severely dehydrated patients has not yet been evaluated. The present study was conducted to determine the effectiveness of this simplified therapy in correcting severe dehydration with a view to recommend this in rural situations where no other treatment is available.

Fifty-six severely dehydrated cases of diarrhoeas including cholera with features of collapse and shock were treated initially with oral fluid only. Dehydration was corrected in 28 out of 56 cases and the remaining 28 cases had to be treated with I.V. fluid. Normal saline was given through the intravenous route along with oral glucose-saline containing bicarbonates. The younger patients responded better than the older patients.

INTRODUCTION

Glucose-electrolyte solution has been found to combat dehydration in severely dehydrated paediatric diarrhoeal cases. It is effective in the treatment of mild and moderately severe cases of adult diarrhoea. But it's role in the severely dehydrated adults has not yet been definitely established The authors took up this study to see how far oral fluid can be used for initial correction of dehydration and maintenance in severely dehydrated patients presenting with features of shock and collapse.

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* Presented by Dr. S.C. Pal

MATERIALS AND METHODS

Fifty-six diarrhoea patients aged between 6-70 years with severe dehydration admitted in the cholera ward of Infectious Diseases Hospital, Calcutta, were selected for this study. The degree of dehydration was assessed by absence of peripheral pulse, unrecordable (35 cases) of very low blood pressure (21 cases).

At the time of admission, the duration of diarrhoea and use of fluid therapy was ascertained and those who received fluid therapy in any form were excluded from this study. The patients were weighed and placed in cholera cots. Clinical examination was done, pulse, P.B., stage od dehydration, muscular cramps at the time of admission and at one hour interval during the whole treatment period were noted in the proforma specially prepared for this study. Plasma specific gracity was determined with total solid meter (American Optical Company). Amount of stool output and vomitus were measured and noted at hourly interval during the whole treatment period.

Treatment

The composition of ORS recommended by the World Health Organization were used. The amount of oral fluid taken varied between 300 ml. to 1000 ml. per hour, depending on the age and weight of the patient. 30-60 ml. were given at a time every few minutes with a feeding cup or a small tumbler. Nasogastric tubes were not used. Intake and output of every patient were recorded and whenever the output (stool and vomitus) exceeded the quantity of intake of oral fluid, normal saline was started intravenously in addition to oral fluid. Only enough I.V. normal saline was given for peripheral pulse to reappear. No alkaline solution was given intravenously.

OBSERVATIONS

The condition of these cases on admission were as follows:

		Mean and Ranges
1.	Body weight	28.3 kg (10-45 kg)
2.	Age	24.5 yrs. (6-70 yrs)
3.	Duration of diarrhoea	8.1 hrs. (3-19 hrs)
4.	Blood pressure (Systo.)	
	(a) 35 cases	Unrecordable
	(b) 21 cases	58.6 mm. Hg. (50-80)
5.	Plasma sp. gravity	1038.1 (1036-1039)

Out of the 56 cases, rehydration with oral fluid was possible in 28 cases only; in the other 28 cases, I.V. fluid was necessary for correction of dehydration, but maintenance could be achieved by oral fluid after initial correction of dehydration. There were no death nor there were complications during the week under observation. The results of treatment is shown in Table 1.

Table 1. Age Distribution of Diarrhoeal Cases

Age in years	Total Cases	Oral fluid only	Oral and I.V. fluid	% success with oral fluid
5 - 10	13	10	3	76.9
11 - 20	10	8	2	80
21 - 30	13	5	8	38.4
31 - 40	8	2	6	25
41 - 50	6	-	6	-
51 - 60	3	1	2	33.3
61 - 70	3	2	1	66.6
	56	28	28	50%

DISCUSSION

The cases selected for the present study had very severe dehydration with features of shock and collapse as evidenced by absent peripheral pulse, unrecordable blood pressure (35 cases) or very low blood pressure (58.5 mm. Hg.) and high plasma sp. gr. (Range 1036-1039). All these cases were treated initially with oral fluid only. In 28 out of 56 cases studied (50%), dehydration was corrected with oral fluid only. The remaining 28 cases required I.V. fluid, which was started between 2-8 hours (average 5.5 hours) after admission. However, the amount of I.V. fluid required by these patients was much less (average 2.7 litres) than those treated by I.V. fluid only. The time taken for the appearance of peripheral pulse was much longer in these cases, (4.7 hrs. and 8 hrs.) than it would have been if initially treated with I.V. fluid. It is not desirable for the hypovolaemia to continue, if facilities for intravenous therapy is available.

Younger patients responded better to oral fluid than older patients. In the 5-10 years age group, the success rate was 76.9% and among 11-20 years the rate was 80%. In older patients with higher body weight, the success rate was considerably less (Table 1.).

Patients with smaller output of stool and vomitus could be maintained in a positive fluid balance only with oral fluid, but those with very large volume of vomitus and stool could not be maintained on oral fluid alone and subsequently required I.V. fluid for correction of dehydration.

The study therefore indicated that oral rehydration therapy can be used as an emergency measure for severe cases of dehydration requiring I.V. fluid, until the patient can be transferred to a health facility (2-8 hours as shown in Table 2). These few hours can be utilized to shift the patients from home environment to a treatment centre.

Table 2. Results of Treatment

	Oral (mean value) (28 cases)	Oral and I.V. (mean value) (28 cases)
1. Time for appearance of peripheral pulse	4.7 hours	8 hours
2. Quantity of stool (12 hours collection)	935 ml.	2175 ml.
3. Quantity of vomitus (12 hours collection)	434 ml.	1420 ml.
4. Total oral fluid required	5.3 litres	5.5 litres
5. I.V. fluid (quantity)	-	2.7 litres
6. I.V. fluid started (hours after admission)	-	5.5 hours (Range 2-8 hours)

CONCLUSIONS

1. Where IV facilities are available, all cases of severely dehydrated diarrhoea should be treated initially with I.V. fluid.

2. If I.V. rehydration is not available patient should be put on oral therapy and sent to a treatment centre.

3. Simultaneous administration of oral fluid with I.V. minimises the requirement of I.V. fluid, I.V. alkaline solution, which is not readily available, is not necessary. Alkali and K^+ is obtained from the oral fluid.

4. Success with oral fluid appeared to be better in younger patients.

ORAL REHYDRATION THERAPY IN AN OUTBREAK OF DIARRHOEAL DISEASE

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ABSTRACT

In October-November 1980, an outbreak of diarrhoeal diseases occured in one of the thanas of Bangladesh. A 3 member ICDDR,B investigation team went and set up an a) improved surveillance of diarrhoeal disease; b) supplied the VHW with packets of ORT and c) trained all the health workers of the thana on diarrhoea, dehydration and ORT. A total of 123 cases were line listed by the surveillance. Case fatality rate before the arrival of the team was 31 in 75 (41%) and 3 in 48 (6%) after establishing intense surveillance and ORT distribution. The difference in mortality ratio is significant. ($P < .01$). Improved surveillance, ORT distribution with training probably accounts for the lower mortality rate during the intervened part of the epidemic. Though prevention of an outbreak of diarrhoeal disease seems remote at the present moment - significant change can be brought about in the mortality pattern by providing simple health education and ORT.

INTRODUCTION

Morbidity and mortality due to acute diarrhoeal illness constitutes one of the major health problems in Bangladesh and other developing countries. Although intravenous rehydration with appropriate fluid and electrolytes may virtually eliminate death from dehydration due to diarrhoea, intravenous fluids are generally difficult to obtain for the rural population. The situation is further complicated during a cholera or diarrhoeal disease outbreak which occurs in this region virtually every year. The development of a glucose or sucrose electrolyte solution for oral rehydration represents a major breakthrough since cost and availability constrains do not post insurmountable barriers to effective treatment of diarrhoea.

The success of oral rehydration therapy to combat dehydration in diarrhoeal illness is now well established. A number of hospital based studies have proved its efficacy and safety and community based studies have shown its impact on mortality though not on morbidity. But how much Oral Rehydration Therapy can help in an outbreak situation?

This paper gives a brief account of one of the diarrhoeal disease outbreaks which the ICDDR,B investigation team attended very recently.

Background

On October, 1980 ICDDR,B received a telegram requesting immediate assistance to help combat a diarrhoeal disease outbreak. The following day ICDDR,B mobile outbreak team left Dacca for the affected thana which is situated in Northwest Bangladesh. Ulipur Thana, an area of 236 square miles, has a population of about 300,000 approximately 50,000 families and distributed in 17 unions.

Medical Facilities

Existing medical services are provided by a central health complex (Thana Health Complex) headed by a doctor (Thana Health Administrator), two government dispensaries, 53 field workers and 21 Thana Health Complex staff. A separate family planning division consists of 121 staff and 7 family welfare centres. The private medical care system has 6 physicians, at least 12 quacks and 12 medicine shops.

An administrative structure gets reports of health problems and deaths via village police. But there is no formalized channel of death reports forwarded to the Thana Health Administrator from the lower administrative structure or family planning field staff.

EPIDEMIOLOGICAL METHODS

Case Definition

A person in Ulipur Thana with 1) onset of diarrhoea or 2) death from gastroenteritis between October 1, 1980 and November 7, 1980.

Case Finding

A line list of all deaths from September 1 through November 7, was reviewed. Thana Health Complex logs were reviewed for diarrhoea cases. Local field staff were trained in taking rectal swabs and instructed to report active and recent cases to the ICDDR,B team who would then visit the scene for detailed epidemiologic investigation of active cases.

Laboratory

Rectal swabs were placed in Cary-Blair media or bile peptone broth. They were then plated on MacConkey agar to identify Salmonella or Shigella organism. Evnrionmental specimens were collected in triple strength bile peptone and plated on TTGA within 9 days of collection.

Therapeutic

Local field staff were trained in oral rehydration therapy and nutrition; they were provided with packets of Oral Rehydration Solution. Posters on oral rehydration were posted in conspicuous places throughout the thana. The mobile team from ICDDR,B gave I.V. fluid in the field and dispensed tetracycline as indicated. It is worthwhile to mention here that only two patients were treated with Intravenous Fluid.

RESULTS

Death surveillance revealed 39 deaths between October and November 7, 1980 and there is no gastroenteritis deaths recorded in September, 1980. Of these 39 deaths, 34 (87%) were gastroenteritis, 23 of which (67%) were females. The mean age of gastroenteritis deaths was 23.7. Eight-eight percent of the gastroenteritis deaths occured within 24 hours or less after onset of symptoms (Fig. 1).

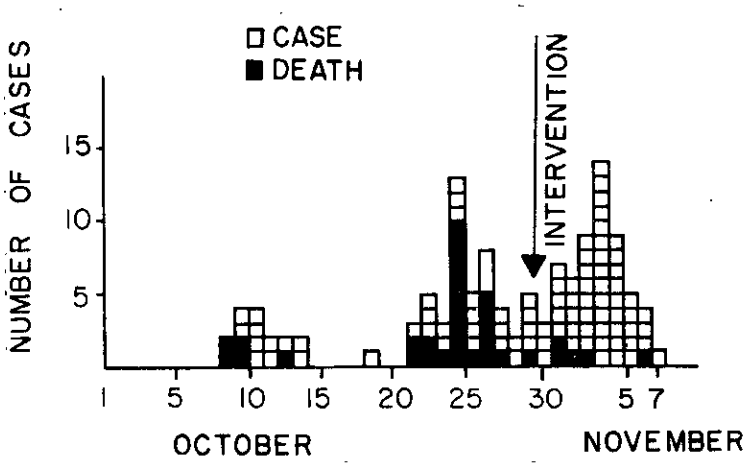

Fig. 1. Incidences of cholera in Ulipur between 1 October to 7 November, 1980. The arrow indicates the medical team's arrival.

Health complex log revealed no active or recent diarrhoea (gastroenteritis) cases. Active field surveillance identified 89 surviving cases. Of the survivors, 60.7% were females and the average age was 26.1. The attack rate for females was significantly greater than males ($P < .01$). The peak attack rate occurred in the 30-39 age group. Before the team's arrival, 31 out of the 75 identified cases died; after the team's arrival 3 out 48 cases identified, died ($P < .01$).

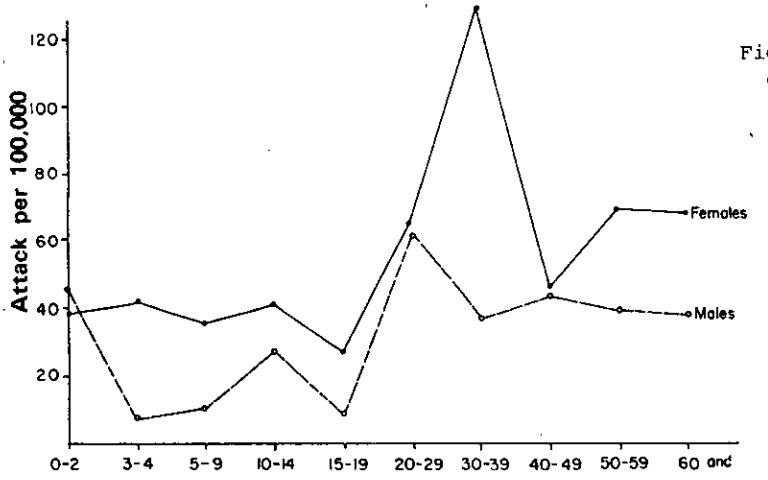

Fig. 2. Attack rate of gastroenteritis in Ulipur by age and sex between 1 October and 7 November, 1980.

Of 24 active cases cultured within one week of onset, 10 (42%) grew *Vibrio cholera* serotype ogawa. One case grew *Shigella flexneri* and one grew a non-agglutinating vibrio. Of the 5 family contacts of a *Vibrio cholera* positive index case, 1 was culture positive for *Vibrio cholera.* All vibrio organisms were tetracycline sensitive.

Only one environmental specimen was culture positive for *Vibrio cholera* serotype ogawa. It came from the pond of two cholera positive females who used the water for bathing and washing. Their water source for drinking and cooking was culture negative. Three other water specimens from culture negative cases gre non-O1 vibrios (group V). Only one of these specimens was a drinking water source.

DISCUSSION

Noteworthy is the fact that none of the cases presented to the health complex. Thus, a hospital based surveillance could not have recorded the outbreak. Active community based surveillance helped define the magnitude and extent of the outbreak as well as helped in providing early therapeutic intervention with oral rehydration to prevent death. Despite the fact that cholera cases were documented to be still occurring, fatality decreased. This underscores the value of oral rehydration therapy and its rapid dissemination throughout the community.

Developing countries often lack laboratories for the identification of cholera organisms. However, this outbreak attests to the fact that a diarrhoeal death occuring within 48 hours of onset in an adult, particularly a female, is a sensitive simple indicator of a cholera outbreak. This particular outbreak could have been recognized as early as October 11, had death surveillance for this indicator been utilized. Earlier therapeutic intervention with oral rehydration therapy may have averted some deaths.

Although epidemiologic investigation failed to incriminate a vehicle or mode of transmission, the value of the therapeutic intervention is clear. (Prevention of cases, especially when the route of transmission is unclear, is exceptionally difficult). But the number of deaths during a cholera outbreak can be reduced with Oral Rehydration Therapy. The value of cholera immunization with its limited efficacy and lag before development of immune response in preventing cases during an epidemic is not very clear. An immediate, cost effective approach to control of a cholera outbreak is dissemination of information and supplies of oral rehydration therapy packets.

EXPERIENCE ON ORS UTILIZATION IN MARIVELES MENTAL HOSPITAL

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ABSTRACT

The efficacy of ORS alone in preventing or reducing death due to dehydration in diarrhoea was compared with the use of ORS and IV, and IV along in an outbreak of diarrhoeal diseases in Mariveles Mental Hospital in the Philippines. It proves not only its effectiveness in preventing and/or reducing death from diarrhoeal diseases in an under-staffed hospital but its simple and easy administration makes it highly recommendable for mentally ill patients because the need of skilled technician or follow-up observation is not necessary. Even the janitors and hospital aides can be utilized in administering the ORS.

INTRODUCTION

The Mariveles Mental Hospital is one of the 39 government hospitals under the direct administration of the Regional Health Office No. 3. It was formerly an extension of the National Mental Hospital from 1957 to 1978. In 1978 it came under the administrative control of the Region.

It is located on a 35 hectare site on the side of a hill facing the China Sea but only about 10% of the area is actually occupied by the hospital buildings and the different facilities.

It's source of water supply is a mountain spring about 10 kilometers away from the hospital. Water is piped from the reservoir and moves to the hospital by means of gravity and pumps. The spring water is more than enough to supply the needs of the hospital, and is also shared by the people living in the town proper of Mariveles. There is no provision for water treatment.

Several septic tanks provided the sewage disposal of the hospital but since the patients are in varying degrees of mental disturbances, the patients

often dispose waste matter on the grounds of the hospital. Garbage is burned but patients sometimes throw their leftovers and garbage in any part of the compound.

The Mariveles Mental Hospital is staffed by one Physician (Chief of the Hospital), ten nurses, a pharmacist, and a number of hospital aides, institutional workers, security guards and janitors. Though the hospital has an authorized bed capccity of 700 beds, but on an average there are 950 in-patients per day almost throughout the year. The great bulk of the cases admitted are adults schizophrenics and in varying types and degrees.

There is a Rural Health Unit about 2 kilometers away from the hospital with full staff complement. These are also often augmented by rural practice physicians and nurses*.

The surrounding area is a rapidly expanding town. People began to build houses and gain access to the spring where the hospital and town proper get their water. This is the setting of the cases to be discussed.

MATERIALS AND METHODS

Attempt has been made to compare the number of deaths from diarrhoeal diseases treated with ORS alone or ORS and IV fluids or IV fluid alone. The study was conducted because of the statements of the Chief of Hospitals in a seminar on diarrhoeal diseases that they could not implement the Oral Rehydrating Solution Project in Region 3 without any proof of its effectiveness because they could not just experiment on the lives of their patients. They were taking the results of the Bacolod studies in the Philippines on the effect of Oresol on cholera cases with skepticism. During such a discussion, the Chief of Mariveles Mental Hospital ventured to offer his experience with Oresol in the diarrhoeal disease outbreak in his hospital and showed that with the use of Oresol alone all deaths could be prevented among the diarrhoea cases in the hospital.

*Rural Practice Physicians and nurses are physicians and nurses who have taken the board examination for licensure to practice but are waiting for the result of the examination. They serve in the rural areas for 4 months as volunteer workers.

Review of Cases

We studied only 67 cases. Although we would like to increase the number of subjects yet interviews among the inmates were unreliable and unintelligent because of their mental conditions, so we agreed to utilize only the subjects that were closely followed up and documented by the staff.

The diarrhoea outbreak was first observed in September 20, 1980 when a hospital staff accidentally discovered a patient in a state of shock wallowing in her watery foul-smelling stool, during the early morning round. Because of her dehydrated condition IV fluids, mainly Ringers Lactate solution was given, 10 litres in the first 4 hours. Inspite of the fluid, antibiotics (chloramphenicol) and anti-shock regimen, she died on September 20, 1980.

On September 22, another female patient was found in an evening round by a nurse to be extremely dehydrated and in shock lying on her watery foul-smelling waste. About 10 litres of IV fluid was given in four hours thereafter hydration was maintained with IV together with antibiotics and anti-shock treatment, but she died on the 27th of September.

On September 24, 1980 two markedly weak women in severe dehydration and shock were discovered during the morning rounds by the hospital nurse in the same pavilions where the previous fatalities were residing. IV fluids and antibiotics were instituted but inspite of the regimen, one died on the 26th and the other on the 27th of September. About 15 litres of IV fluids were consumed by both patients in the first twenty-four hours of treatment.

The discovery of the two cases on the 24th caused alarm among the hospital staff. Everybody among the staff was mobilized and instructed to closely observe the inmates for signs of diarrhoea or dehydration. All wards were cleaned with water and lysol solution. Beds were also washed and exposed to sunlight. Bedding was changed daily and soaked in lysol solution before laundering. Inmates were closely supervised while bathing and washing their hands before eating. Nobody was allowed to go out of the hospital so no food from outside the compound will be consumed, and everything to be eaten was properly cooked. Drinking jars with chlorinated water was provided in all the wards instead of the previous unchlorinated water from the faucets.

There were five fatalities on the 27th of September due to diarrhoeal diseases. All of them were given IV fluids consuming 10 to 15 litres per patient per day in the first day and later maintained on IV for the losses.

Towards the end of September, reports about the diarrhoeal deaths reached the Regional Health Office and the Epidemiologist designate was sent to the Mariveles Mental Hospital together with a Sanitary Engineer to find out what preventive measures were undertaken by the hospital staff. Oresol;

transport media for rectal swab (Carey and Blair media) and more calcium hypochlorite were also brought tc the hospital. Instructions on the use of the Oresol and calcium hypochlorite were given to the hospital personnel.

Rectal swabs on the diarrhoea cases and suspects were done. Water samples from the different faucets of the hospital were taken for bacteriological examinations, at the Regional Office and Bureau of Research and Laboratories. Water samples showed contaminations with faecal coli.

Oresol together with IV fluids were given to patients from October 3, 1980 to October 13, 1980. After October 13, only Oresol was given to diarrhoea patients because the hospital ran out of stock of IV fluids. The hospital staff prepared ORS and it was found to be simple to administer even to mentally ill patients.

Out of the 67 subjects, 11 patients were given only IV fluids and were in moderate to severe dehydration. Out of these 11 cases on IV fluids along, 7 died (63% fatality rate).

Out of the 67 patients with diarrhoea under study 34 were given IV and Oresol for correction of dehydration. There were no fatalities among them, although 32 were in severe and moderate degree of dehydration and two in mild dehydration.

There were 22 patients on (Oresol) oral rehydration alone, 5 were in mild degree of dehydration and the rest were from moderate to severe dehydration. There were no fatalities among these cases.

CONCLUSIONS

Based on the observation in the hospital it was concluded that the number of deaths can be reduced or prevented with the early administration of the oral rehydrating fluid, since all other conditions the patients were exposed to, and the medicines given to all the 67 patients were the same except for the difference in the type of rehydration.

From this experience we can recommend use of ORS for mental patients. The following were our observation on ORS use:

Fatality is greatly reduced or prevented.
Needs less skilled personnel to administer.
Economical - no expensive gadgets used.
Easily accessible - supply always available.
Easy to prepare.
Less care during administration.
Acceptable by patients.

BASIS FOR ORAL REHYDRATION AND SOME GROWING AREAS

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ABSTRACT

A major advance in the treatment of diarrhoea has been the use of oral glucose-electrolyte solutions to replace diarrhoeal fluid losses. Although these solutions successfully restore and maintain hydration, they do not however reduce the magnitude and duration of diarrhoea. In cholera patients one study demonstrated that a hyperosmotic oral hydration solution with a combined substrate of glucose and aminoacid (glycine) could substantially reduce the magnitude of diarrhoea compared to controls treated with conventional oral rehydration solution. A fresh look at the various substrates that enhance absorption of sodium and water may lead to the formulation of an ideal oral hydration solution. Also practical problems in terms of delivery of health care such as unstable property of bicarbonate glucose combinations and universal availability of ingredients required for standard oral hydration solutions deserve further study. A few preliminary observations on some of these areas of interest will be presented. By an invivo perfusion technique using the whole of jejunum and ileum in adult albino rats we have done a comparative study of glucose and glycine mediated absorption of sodium, potassium, chloride and water. The results show that glycine is as good as glucose in enhancing net absorption of the aforesaid substances. Addition of bicarbonate has an additive effect on both the glucose and glycine mediated enhancement of salts and water absorption. In a similar series of experiments we studied the sucrose mediated absorption of salt and water and compared it with the absorption of these substances by glucose and mannitol. Sucrose stimulates significantly higher absorption of sodium, chloride, potassium compared to those by glucose whereas net water absorption by sucrose is significantly lower compared to that by glucose. We have also done a controlled trial of acetate and bicarbonate containing standard oral rehydration solution in order to find a suitable base precursor as an alternative to the traditionally used bicarbonate for the correction of acidosis. Forty patients with moderate to severe dehydration in the age group of 4 months to 5 years were studied. Our preliminary observations suggest that acetate is as good as bicarbonate in correcting acidosis. The acetate containing solution is palatable and well tolerated. It was possible to achieve rehydration by feeding acetate containing solution with cup and spoon.

COMMON SALT AND MOLASSES WITH AND WITHOUT SODIUM BICARBONATE AS AN ORAL REHYDRATION SOLUTION IN CHILDREN

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ABSTRACT

The effectiveness of crude common salt and molasses or gur with and without addition of sodium bicarbonate as a simplified oral solution for fluid replacement in cholera and other non-cholera diarrhoeal diseases of children under 12 years of age (mean 6.35 yrs) was prospectively compared in a randomized double-blind trial. After initial standard intravenous rehydration with cholera saline, patients were randomly assigned to receive either solution by mouth. Both groups were comparable as to age and initial severity of dehydration (mean pl. Sp. Gr. 1.030). If the child failed to maintain hydration or develop serious electrolyte imbalance during treatment, we considered this as failure of oral therapy. Overall success of oral therapy in both treatment groups was about 65%. Significant differences in success rate (50% vs 88%) was observed between cholera and non-cholera patients. It has further been observed that rate of oral failure varies directly with high stool output (100 ml/kg/24 hrs). Younger children suffering from actively purging cholera are difficult to treat with either solutions. There was little correction of acidosis in salt-sugar group and sustained acidosis was found particularly related to oral failures. Although molasses contributes some natural potassium, it failed to maintain potassium balance. In fact, salt-sugar failure group showed severe hypokalaemia after 48 hours of therapy. We conclude that (1) neither solution can effectively treat all patients with high stool output (2) oral solution without sodium bicarbonate cannot fully correct acidiosis (3) potassium depletion developed in both groups but was more pronounced in the salt-sugar failure group.

RECURRENT ACUTE DIARRHOEA SYNDROME OF THE GANGES DELTA ASSOCIATED WITH MALABSORPTION

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ABSTRACT

Acute watery diarrhoea due to enterotoxigenic bacteria particularly *E.coli* is well established. However chronic colonisation of distal samall bowel by enterotoxigenic bacteria has not been reported. We have studied a group of patients who, clinically, have recurrent watery diarrhoea or liquid stool with intervening periods of constipation. These patients were found to have two or more standard parameters of malabsorption. They were intubated and small bowel fluids were sampled at various levels for microbiological studies. The selected organisms were tested for enterotoxin production. The findings and their relevance will be discussed.

CHAPTER 4

PREVENTION AND CONTROL

KNOWLEDGE AND ATTITUDES OF HEALTH WORKERS IN THE COMMUNITY REGARDING TREATMENT OF ACUTE DIARRHOEA IN CHILDREN

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ABSTRACT

The knowledge and attitudes of health workers trained on various aspects of diarrhoea was assessed by an interview schedule. Knowledge of community health volunteers (CHV) who were trained repeatedly was better than those who received minimal training. The performance of male multipurpose health workers was poorer in comparison to female multipurpose health workers: There was considerable variation amongst different categories of health workers on their ability to diagnose malnutrition. Inspite of repeated training, the health workers did not remember the signs of severe dehydration and a large number of them continued to restrict food and breast feeding during and after diarrhoea.

INTRODUCTION

In recent years a single formulation of oral rehydration solution (ORS) has been shown to be effective[1] in the treatment of diarrhoea irrespective of age and etiology[2]. As a result of the introduction of ORS, significant reduction in the mortality rate from diarrhoea has been demonstrated[3,4]. Prolonged or severe food restriction is no longer advised, and the stress on chemotherapy or symptomatic drugs is less than in the past[5]. Though this breakthrough has been achieved in the management of acute diarrhoeal disease, for successful implementation of ORS programme it should be easily available and the people should be properly advised by the health care providers on its correct use.

Health care in India is provided by a network of more than 5000 primary health centers, one in each community development block. Each primary health

center has two medical officers and a number of health aurilliaries. Recently the Government of India has launched the multipurpose health worker scheme, community health volunteer scheme and integrated child development scheme to increase the outreach of health care to the community. Under these schemes, the health auxilliaries have been trained to provide first-aid and simple medicines for the treatment of minor illnesses. The use of ORS was introduced in a community development block in Haryana State, India in 1975. This was combined with training of the health functionaries on various aspects of management of diarrhoea. The present study was carried out to determine the knowledge and attitudes of different categories of health workers regarding treatment of diarrhoea. The study has shown that repeated training is important in improving the knowledge of health workers and that the training requirements of different categories of health workers are different, which in turn, depend on their job expectations and educational background.

MATERIALS AND METHODS

Study Area

The study was undertaken in a backward community development block in Haryana State, India, with a population of 112,000 in 168 villages. Farming is the main occupation, land holdings are small and most of the land is unirrigated. About 25% of the villages are cut-off from the rest of the block during the rainy season. Only 16% of the villages have postal facility.

Health care is provided by the primary health centres located in the block headquarter village, three rural dispensaries and 17 subcentres. In addition, part-time community health volunteers and Anganwadi workers are available in the villages to improve the outreach of health care system.

Postgraduate Institute of Medical Education and Research (PGI), Chandigarh, India, adopted this block for collaborative work with Haryana State in 1974 to strengthen the health care services.

Oral rehydration solution therapy was introduced in 2 villages in the block in 1975. It is not available in all the 158 villages. The health workers have combined treatment of diarrhoea by ORS alongwith education of the community in the treatment of diarrhoea, specially in use of ORS. In the block, 94 community health colunteers (CHV), 114 Anganwadi workers (AWW), 45 multipurpose health volunteers (MPW) and 15 health supervisors have been trained on various aspects of therapy of acute diarrhoea. The training programme comprised of teaching the preparation of oral rehydration salts, constitution of ORS, recognition of dehydration and malnutrition, safety features to be observed during ORS therapy and advice to be given regarding feeding during diarrhoea. Appropriate teaching aids have been developed. All

the health workers have been trained repeatedly in small groups, using different techniques like lecture, demonstration and use of appropriate teaching aids. The teaching has been strengthened by encouraging the health workers to give ORS therapy. The teaching of health workers was undertaken in 12 different centres spread all over the block.

The knowledge of health workers was assessed using a pretested interview schedule comprising of questions on various aspects of management of diarrhoea in the community. The impact of training was evaluated by assessment of 30 CHVs before training, 31 CHVs trained on treatment of diarrhoea according to national guidelines, 30 CHVs who were trained in ORS therapy for a period of less than 1 year (a minimum of 3 teaching sessions) and 34 CHVs who have been trained repeatedly for longer than 1 year. The variation in the knowledge of ORS therapy in different categories of health workers was studied by interviewing 34 CHVs, 38 AWWs, 11 female MPWs and 9 male MPWs.

Observations

Community Health Volunteers who were trained repeatedly on treatment of diarrhoea had better knowledge about correct use of ORS as compared to those who were trained according to national guidelines of those CHVs who had not received any training. The performance of all four groups of CHVs were poor when questioned about the method of preparing home made rehydration solution (Table 1). Excessive thirst, sunken eyes, dry mouth and poor skin elasticity were commonly recognised to signify dehydration; excessive crying or drowsiness and depressed anterior fontanel were not mentioned frequently. Manifestations of severe dehydration were not remembered at all (Table 2). Community Health Volunteers with repeated training relied on ORS therapy, knew the importance of monitoring dehydration and believed that diarrhoea should be treated early. Restriction of food and breast feeding during diarrhoea was recommended less often by CHVs with repeated training compared to those who were not trained at all or were educated according to national guidelines (Table 3).

When the knowledge of different categories of health workers was compared, the performance of male MPWs was significantly poor in comparison to other categories regarding correct use of ORS therapy (Table 1). Only small percentage of AWWs and female MPWs were familiar with the signs of severe dehydration like acidosis, anuria and shock, while other workers made no mention of these signs (Table 2). While more than 50% AWWs and female MPWs could enumerate more than 3 signs of protein energy malnutrition, the knowledge of male MPWs and CHVs in this respect was poor. The importance of weight for age was not recognised by most CHVs and male MPWs. The knowledge of prevalent feeding practices was poor amongst male MPWs (Table 2). More

Type of worker	CHV	CHV	CHV	CHV	AWW	MPW (Female)	MPW (Male)
Training	Nil	As per national guidelines	/ 1 year		1 year		
Number	30	31	30	34	38	3	9
1. Composition of ORS	0(0)	2(6.4)	9(30.8)	30(88.2)	30(78.9)	9(81.8)	6(66.6)
2. Reconstitution of ORS	0(0)	3(9.6)	10(34.6)	32(94.1)	30(78.9)	9(81.8)	5(55.5)
3. Volume measure accuracy	0(0)	11(35.5)	10(34.6)	32(94.1)	36(94.7)	8(72.7)	4(44.4)
4. Quantity of ORS needed	4(13.3)	14(45.1)	17(57.1)	30(88.2)	27(71.0)	9(81.8)	3(33.3)
5. Method of preparing HRS*	0(0)	2(6.4)	9(30.8)	12(35.3)	11(28.9)	3(27.3)	1(11.1)
6. Storage of ORS	0(0)	3(9.7)	14(48.4)	30(88.2)	37(97.4)	11(100)	6(66.6)
7. Literature on ORS	0(0)	1(3.2)	15(50.0)	30(82.3)	25(65.8)	9(81.8)	6(66.6)

*HRS - Home made rehydration solution

Table 2. Knowledge about Various Signs of Dehydration and Malnutrition amongst Health Workers

Type of worker	CHV	CHV	CHV	CHV	AWW	MPW (Female)	MPW (Male)
Training	Nil	As per national guidelines	/ 1 year		1 year		
Number	30	31	30	34	38	3	9
Dehydration							
1. Thirst	4(13.2)	18(58.0)	15(50.0)	32(94.1)	15(39.5)	8(72.7)	4(44.4)
2. Sunken eyes	2(6.6)	23(74.2)	23(76.9)	32(94.1)	13(34.2)	9(81.8)	4(44.4)
3. Dry mouth	0(0)	12(38.7)	15(50.0)	32(94.1)	12(31.6)	8(72.7)	4(44.4)
4. Poor skin elasticity	1(3.3)	9(29.0)	23(76.9)	32(94.1)	17(44.7)	9(81.8)	5(55.5)
5. Excessive crying/ drowsiness	0(0)	0(0)	7(23.1)	12(35.3)	18(47.4)	9(81.8)	4(44.4)
6. Depressed anterior	2(6.6)	0(0)	8(26.9)	24(70.6)	8(21.0)	9(81.8)	3(33.3)
7. Acidosis	0(0)	0(0)	0(0)	0(0)	4(10.5)	2(81.2)	0(0)
8. Anuria	0(0)	0(0)	0(0)	0(0)	0(0)	4(36.4)	0(0)
9. Shock	0(0)	0(0)	0(0)	0(0)	0(0)	1(9.1)	0(0)
Malnutrition							
1. Names more than 3 signs	0(0)	0(0)	0(0)	1(2.9)	21(55.3)	7(63.6)	2(22.2)
2. Knows weight for age	0(0)	0(0)	0(0)	2(5.8)	36(94.1)	7(63.6)	0(0)
3. Knowledge of feeding practices	5(16.6)	10(32.2)	15(50.0)	18(52.9)	24(63.1)	7(63.6)	1(11.1)

Table 3. Knowledge and Attitudes about Treatment of Diarrhoea amongst Health Workers

Type of worker	CHV	CHV	CHV	CHV	AWW	MPW (Female)	MPW (Male)
Training	Nil	As per national guidelines	/ 1 year	1 year			
Number	30	31	30	34	38	3	9
1. Treats diarrhoea early	2(6.6)	13(41.9)	19(65.4)	18(53.0)	18(47.4)	7(63.6)	3(33.3)
2. Prefers ORS to other modes	4(13.3)	14(45.2)	25(84.6)	34(100)	36(94.7)	9(81.8)	3(33.3)
3. ORS produces vomiting	18(60.0)	12(38.7)	10(34.6)	5(14.7)	8(21.0)	1(9.1)	3(33.3)
4. Monitors dehydration	0(0)	12(38.7)	25(84.6)	24(71.9)	36(94.7)	8(72.7)	3(33.3)
5. Community demands drug for diarrhoea	30(100)	31(100)	27(90.3).	21(61.7)	24(63.1)	8(72.7)	
6. Restricts foods	24(80)	23(74.2)	14(46.1)	16(47.1)	27(71.0)	8(72.7)	7(77.8)
7. Restricts breastfeeds	28(93.3)	18(58.0)	18(53.6)	10(29.4)	14(36.8)	7(63.6)	5(55.5)

Table 1. Knowledge about Use of Oral Rehydration Solution amongst Health Workers

than 70% AWWs and MPWs advised stoppage or restriction of breast feeding during or after an episode of acute diarrhoea (Table 3). Only 33.3% of male MPWs knew the importance of monitoring dehydration.

DISCUSSION

Several studies conducted in the hospitals and in the community have demonstrated the efficacy of ORS therapy in the treatment of diarrhoea[3,4]. However, for effective reduction in mortality it is important that this mode of therapy is accepted by the people in the community. Recognising the importance, the WHO is planning a global programme for training and orientation of doctors and health workers so that they can in turn educate the people regarding correct management of diarrhoea. This study highlights the importance of repeated training and orientation of health workers in improving their knowledge because a single training session was found to be insufficient. In our set up, training was done in small groups and lecture material was backed up by visual aids, practical demonstration and opportunities for prescribing ORS. A greater stress on teaching of the skills may be particularly useful for health workers who have limited educational background.

In spite of repeated orientation and using several different techniques of training, certain deficiencies in knowledge or wrong attitudes persisted. This may be related to the difficulties in comprehension about aspects which cannot be easily demonstrated. The knowledge about manifestations of severe dehydration was poor because severe dehydration was rare in the community and hence inability to demonstrate the signs of severe dehydration. The inadequate knowledge about various aspects of malnutrition was related to the fact that CHV and male MPW do not routinely weigh the children (female MPWs and AWWs are responsible for this job) and severe malnutrition is uncommon in the community. Some aspects of deficiencies are related to prevalent cultural beliefs. Many of the health workers believed that restriction of food and breast feeding during and after diarrhoea should be practiced in spite of our instruction during training that food restriction is harmful. The widespread prevalence of these beliefs in the community has been reported. Health workers who belong to the same area where the above study was done, have similar beliefs. It is well known that beliefs are very difficult to change particularly in traditional societies. A large percentage of AWWs and female MPWs believed in restricting food during and after diarrhoea. This may be related to the influence of elders in their home. Similarly, the demand by the community for drugs in the treatment of diarrhoea appears to be deep rooted and reflects the belief that only drugs can cure illnesses. This is likely to be removed by concerted efforts by the health care providers which aim at removing harmful beliefs.

Different categories of health workers showed considerable variation in their knowledge about certain aspects of management. Male MPWs poor performance

may be partly because they could not accept therapy of diarrhoea as a part of their overall job description and partly because they have not been adequately prepared to treat illnesses or symptoms other than malaria. The knowledge of CHVs and male MPWs regarding signs of malnutrition and weight-for-age criteria was poor because they probably considered this to be the job of AWW or female MPW and therefore did not pay enough attention to this aspect during their training. The better performance of female MPWs is partly related to their longer pre-service training (1½ years - 2 years as compared to 6-16 weeks for others) and also because they are expected to treat minor ailments.

Future training programmes for health workers should provide for repeated training and orientation. Prevalent beliefs of the community in their training programme will have to be given a prominent place in training of health workers to overcome harmful beliefs and practices. The present study also highlights the importance of training different categories of health workers according to their previous educational background, training needs and job expectations.

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Discussion

Dr. Nigar Shahid

Q. Who were the CHWs and how were they accepted in the community?

A. They are part-time workers. They are well accepted by the community for treatment purposes but not so well accepted for preventive aspects or environmental sanitation measures.

Dr. Deb

Q. In one of the slides, you have shown that CHW couls identify dehydration better than CHW. How do you explain that?

A. CHWs were trained in 1977 but MPW (M) orientation in 1975. CHWs were trained for 3 months, MPW (M) for a few days to 6 weeks.

Dr. Eusof

Q. Do you have a standard teaching module for the community health worker? How was the training impact evaluated?

A. Two teaching sessions were prepared. The questionnaire was pretested, 42 questions were administered. They were mostly structured for specific responses to measure the impact of training.

Comments: Dr. Greenough

Some current practices in Matlab households which have mothers with an education upto sixth class must be very effective since there is a much lower mortality among children in such households. We may gain important clues for health education by investigating practices in such families.

PARDĀ AND SOME HEALTH PRACTICES IN TWO CONSERVATIVE RURAL COMMUNITIES OF BANGLADESH

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ABSTRACT

This article, based on field observation and indepth interviewing in two rural communities of Teknaf *thānā* of the coastal area of Bangladesh, focuses on *pardā* and some health practices with a special reference to defaecation habits and water use pattern. Viewing defaecation habits and water use pattern as an expression of social norms, both women and men are analysed from the point of view of health practices. This analysis has shown that the practice of *pardā* has been responsible for differential defaecation habits and water use pattern among males and females. The study revealed that the males defaecate following rising from the bed in the morning. The timing for such defaecation may be either before sunrise or following sunrise according to his need. But women defaecate immediately following sunset and sunrise. For the sake of the observance of *pardā* women since their childhood develop the habit of defaecation following sunset or before sunrise. Indepth interviewing revealed certain unconventional practices of hasty defaecation and cleaning of bottom, when emergency need for defaecation by women arose during day time. It was found that for the sake of observance of *pardā* women mainly carried water from the tubewell following sunset and before sunrise. When there was need for water during day time women had to frequently depend on their minor children for carrying water from the tubewell. Observation revealed that the children were much less careful than the women in maintaining cleanliness of water from water collection point to home.

INTRODUCTION

The World Health Organization has adopted the goal of health for all by the year 2000[1,2]. This goal has been subscribed by the government of Bangladesh also. The programme of health for all by 2000 includes the

provision for an adequate supply of safe water and basic sanitation. In two rural communities of Teknaf *thana* in Bangladesh observations were made on sources and uses of water, and defaecation habits. Some field observations and indepth interviewing were made to investigate the role of *pardā** in relation to water use and defaecation practices. This study has policy implications in respect of implementation of programmes for adequate supply of safe water and basic sanitation for the conservative rural communities which strictly adhere to the observance of *pardā*. Traditionally the practice of *pardā* required women to be confined within the four walls of the home[3,4]. Even today in the study areas *pardā* imposes such confinement to the homestead since women are permitted to go out of the house mainly following sunset and before sunrise.

The two study communities are situated in Teknaf *thānā* in the coastal area of Bangladesh. The people of the study areas depend entirely on ditch and tubewell water for all purposes. Most of the households have no fixed latrine (Table 1).

The study communities have got a population of 4,242. They are almost entirely Muslim. The major occupation in one community is fishing, and that in another fishing and agricultural activities. The people of both the communities are mostly illiterate (Table 2).

METHODS

The methods in this study were field observations as participant observer and indepth interviewing with a special reference to water use and defaecation habits. Ten males and 10 females were selected based on purposive sampling for indepth interviewing. Only willing persons were interviewed. Women who crossed their childbearing age volunteered for interview. The observations and interviews were carried out during the end of 1980 and early 1981.

RESULTS

In the study communities it was observed that women adhere to strict *pardā*. It was found that during day time women passed most of their time inside the dwelling houses. The dwelling houses mostly have bamboo walls of low height and leaf roofs. Most of the houses have no windows and have one

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*Seclusion of women from male strangers

Table 1. Place of Defaecation for Males and emales above 5 Years of Age in the Study Communities

Sex	Total No. of Households	Water Seal Latrine %	Fixed Open Pit %	Bush %	Bank of River %	Open Field %	Not Reported %
Male	685	4.4	6.7	63.7	9.8	12.7	2.6
Female	685	5.7	10.7	67.6	9.5	6.0	0.6

Table 2. Literacy Level in the Study Communities by Age and Sex

Age Group	Male				Female			
	Total No.	No Education %	Maktab %	Primary+ %	Total No.	No Education %	Maktab %	Primary + %
All ages(5+)	1701	62.6	29.8	7.6	1704	62.2	36.9	0.9
5-9	310	55.8	40.3	3.9	309	46.9	51.8	1.3
10-14	318	50.3	40.3	9.5	304	43.0	55.9	1.0
15-24	364	67.0	14.2	8.7	399	61.6	37.6	0.8
25-44	415	66.5	24.1	9.4	414	72.5	76.3	1.3
45+	294	71.8	22.4	5.7	278	85.6	14.4	-

to two doors. The dwelling houses are divided into male and female sections. In some of the houses the female section has a door leading to a small backyard mainly used by females. Dwelling houses are usually dark inside particularly the women's sections. Often the kitchens are situated in the women's sections. This section is also used for eating and storing of drinking and cooking water. The homesteads consist of one or more households and are surrounded by high bamboo walls with a gate. Males outside of the family cannot enter the homestead without prior permission. Such entry is not permitted when male members are not present. When male outsiders are within the compound of the homestead the women strictly remain inside the house and keep silent so that their voices cannot be heard by the visitors.

It was observed that women usually do not carry water during the day time. Due to *pardā* they fetch enough water to last during the day before sunrise, and also after the sunset. If the water runs out during the day time, then they ask the children to fetch water either from the tubewell or the ditch. It was observed that when the children carry water they do not pay particular attention to cleanliness.

It was found that sometimes elderly women fetched water during day time when no males were around the water source. In such cases water is collected quickly as a precaution against breaking *pardā*. The males and children take their bath at the water source. The female do not do it there.

It was observed that most of the defaecation sites for women were located in the nearby bank of the river and bushes. The defaecation sites for males were located in similar places further away. The defaecation sites showed that it was done in different spots indiscriminately. It was found that there were defaecation sites for women within the homestead compound.

Indepth interviewing revealed that there were different time schedules for water fetching and defaecation for male and female which are linked with the observance of *pardā*. Most of the respondents stated that females fetch water and have their bath either before sunrise or after sunset. They stated that some women have their bath during day time in a fenced place located inside the homestead. All the respondents stated that due to the practice of *pardā* children are frequently utilized to fetch water during day time. They also stated that as a consequence of *pardā* resulting in restricted movement of the women during the day they use far less water for domestic and personal purposes than is actually required.

Most of the respondents stated that women usually defaecate after sunset and before sunrise. They stated that women have defaecation sites near their houses which were generally surrounded by bushes. They said that men go for defaecation further away from the women's defaecation sites.

It was stated that generally men defaecate after waking up in the morning and the timing for the defaecation may be either before sunrise or following sunrise according to their natural requirement.

Most of the respondents stated that women usually go in groups for defaecation either before sunrise or after sunset with a pot of water each, which holds one-third to half a gallon of water.

Some respondents stated that when women felt the need for defaecation during day time 'they try to hold it with difficulty' till sunset. Sometimes such women skip lunch so that they can delay the movement of the bowel. Skipping lunch for these reasons sometimes occur several times in a month for most women. When they fail to hold the motion they defaecate hastily in the backyard.

DISCUSSION

The study communities are prone to frequent incidences of dysentery and diarrhoea. It has been found out that most of the dysentery cases were caused by shigella. A study by Rahaman and Aziz[5] in and around the study area showed that during 1976 to 1978, 5,283 patients were treated with diarrhoeal symptoms. More than 80% of these patients gave clinical history suggestive of dysentery and the remaining were patients with diarrhoea. Among these patients 1,863 isolation of various serotype of Shigella were made which is 35% of the total dysentery and diarrhoeal cases. Whereas in 1968 Khan and Mosley[6] reported that shigellogis accounted for 4.4% of all grades of diarrhoeas based on results from an intense community study in Dacca. The significantly high incidence of shigella in and around the study communities are related to scarcity of water for personal and domestic purposes. The findings of this paper give some evidences of relationship between Shigellosis and scarcity of water in the study area and that the strict observance of *pardā* has made the situation worse.

In the study communities women cook inside the house which is dark and offers limited visibility while washing, preparing food or serving. Due to the strict observance of *pardā*, the children frequently fetch water. These children often do not clean their hands adequately following defaecation after cleaning their bottoms.

At the time of defaecation a pot of water is carried to wash the bottom and hands afterwards which is not sufficient for proper cleaning. Proper washing of hands before eating and handling food is also not done frequently mainly due to the scarcity of water at home. Moreover, when one defaecates in haste during the day thereis little scope for proper washing of hands after washing the bottom. Last but not the least we want to emphasize here

that this preliminary study on the influence of *pardā* on health habits indicate the need of further research on the behavioural aspect of the custom of *pardā*.

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INTERVENTION OF SHIGELLOSIS BY HAND WASHING

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INTRODUCTION

Shigellosis though a world wide problem is a great concern of most of the developing countries. It spreads very easily among the contacts. Secondary infection rates of shigellosis varies from 15-33%. The death rates under hospital treatment ranges between 5 and 15%. Immunization has not been found promising. Resistance of *Shigellae* to multiple antibiotics is building up steadily. A study was therefore essential to find out some sort of simple intervention.

We decided to use a simple procedure of washing hands with soap and water after defecation and before meals to attempt to prevent the spread of shigellosis. Health workers were employed to educate families and maintain surveillance to make sure that people washed their hands after defecating and before eating food.

METHODS

In this prospective study, study and control groups were all culture confirmed shigellosis cases from the ICDDR,B clinic, matched for age, sex and socioeconomic status. The cases were alternately selected for both study and control groups.

The study families were provided with 2-4 pieces of soap and 1-3 water pitchers for storing enough water and were urged to wash their hands after washing the anus following defecation and before taking any food. Quantity of soap and left over water were checked daily by observing the size of soap and measuring the water to determine usage. A further 50 families provided with soap only and another 50 with water containers only were also studied to compare the efficacy of soap and water independently. Control families were not provided with any intervention.

Rectal swabs of family contacts of study and control cases were obtained daily for 10 days for culture. Left hand washings were cultured in gram negative broth (G.N. broth). Domestic water was cultured after millipore filtration. A history of dysentery was obtained from cases and contacts.

The differences in secondary infection and case rate between study and control groups were tested for statistical significance by the Chi Square test.

RESULTS

Table 1 presents data on the essential comparability of the groups under study. We have used family size, crowding, income, water and latrine use pattern as indexes. The data shows that the groups were quite comparable.

The age specific secondary infection rates among the family contacts are shown in figure 1. The overall infection rate was 10.1% for the study and 32.4% for the control group. Total reduction in secondary infection was 67%. This difference was highly significant ($P < .01$).

The overall secondary case rate was 2.2% for the study and 14.2% for the control group. The overall reduction in secondary case rate was 84%. This difference was also highly significant ($P < .01$).

The effectiveness of the intervention in the case of different types of *Shigellae* is seen in figure 2. *Shig. flex.* could be reduced by 67%, *Sh. dyst.* type I by 33% and other *Shigellae* by 87%. Even if the possible co-primary positive isolates were excluded the difference was statistically significant.

The relation between quantity of water used per person per day and rates of secondary infection is shown in Table 2. In the study group, the secondary infection rates were independent of the amount of water used for drinking and cooking. But were associated with the amount of water used for bathing and washing ($P < .01$). This association did not persist when all were combined. Among the controls however, there was no association with the amount of water used and secondary infection rates.

Rates of infection in families who were provided with only soap or water pitchers are shown in figure 3. The *Shigellae flexneri* secondary infection and case rates for the soap using group were significantly lower than the water using group ($P < .01$). There was no significant difference for *Sh. dyst.* type 1 in this regard. This might be due to small number of families in this group.

Out of the 579 study and soap group individuals whose left hand washing were cultured none was positive for *Shigellae*. Out of the 617 control and water group individuals 7 were positive for *Shigellae*. This was significant ($P < .025$).

TABLE I--STUDY AND CONTROL GROUPS COMPARED

Groups	Household population excluding index	Percent ≤ 5 yrs.	Average room/ family	Average member/ family	Average member/ room	Drink TW/Tap water	Used mixed sources of water for domestic use	Used Sanitary latrine
Study (Soap + water)	279	23.3	1.56	5.6	3.1	94%	26%	36%
Control (no soap or water)	318	24.8	1.78	6.4	4.0	98%	20%	42%
Only soap	300	21.6	1.58	6.0	3.7	92%	32%	50%
Only water	299	23.1	1.72	6.0	3.3	93%	28%	56%

Fig 1. Age specific subsequent infection rates in study and control groups in percent

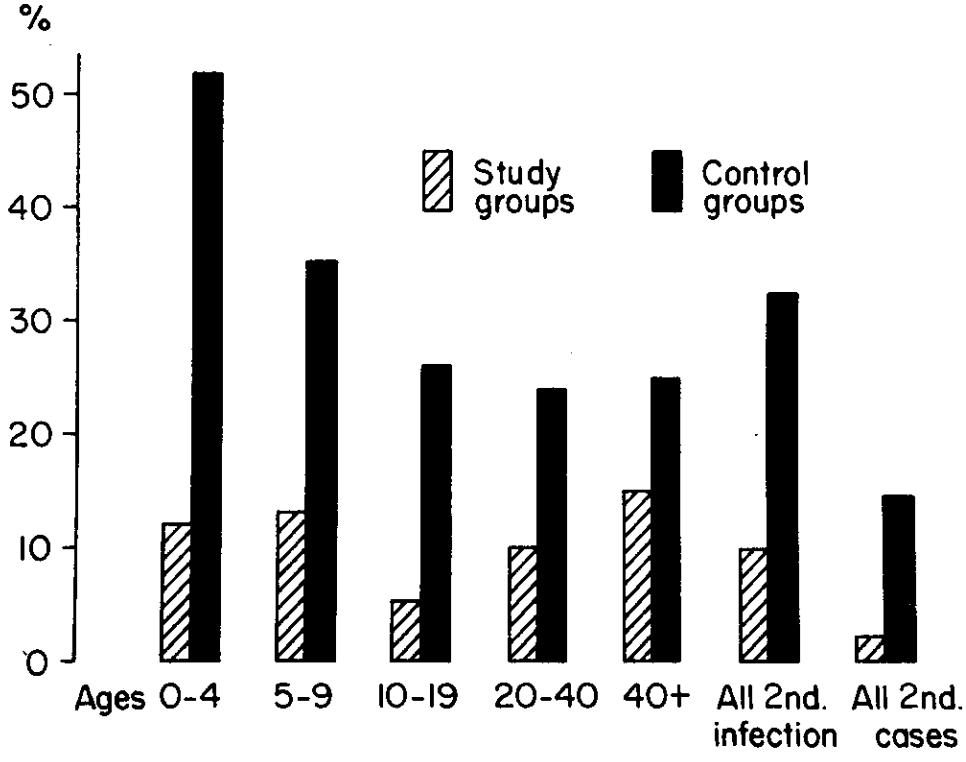

Fig 2. Secondary infection rates in study and control groups by types and percent of Shigellae

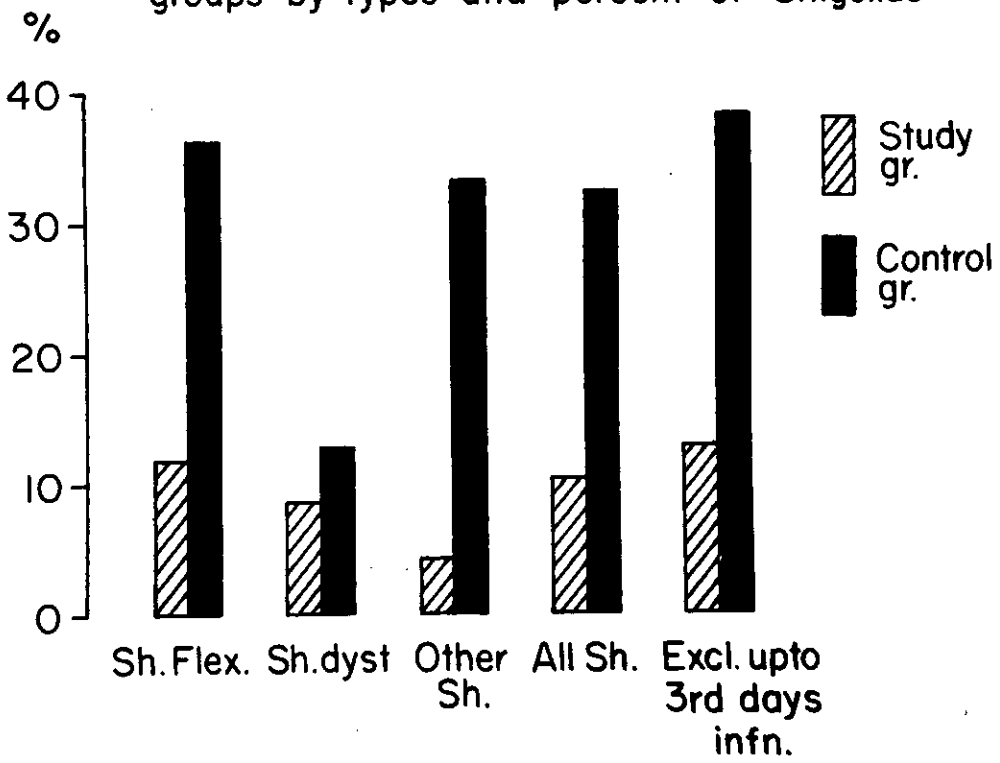

TABLE II--SECONDARY INFECTION RATES ACCORDING TO QUANTITIES OF WATER USED

Average quantity of water used	Study			Control		
	No. of contacts cultured	No. infected	Secondary infection rate	No. of contacts cultured	No. infected	Secondary infection rate
Drinking $\leq$ 4.5 kg. and Cooking $\geq$ 5.5 kg.	25[a]	2	(8.0)	101[e]	35	(34.6)
	165[b]	7	(4.2)	50[f]	15	(30.0)
Bathing $\leq$ 20 kg. and Washing $\geq$ 25 kg.	41[c]	6	(14.6)	95[g]	21	(22.1)
	66[d]	1	(1.5)	21[h]	3	(14.3)

a vs b N.S. e vs f N.S.

c vs d (P <.01) g vs h N.S.

Fig 3. Secondary infection of Shigellosis in groups who used only soap and only water in percent

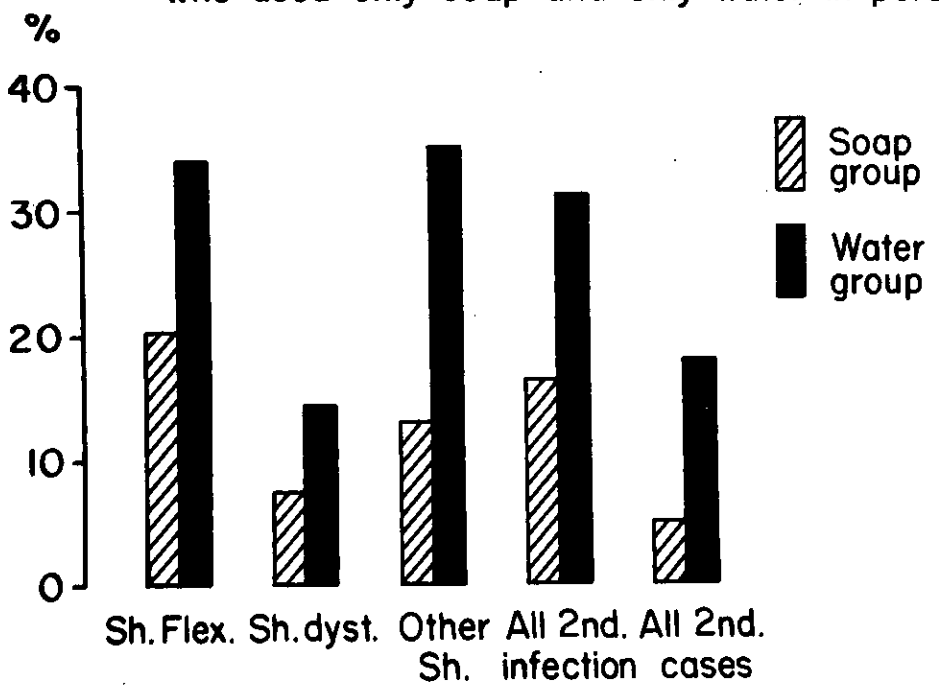

Table 3. Incidences of Diarrhoea of Unknown Origin

Groups	No. of contacts who had diarrhoea of unknown cause	No. of contacts who had no diarrhoea of unknown cause	No. of Total
Soap + water group	16[a]	213	229
Control group	30[b]	238	268
Total	46	451	497

X^2 of a vs b = 2.6 = N.S.

In the study group 7% contacts and in the control group 11.2% contacts developed diarrhoea of unknown etiology (Table 3). The differences (37%) were not significant, but if the trend was maintained a larger sample size might reveal a significant difference.

DISCUSSION

The occurrence of shigellosis is directly related to sanitation. Still even in developed countries it has yet to be eradicated.

Practical solutions are needed when proper sanitation is not feasible and immunization does not help. Antibiotics are expensive and changing resistance patterns makes drugs less efficacious. People use surface water which may transmit the organisms of diarrhoeal disease. Hand pump tubewells for drinking alone did not help in reducing diarrhoeal disease and cholera since other sources are used for bathing and washing. Although shigellosis is said to be a water wash disease, this study could not prove it. However, much larger quantity of safe water may have some effect.

Handwashing seemed an effective and practical alternative. It is most effective in vulnerable younger groups possibly because mothers feed them. Adults often go out and do not have soap available for washing. The effects were most significant in all *Shigellae* species except *Shigellae dysenteriae* type 1. Minimum difference in the case of *Sh. dyst.* type 1 might be due to smaller number of cases, its greater virulence and smaller dose requirement for infection. Diarrhoeas due to other causes were also reduced (37%) by the use of soap and water.

The results of the hand cultures show the distinct possibility of contamination of food or the right hand.

We conclude that adoption of this intervention, would result nearly in 80% reduction of hospitalization of dysentery cases due to shigellosis and a 37% reduction in occurrence of other diarrhoeas as well as significant savings. Pre-requisites are proper motivation and easily available soap. The practice needs to be implemented as a part of the national diarrhoeal disease control program.

Discussion

Brigadier Chowdhury

Q. What type of soap was used? Which hand i.e. right hand or left hand was washed, were there any difference in the isolation rates?

A. Washing soap was used. Only left hand was washed.

O. P. Ghai (Comment)

Some unexpected and disturbing finding in an old epidemiological study carried out by us showed a higher prevalence of diarrhoea amongst children of school teachers of the village compared to other categories. In the houses where cattle was in the midst of the house had less infection. There was relationship between diarrhoea incidence and nutrition but no such relationship could be found with sanitation.

Dr. G.H. Rabbani

Q. Why was the difference found in *Shigella dysenteriae* not significant compared to *S. flexneri infection?* What is the definition of secondary case?

A. In shigella dysentery, even a low dose can produce significant infection, higher virulence may be the cause for this lack of 7 significant difference. Symptoms of dysentery absent in the family contacts of index cases but shigella could be isolated.

Dr. Deb

Q. How do you assure that soap is being actually used? It may have been used for other purposes?

A. Three day observation showed that soap was actually used and that the amount of water leftover in the pitchers was recorded.

Nigar Shahid

Q. Have you considered the use of "ash" as soap, is it still very expensive?

A. Study was done in an urban setting where ash is difficult to come by, gas and kerosene is used as fuel.

VARIATION IN GLUCOSE AND ELECTROLYTE CONTENT OF ORAL REHYDRATION SOLUTIONS PREPARED AT HOME

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ABSTRACT

As part of the follow-up substudy of the 1979-1980 Matlab field trial of home-prepared oral rehydration solution (ORS) 880 samples of ORS actually being used by patients were collected. Four hundred and fifty-nine of these samples had been prepared from packets provided by ICDDR,B; the rest had been made using local sugar (gur) and salt (lobon) measured using a special spoon. Glucose, sodium, potassium and bicarbonate contents were measured using standard laboratory procedures. The contents of most samples of both types of solutions were within acceptable limits, although there was more variation in the contents of solutions prepared from lobon and gur. There was no apparent trend in the accuracy of solution preparation during the two years of the study.

INTRODUCTION

Oral rehydration solution (ORS) prepared and used in the home has been widely advocated as a simple, cost-effective and efficient way to reduce morbidity and mortality from diarrhoea[1]. One question about this approach, however, is whether individuals without special medical training, who in fact may be illiterate, can prepare the solution properly[2]. A second issue is whether solution can be prepared as accurately from local sugar and salt as from the more expensive packets. The field trial of oral therapy conducted by the International Centre for Diarrhoeal Disease Research provides some data to answer these questions.

METHODS

The field trial was conducted from January 1979 through December 1980 at the ICDDR,B Matlab field station.

Two "treatment areas" were designated, each with a population of about 40,000; in one WHO-formula packets were provided and in the second, crude brown sugar (gur) and salt (lobon) with a special measuring spoon. One woman from each bari-clusters of about 6 households belonging to an extended family was chosen by the ICDDR,B community health worker to be the "bari mother" and was trained to prepare ORS using either the packet or the spoon-lobon-gur method, and given basic education about diarrhoea and dehydration.

As follow-up, supervisors visited the bari-mothers and observed while they made the solution, and corrected any failure of technique. This spot-checking was continued, though at a lesser frequency, throughout the study.

All bari mothers were given a used 1-litre intravenous fluid bottle to measure water. In addition, women in the lobon-gur area were given special two-ended measuring spoons. Supplies of lobon, gur and packets were replenished regularly during the female village workers' biweekly visits. Lobon and gur were purchased in bulk from local suppliers. Packets were prepared in Matlab from locally purchased glucose and salts. Ingredients were first weighed and mixed in quantities sufficient for 100 packets, then the amount sufficient to make 1 litre of solution was measured volumetrically and sealed in plastic polythylene bags with a spirit lamp. All supplies were free to bari mothers - patients were not charged for ORS.

The sample studies were collected by diarrhoea surveillance workers who visited each household bi-monthly from ORS prepared by bari-mothers for treating diarrhoea patients. They collected 6-ml samples in screw-capped vials. Samples were brought to the Matlab Treatment Centre within 4 hours and refrigerated until taken to Dacca for analysis.

The longest interval between collection and analysis was 4 days. Standard methods were used for analysis. Flame photometry was used for sodium and potassium. Glucose was measured by the Nelson and Somogyie method[3]; before this measurement lobon-gur solutions were hydrolized in hydrochloric acid for 2 hours. An IL analyser was used for chloride and bicarbonate determinations. Unfortunately, this machine cannot measure bicarbonate concentrations of less than 8 mmol/litre with accuracy. The laboratory participants in the WHO international quality control scheme; the performance for electrolytes and glucose is good. In addition, check samples were analyzed - at least 2 samples per lot.

RESULTS AND DISCUSSION

Four hundred and fifty-nine samples were collected from patients in the packet area, and 421 from patients in the lobon-gur area. Because they were collected on opportunity by the diarrhoea surveillance worker, they do not constitute a random sample, although probably they are fairly representative. There are about 1400 baris in the treatment areas: (determined by checking the census numbers of patients providing the solutions against demographic records) the samples analysed came from 669 baris, about half providing more than one sample. Thirteen patients provided two solutions each during the two years of the study.

The 459 samples from the packet area had a mean glucose content of 101.5 ± 23.4 mmol/litre, and a mean sodium content of 84.3 ± 20.6 mmol/litre; the corresponding values for lobon-gur samples are 119.0 ± 46.0 mmol/litre for glucose and 96.0 ± 34.4 mmol/litre for sodium (Table 1, Table 2). The mean chloride content of the lobon-gur solutions is very close to the sodium values 95.1 ± 33.2 mmol/litre, while in the packet samples it is much lower - 71.5 ± 18.3. As expected, there is more bicarbonate in the packets (26.8 ± 7.3 mmol/litre) than in lobon-gur (8.8 ± 5.4); in fact, it is surprising that there is any bicarbonate in lobon-gur, which probably comes from bicarbonate added during processing of the gur. Because gur also contains a fairly large, though not constant, amount of potassium, there is almost as much potassium in the lobon-gur samples (14.1 ± 6.3) as in the packet samples (17.3 ± 5.1 mmol/litre).

Table 1. Glucose and Electrolyte Concentration (mmol/litre) in Samples of ORS Collected from Patients' Homes - Packet

	Glucose	Sodium	Chloride	Bicarbonate	Potassium
WHO Formula	110	90	80	30	20
Packet					
N	459	459	459	457	459
Mean	101.5	84.3	71.5	26.8	17.3
SD	23.4	20.6	18.3	7.3	5.1
CV%	23	24	26	27	29
Median	104	85	71	27.2	17
Number high (%)*	-	11(2.4)	11(2.4)	-	-

*Defined as > 120 mmol/litre of sodium

Table 2. Glucose and Electrolyte Concentration (mmol/litre) in Samples of ORS Collected from Patients' Homes - Lobon-Gur

	Glucose	Sodium	Chloride	Potassium
WHO Formula	110	90	80	20
Lobon-Gur				
N	421	348	421	348
Mean	119.0	96.0	95.1	14.1
SD	45.0	34.4	33.2	6.3
CV%	38	36	35	45
Median	117	93	92	14
Number high (%)*	-	31(8.9)	37(8.8)	-

*Defined as > 120 mmol/litre of Sodium or Chloride.

Frequency histograms in 5 mmol intervals of sodium, glucose and potassium confirm the greater variation in the contents of lobon-gur solutions (Figures 1-3). For sodium, both types of solution show fairly well-defined peaks, but for glucose and potassium the lobon-gur curves are flatter than the packet curves. This reflects not only the variation in measuring the gur, but also the normal variation in the glucose and potassium content of gur.

The mean amounts of all constituents in the packet mixtures are slightly lower than those recommended by WHO; while in the lobon-gur samples the mean values for glucose, sodium and chloride are slightly higher, the potassium slightly lower and the bicarbonate much lower than the recommended ones. The variation in the solution contents of the major ORS constituents - glucose, sodium and chloride - as measured by the coefficient of variation (SD÷mean) is very stable within solution type: 23-26% for packet and 35-38% for lobon-gur. This is expected for the packet with premeasured constituents, the stability for the lobon-gur solutions suggests that the same type and amount of heaping or packing occurs at both ends of the spoon.

Solutions were defined as "high" if they contained more than 120 mmol/litre of sodium, or in the case of lobon-gur solutions more than 120 mmol of sodium or chloride. Using this criterion there were significantly more high lobon-gur (37,8.8%) than packet solutions (11,2.4%; difference significant at $p<<.00005$. X^2 test). Of all the 11 high packet solutions, 7 probably represent dilution problems, that is, all constituents are high. In the remaining 4 (sodium 122, 125, 127 and 156 mmol/litre), glucose concentrations are not proportionately high (128, 128, 119 and 113 mmol/litre respectively). As the

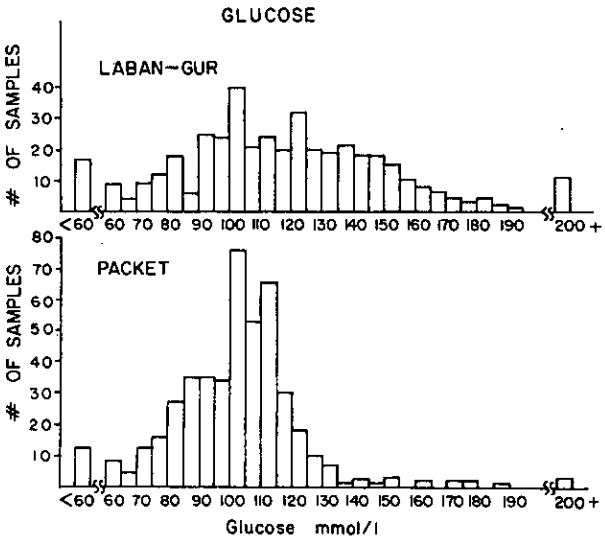
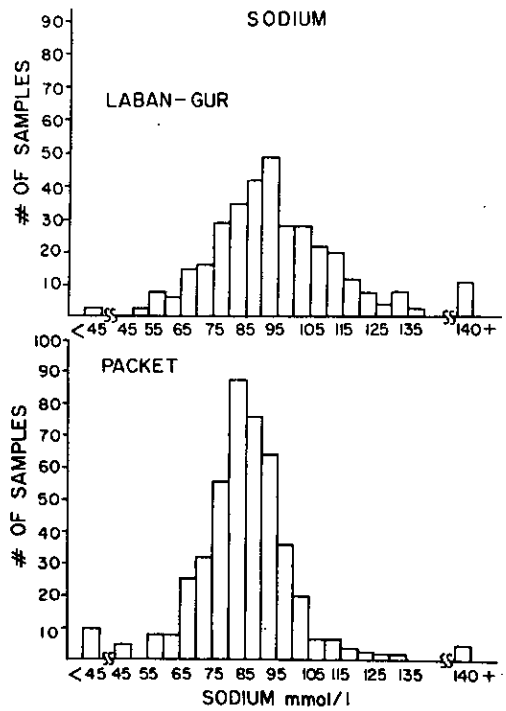


Fig. 1: Glucose concentrations (mmol/litre) of samples of ORS collected from patients' homes.

Fig. 2: Sodium concentrations (mmol/litre) of samples of ORS collected from patients' homes.



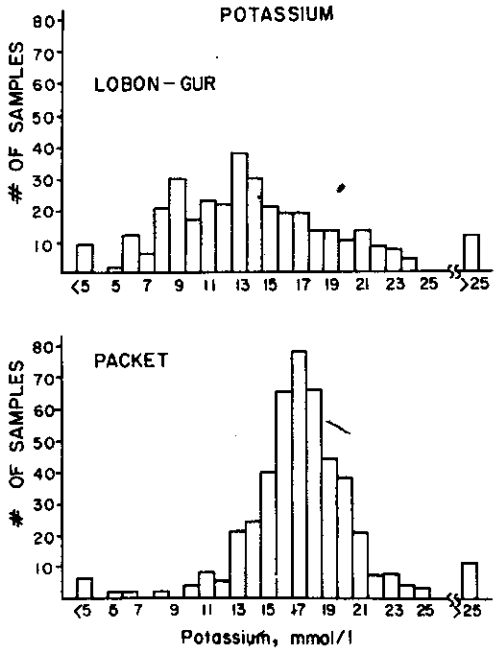


Fig. 3: Potassium concentrations (mmol/litre) of samples of ORS collected from patients' homes.

other constituents are also close to the standard concentrations, this suggests either an error in packet preparation extra salt added by the mother, or solution made from ingredients not supplied by ICDDR,B. In addition, one solution from the packet area contained adequate amounts of all constituents except glucose and two solutions were received which are essentially water. We are currently examining quality control records for the packets, and will report results elsewhere.

Of the 37 high lobon-gur solutions, 12 probably represent dilution problems; in 5 it seems likely that the wrong end of the spoon was used to measure salt. It must be noted that no hypernatremia attributable to ORS use was detected clinically or in serum samples either in these patients or in those coming to Matlab Treatment Centre from the trial area.

The plot of monthly means and standard deviations (Figure 4) for glucose also show that packet concentrations tend to be low. It is interesting that

this seems to be greater during the humid monsoon season. It is possible that this reflects packet ingredients sticking to the packet and not getting shaken out into the water. The packet glucose values may tend to be low overall because the glucose used is not anhydrous. Examination of the distribution of solution means and standard deviations over time demonstrates no apparent overall trend in either accuracy or precision of solution preparation.

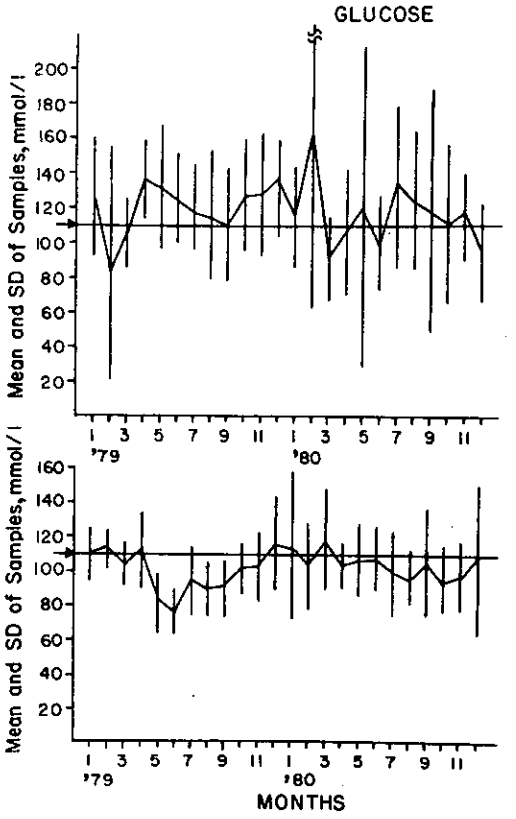


Fig. 4: Glucose concentrations (mean ± 1 standard deviation) of samples collected during each month. The top panel represents lobon-gur samples; the bottom panel, packet samples.

CONCLUSION

From these results it is clear that rural women when properly trained and given adequate measures can prepare ORS accurately, and maintain this accuracy for two years. There is more variation in the glucose and electrolyte contents of solutions prepared using the spoon-lobon-gur technique; some of this is due to measurement variations, but some is probably also attributable to variation in the normal constituents of the ingredients, particularly the gur. For both techniques, dilution problems were a factor in high sodium solutions. The proportion of high-sodium solutions is, from clinical and serum Na^+ evidence, apparently not a problem. Choice between the two techniques must take into account other factors such as access, cost and acceptability.

A STUDY OF ACUTE GASTROENTERITIS IN CHILDREN IN KUWAIT

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This study was done in the paediatric department of Al Sabal Hospital, Kuwait. One of the main General Hospital with a Paediatric Department consisting of 9 wards.

In 1980, there were 16814 children admitted in the paediatric ward out of these 2974 were due to acute gastroenteritis (Table 1) i.e. 18% of total admission or nearly one fifth of the total number. This constituted the largest single cause of admission. Out of the 2974 cases, 500 cases have been analysed(almost every fourth case was analysed).

Table 1. Paediatric Department, Al-Sabah Hospital, Kuwait, 1981

Total Admissions	Gastroenteritis	% with GE
16814	2974	18%

Table 2 shows the overall mortality from enteritis. It can be seen mortality was also very high from gastroenteritis.

Table 2. Mortality

Total Mortality	%	G.E. Mortality	%
341	2.1	89	3

Table 3 shows the mortality rate, this was low in male patients but higher in females. This is considered to be due to the fact that female patients are brought to the hospital in a late stage of the disease.

Table 3. Mortality

Total	Male	Female
89	35 39.32%	54 60.67%

Table 4 shows that the cases were divided into 3 groups depending upon the duration of symptoms. The largest group was in children who were brought to the hospital on the 1st or 2nd day of the onset of symptoms.

Table 4. Duration of Symptoms

< 3 Days	3-7 Days	> 7 Days
215 43%	192 38.4%	93 18.6%

Table 5 presents the results of the stool cultures.

Table 5. Stool Cultures

Positive	138 = 27.6%
Sterile	187 = 37.4%
Results not Received	175 = 35%

Organisms:

Salmonella	= 14.4%
E.coli	= 8.4%
Shigella	= 4.8%

Table 6 shows that a very high percentage of these children are bottle fed and the practice of bottlefeeding directly co-relates with incidences of diarrhoea among the under 6 month old babies.

Table 6. Feeding

Total No.	232
Bottle fed	201 i.e. 86%
Breastfed	31 i.e. 14%

Figure 1 shows the seasonal incidence of gastroenteritis very clearly. Two distinct peaks can be seen one in April-May and another in October-November.

Fig. 1

SEASONAL INCIDENCE

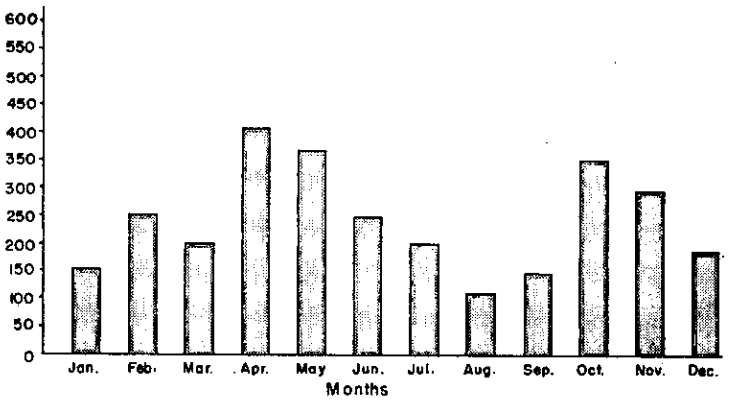


Figure 2 represents the age incidence of G.E. highest peak was among the 6 month olds, which falls by the age of 2 years. This becomes negligible at 3-5 years. When analysed, patients over 5 years reporting to the hospital with gastroenteritis after 5 years of age were almost all due to food poisoning with history of having ingested contaminated food, other members of the family also being affected. Symptoms generally start with abdominal pain and vomiting after a few hours of food consuption. It is of interest to note that such incidences in babies aged below one month is very low (babies under 10 days not included in this study) by the age of three months, the incidence of G.E. rises.

Fig. 2
AGE INCIDENCE OF GASTROENTERITS

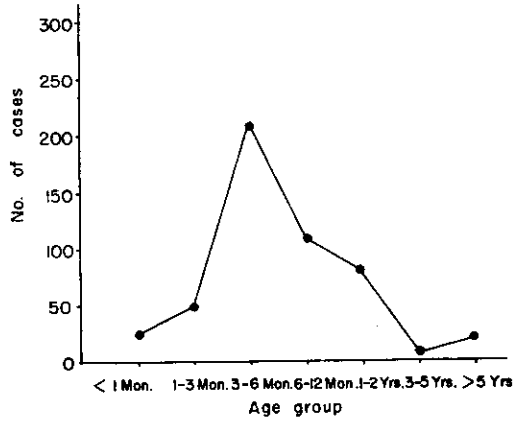


Figure 3 presents the symptoms of the hospitalized cases

Fig.3
PRESENTING SYMPTOMS

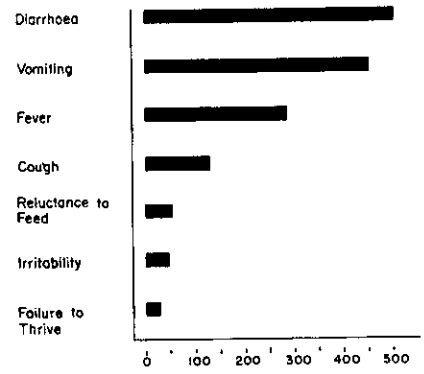


Figure 4 presents the associated complications seen among the study patients.

Fig. 4

ASSOCIATED CONDITIONS INCIDENCE

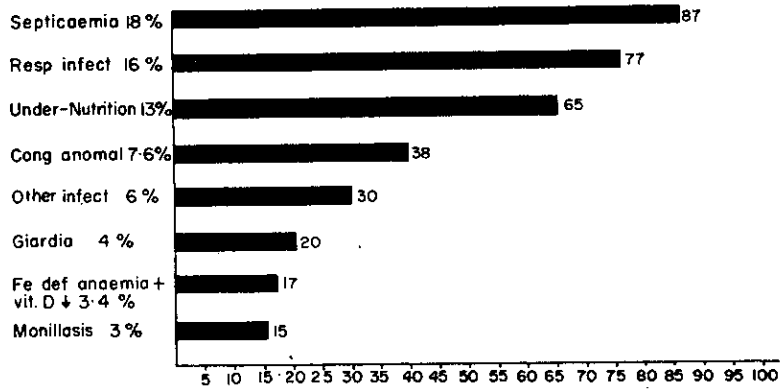


Figure 5 shows isolation of salmonella among the patients under study, the highest incidence is around 1-6 month old and co-relates with incidences of G.E.

Fig. 5

SALMONELLA GASTRO-ENTERITIS AGE INCIDENCE

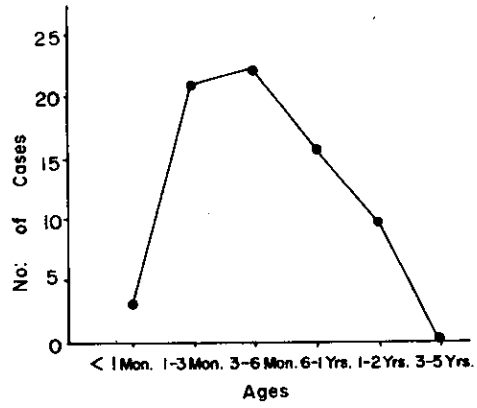


Figure 6 presents the age wise mortality: Again the highest number of deaths are among the 1-6 month olds.

Fig. 6

AGE- WISE MORTALIT

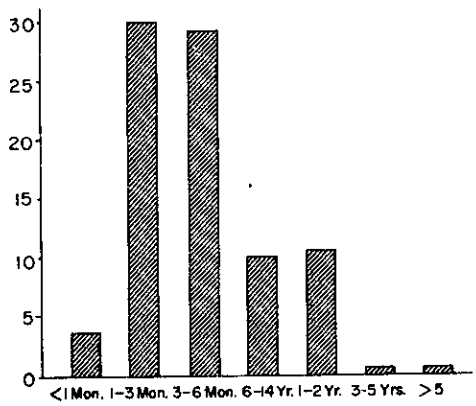


Figure 7 gives the number of fatality of cases with duration of symptoms during diarrhoea.

Fig. 7

DURATION OF SYMPTOMS & CO-RELATION OF MORTALITY RATE

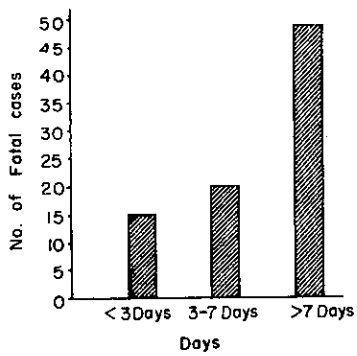
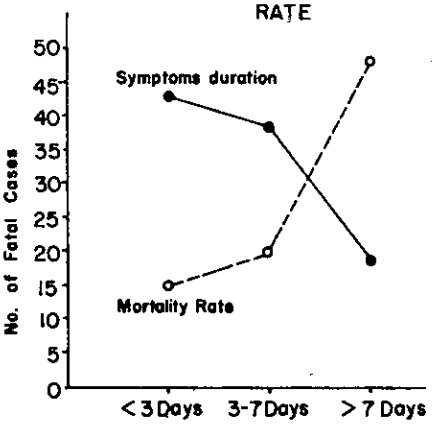


Figure 8 co-relates the mortality rate with duration of symptoms.

Fig. 8

DURATION OF SYMPTOMS MORTALITY RATE



TRAINING THE VILLAGE PRACTITIONERS AS AN INTERVENTION STRATEGY FOR DIARRHOEAL DISEASES: REPORT FROM A PILOT PROJECT

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ABSTRACT

Village practitioners, identified during a census in the project area, formed one of the groups who were selected for training on diarrhoeal management with particular stress on preparation and use of ORS prepared from locally availanle ingredients. This report describes some of the characteristics of these practitioners found in a pre-training survey. The post training effectiveness and the impact of the group in the project area, as regards diarrhoea management and ORS utilization found through the surveillance system is also reported. The project findings indicate that although most of the practitioners practice in Allopathic medicine (83%), only about 12% have any medical education; more than 60% have practising experience of 10 years or more, but only 40% of the practitioners treated on an average 6-10 diarrhoea cases per month. The rest only treated less than 5 diarrhoea cases a month. About 60% had no knowledge about oral rehydration and the rest had unacceptable ideas about its use. Within one year, after training, 109 trained practitioners treated 17% of the diarrhoea cases occurring within the project area. Of the patients treated by them, 84% were treated with ORS only. Hospital referral and case fatality were negligible.

KNOWLEDGE ATTITUDES AND PRACTICES CONCERNING DIARRHOEA IN A RURAL AREA OF BANGLADESH

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ABSTRACT

From January 1979 through December 1980 at its Matlab field station the ICDDR,B carried out a field trial of home-delivered oral rehydration solution (ORS). There were three study areas: one comparison area and two in which ORS was provided, in one in the form of packets of glucose and electrolyte salts and in the other as simple salt (lobon) and crude sugar (gur). At the beginning of the trial and one year later a knowledge, attitudes and practices (KAP) survey questionnaire was administered to a sample of about 700 women from the three areas. The questionnaire concerned three subject areas: (i) general information about diarrhoea, (ii) treatment, and (iii) diet. We have present in detail analysis of the of the first part of the questionnaire = information about the definition of diarrhoea, its causes, and its harmfulness. We also briefly describe some of the major findings concerning treatment and diet, and discuss the implications of these results for implementation of oral rehydration projects.

CHAPTER 5

ENVIRONMENTAL HEALTH AND COMMUNITY IMPLEMENTATION

DIARRHOEA IN INFANTS AND CHILDREN - AN OVERVIEW

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INTRODUCTION

Diarrhoea in early childhood is a major public health problem in the developing countries. According to the 1979 survey of infant and child mortality in India, diarrhoeal diseases were cited as cause of death for 1343 infants, of which 389 were among 2 year old child and 279 among three year old children per 100,000 rural children of the respective ages[1]. Diarrhoea is an indirect cause of death in larger number of children, since it precipitates or aggravates malnutrition in children and makes them more vulnerable to other infections.

Epidemiology of Diarrhoea

In an epidemiological survey of a rural community near Delhi[2], preschool children suffered 1.38 episodes of diarrhoea per child per year. Over 72% of the cases occurred in infants and children below the age of 2 years. Majority of the cases were aged between 6 and 18 months, with the peak incidence between 7 and 12 months. The incidence was high from the month of April to October. Children from lower socioeconomic class suffered an average of 1.55 episodes as against 0.85 episodes in children from richer families. Surprisingly, the incidence of diarrhoea among children of school teachers was as high as those of uneducated artisans and farmers.

In a subsample of population, undernourished children below the age of 18 months suffered 3.1 episodes of diarrhoea per year as compared to 1.2 episodes in well nourished children. In older children (19-60 months) with weight below 3rd percentile suffered 2.1 episodes per year as compared to 0.6 episodes in those whose weight was above 3rd percentile.

There was little difference in the incidence of diarrhoea between the entirely breastfed and the partially weaned infants in age group 6 to 8 months both in the urban and rural areas. However, in the age group 9 to 11+ months, there was distinct increase in the indicence of diarrhoea in the artificially

fed babies (6.6 episodes 52 weeks) as compared to breastfed babies (8.2 episodes 52 weeks). Similar trend was observed in urban infants of the corresponding age (artificially fed 2.7 breastfed 1.2). Surprisingly, the percentage of urban infants who suffered from diarrhoea was lower in the partially weaned (Breast milk + artificial feeds) as against those entirely breastfed[3].

Etiology of Diarrhoea

In our studies in the rural communities, Enteropathogenic Escherichia coli (EPEC) were recovered from 8.8% of 921 patients[4]. However, isolation rates of EPEC from diarrhoeal stools varied widely in Indian Studies and could be related to ecological factors such as quality of water supply, sewage disposal, chances of fecal contamination of food, standards of sanitation and climatic conditions. Ganguly et al (1980)[6] from Calcutta also reported a low EPEC prevalence rate of 8.4% Behera et al (1979)[7] from Orissa and Shehaad Javid[8] from Kashmir reported higher prevalence rates of 25.5% and 27.2% respectively. A recent report from Bikaner[9] in West India an unusually high frequency of 73.1% of EPEC was reported. They attributed it to intensive faecal contamination of water in the study area. The serotypes of EPEC found to be common in India include O_{55}, O_{111}, O_{126}, O_{127}, O_{128}, O_{86}, O_{123}, O_{18}.

Enterotoxigenic Escherichia coli (ETEC)

In recent study of 104 cases of diarrhoea in preschool children[10] we could demonstrate ETEC in 9 cases; stable toxin (ST) + strains in 7, labile toxin (LT) + in 1 and both LT and ST+ in one case. Presence of LT toxin was demonstrated by distension of rabbit ileal loop in 18 hrs. and cytotoxic effect of culture filtrate on tissue culture of Vero cells. For demonstration of stable toxin the infant mouse model and 6 hour rabbit ileal loop assay were employed. More recently, using CM_1 ELISA and Y-1 adrenal cell tissue culture system, we detected LT+ETEC in 10% of cases of diarrhoea in the preschool children in an urban slum in Delhi, Vero cells and Y-1 adrenal cells gave almost identical results[11].

Enteroinvasive E.coli (ETEC)

Enteroinvasive property of *E.coli* isolates from diarrhoeal stools was demonstrated by Sereny's test and the effect on Hela tissue culture cell line. Two strains of *E.coli* out of 32 *E.coli* isolates in 104 cases of diarrhoea showed enteroinvasive property[11]. Sarkar et al (1980)[12] demonstrated enteroinvasiveness in strains of *E.coli* serotype O124 from among 50 cases with EPEC of different serotypes.

Adhesive property of *E.coli* (pure growth)

Twelve of the 32 strains of *E.coli* obtained as pure growth from 104 cases of diarrhoea showed mannose resistant hemagglutination[11]. This is an indirect evidence for the presence of colonisation factor antigen. Four of these adhesive strains were toxigenic. (MPH) mouse resistant hemagglutination
ST+ - 17
LT+ - 1+1

Klebsiella

An epidemic of diarrhoea in our newborn nursery was found to be caused by Klebsiella[14]. Thirteen out of the 25 strains of Klebsiella isolated from stools of affected neonates were found to be toxigenic (LT). We isolated Klebsiella in 9.6% of 180 sporadic cases of diarrhoea, of which 53.8% elaborated labile toxin[15]. Recently, we isolated toxigenic Klebsiella from stools, duodenal aspirate and feeding bottle of a 3 months old infant with diarrhoea, incriminating this organism in the pathogenesis[11].

Shigella

Shigella was cultured in 1.4% of cases in the community[4] and 4.8%[5] of hospitalized patients.

Yersinia enterocolitica

Employing a modified Y-media, we have not been able to isolate Yersinia enterocolitica from a small sample studied so far.

Lactose Malabsorption following Gastroenteritis

Eighty-seven preschool children with acute diarrhoea were kept under surveillance for lactose malabsorption and milk intolerance[16]. Of these, 29 showed reducing substance in the stools or pH less than 6. Milk tolerance returned within 4 days in 55% and within 5 to 10 days in 38%. Only one patient started tolerating milk after 16 days. Forty-two patients (47.2%) recovered from diarrhoea within 3 days while on usual diet. Fourteen of them (33.3%) showed reducing substance in the stools and in 12 (28.6%) pH of stools was less than 6. Forty-seven patients who continued to have diarrhoea for more than 3 days after first contact, 25 (53.2%) had reducing substance in stools and pH was below 6 in 27 (57.4%). The body weight of the early recovery group was more than 80% of the reference standard in 69% and between

60 and 80% in 26.2%. In late recovery group 78.7% weighed more than 80% of reference weight and 17% weighed between 60-80% of expected weight. There was no difference in the two groups. Out of 40 patients in the late recovery group available for follow-up, 35 (87.5%) improved on milk withdrawal for 24 hours, but in 11/31 (35.5%) diarrhoea recurred on reintroduction of milk.

Lactose malabsorption in Persistant Diarrhoea[16]

Among another set of 25 patients with persistent diarrhoea of more than 2 weeks duration reducing substance (0.5%) was present in the stools of 11 patients. Oral lactose tolerance test was normal in 10 patients within 4-6 weeks. Eight of these started tolerating milk in this period. Two did not tolerate milk till 8 and 10 weeks. These cases probably represent post enteritis milk protein intolerance. Both these infants were less than 3 months old at onset of symptoms.

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Discussion

Dr. M.U. Khan

Q. Since your data seems to be quite different from our observation, I would like to know from which area this data was derived and was this was cross sectional data or an average of data covering all seasons?

A. Isolation rates vary from area to area and from time to time due to ecological factors. The incidence has been reported to be 73% to 88% in different parts of India. We initially had low LT+ later we found higher LT cases. This is a hospital study. Observations and cross section for seasons.

Dr. Rabbani

Q. Do you incriminate Klebsiella and Pseudomonas as the cause of diarrhoea according to Koch's criteria in these patients?

A. All *E.coli* do not cause disease, all Klebsiella do not cause diarrhoea. It is only those strains which are toxigenic that cause diarrhoea. We are aware of the skepticism of many workers. However, Klebsiella culture lysate showed capacity to distend rabbit illeal loop.

Dr. Sharma

Q. It was not clear whether all Klebsiella strains produced enterotoxin. If yes, was it resembling LT or ST?

A. LT in 53% of cases.

Dr. Sanyal

Q. Did you get positive results with Klebsiella in GM_1 ganglioside ELISA and Y_1 adrenal cells and CHO-cell cultures?

A. Not done so far except on verrow cell.

Increased number of Klebsiella isolation for diarrhoeal stool is common. We have also got similar isolation in Benaras.

Dr. Ganguly

Q. Klebsiella toxigenic strains have been reported from Chandigarh. Your ST strains were seen in children or adults and what was your correlation berween sereny and Hela cell tests?

In children and the correlation was good.

Q. What was the age group of the patients studied?

A. Most patients were under 18 months.

AN EPIDEMIC OF ROTAVIRUS DIARRHOEA IN MANIPUR, INDIA

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INTRODUCTION

Since 1969, recurrent epidemics of acute diarrhoea in children below 2 years have been reported from Manipur, a north-eastern state of India. The epidemics start in September-October every year and usually subside by the month of January. The patients were admitted mainly in the Regional Medical College Hospital, Imphal. During the winter, about 80 percent of the children's ward was occupied by diarrhoea cases. The epidemics were investigated twice during 1974[1] and 1976 when enteropathogenic *E.coli* and shigella were isolated from about 30% of diarrhoeal children. Facilities for the detection of rotavirus were not available.

The present report describes the results of investigations of a similar epidemic of acute diarrhoeal diseases during November, 1979.

Demographic Features

The state of Manipur comprises of 4 hill districts and a Central district, the Imphal valley. Sixty-five percent of the total population (1073000) live in the central valley and the density of population was 48 per sq. km. according to 1971 census. The yearly rainfall is 150-200 cm. and the winter is fairly severe. Parts of Imphal town is supplied with piped chlorinated water which is intermittent and inadequate. Rest of the state depend on surface water for drinking as well as for domestic purposes. This surface water also becomes very scarce during the winter months. Night soil disposal system is grossly inadequate in Imphal town and people at large use open land for defaecation.

METHODS

Clinical and epidemiological data were collected from the case of gastroenteritis admitted at the Regional Medical College (R.M.C.) Hospital, Imphal and the Thoubal, Kakching, Moirang and Bishenpur Primary Health Centres (PHCs). A total of 59 patients admitted during the visit of the investigators were included for microbiological studies, 422 patients admitted outside this period could not be included.

Rectal swabs and catheter specimen of stools were collected in phosphate buffered saline from 51 cases of gastroenteritis admitted in the paediatric ward of R.M.C. Hospital and 8 hospitalized children in Moirang and Thoubal PHS. Rectal swabs were also collected in Carry & Blair's transport media and screened for the presence of *V. cholerae*, *V. parahaemolyticus*, shigella, salmonella and *E.coli* using standard laboratory techniques[2,3]. The stool specimens collected in phosphate buffered saline pH 7.4 were kept at 4°C and examined by Enzyme Linked Immunosorbent Assay (ELISA) within a week. The procedure followed was that of Yolken et al, 1977 with certain modifications as adopted by NIH, USA (Yolken - personal communication)[4]. Each well of polyvinyl microtitration plate (Dynatech Laboratories) was coated with 100 µl of diluted goat anti-rota serum (1:10,000 in carbonate buffer pH 9.6) and incubated at 4°C for 24 hours. The plate was then washed 3 times with phosphate buffered saline pH 7.4 containing 'Tween 20' at a concentration of 0.5 ml/l (PBS-tween). Fifty µl of a mixture of PBS-tween containing 1% foetal calf serum (FCS) and 0.5% normal goat serum (GS) was added in each well and then 50 µl of stool suspension 2-10% diluted in PBS buffer was added. The plate was incubated at 4°C overnight and washed 3 times with PBS-tween. To each well 100 µl of guineapig anti-rota serum diluted 1:700 in PBS-tween-FCS-GS mixture was added and incubated at 37°C for 1 hour. The plate was washed 3 times with PBS-tween and 100 µl of alkaline phosphatase labelled goat anti-guineapig serum diluted 1:400 in PBS-tween FCS-GS mixture was added, incubated at 37°C for 1 hour and was washed 3 times with PBS-tween. Finally 100 µl of p-nitrophenyl phosphate substrate (Sigma) at a concentration of 1 mg/ml in 10% diethanolamine buffer pH 9.8 was added and the plates incubated at room temperature until yellow colour appeared. The colour was matched with known weekly positive and negative controls. The positive controls consisted of Nebraska calf diarrhoea virus and the negative control was a rota negative stool sample by electron microscopy.

The unknown stool samples as well as the positive and negative controls were tested in duplicate. All positive samples were confirmed by blocking tests using bovine anti-rota serum. Reagents employed for ELISA including blocking test and positive control were kindly supplied by Dr. Kapikian of NIH, USA.

RESULTS

A total of 481 cases of acute diarrhoea were admitted in the R.M.C. Hospital, Imphal, during October-November, there were 4 deaths, thereby giving an overall case fatality rate of 0.8% (Table 1). Number of cases admitted in October was 145 and in November 336 with cast fatality rates of 1.4% and 0.6%, respectively. 93.3% of these cases ere below two years of age of which 76.5% belonged to the age below 1 year. Proportion of male and female cases was 1.9:1 (Table 1).

Admissions of acute diarrhoea cases in R.M.C. Hospital, Imphal started increasing from the last week of October and the peak incidence was noted during the week ending 13 November. Thereafter the epidemic gradually declined. No adult cases of gastroenteritis were admitted either in the R.M.C. Hospital or in any of the Primary Health Centres during this period.

The important clinical features included watery diarrhoeas (100.0%), vomiting (90%), fever (46.7%), cervical lymphadenitis and phanyrgeal erythema (43.3%). Abdominal distension was noted in about 30.0% of the cases. Mild to moderate degrees of dehydration only about 5.0% was seen in 95% of the children had severe dehydration. The mean duration of diarrhoea (from the onset of clinical symptoms till the stoppage of diarrhoea) was 68 hours. 93.3% children were free from any clinical signs of malnutrition. 63.3% cases were treated before admission by a wide range of antibiotics including streptomycin, tetracycline, chloramphenicol, neomycin and sulfa drugs either singley or in combination.

All these cases were single single cases in their families. Percentage distribution of the affected families of 60 cases, studied by the team, showed that 46.7% were drinking chlorinated tap water, 40.0% pond water and 13.3% river water. Forty-five percent of the families used open field for defaecation and 33.3% used dug well, 16.7% sanitary and 5% service type latrines. Majority of the families (63.4%) had a monthly income of below Rs. 500/- per month.

Rotavirus was detected in 53 out of 59 stool samples examined by ELISA technique thereby giving a detection rate of 89.8% (Table 2). Enteropathogenic *E.coli* (EPEC) was isolated from 7 stool samples which were also positive for rotavirus. A total of 29 strains of *E.coli* isolated from these specimens were tested for their enterotoxigenicity (both LT and ST) in rabbit ileal loop and suckling mice models. None of the strains were found to be toxigenic (ETEC). One sample yielded *Shigella flexneri* type 2 in addition to rotavirus.

Table - 1

Distribution of cases hospitalised during the two months with their case-fatality rates.

Age Groups. (months)	Number of cases hospitalised			
	Admissions	Cumulative percentage	Deaths	Case-fatality rate (percentage)
< 12	368	76.5	2	0.5
12 - 23	81	93.3	2	2.5
24 - 35	10	95.4	Nil	-
36 - 47	7	96.9	Nil	-
48 - 59	3	97.5	Nil	-
≥ 60 *	12	100.0	Nil	-
Total	481	100.0	4	0.8

* Between 5 and 13 years of age.

Table 2. Pattern of Isolation of Enteropathogens in 59 Paediatric Diarrhoea Cases in Manipur during November, 1979

Enteropathogen Isolated	Number (N = 59)	Percent
Rotavirus only	45	76.3
Rotavirus + EPEC[a]	7	11.9
Rotavirus + Sh. flex.[b]	1	1.7
ETEC	Nil	-
Total	53	89.9

[a]Serotypes - O44:K74, O86:B7, O12:B16, O142:K86

[b]Type 2.

DISCUSSIONS

Rotavirus has emerged as the most important viral agent etiologically associated with severe diarrhoeal diseases in infants and children. In a number of hospital-based studies carried out mostly in developed countries, rotavirus has been detected in approximately 50% of infantile diarrhoea cases. However, there is little information about their importance and prevalence in the developing countries with tropical climates, where diarrhoeal diseases represents one of the most important causes of morbidity and mortality among infants and younger children. Similarly, there is lack of information regarding the natural history of the disease such as principal reservoir of human rotavirus, their mode of transmission, seasonality, survival in different environmental conditions etc.

In the present study rotavirus has been detected in the faecal samples of 89.8% of diarrhoeal children aged under one year. It is most interesting to note that epidemics of similar clinical entities have been recurring every year in Manipur since 1969. Availability of a fresh group of susceptible population (children under 1 year) coupled with favourable environmental factors that exist in the area during the winter months, probably sparked off recurrent epidemics every year. This exact cause of recurrence, the natural reservoir of rotavirus, the mode of transmission and

the triggering environmental factors are yet to be determined.

Cervical lymphadenitis, pharyngeal erythema accompanied with fever in a large proportion of cases may indicate involvement of a respirtory system as well.

ACKNOWLEDGEMENT

We thank both the Principal and the Superintendent of the Regional Medical College Hospital, Imphal for their active cooperation and support and Dr. P.K. Singh, Director of Health, Government of Manipur, for his interest and advice. The excellent assistance offered by the doctors in the Paediatric ward of RMC Hospital and the laboratory staff of NICED, Calcutta is also thankfully acknowledged.

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LONG TERM COMPLICATIONS OF MEASLES IN RURAL BANGLADESH

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ABSTRACT

A retrospective case-control study carried out in a rural area of Bangladesh identified respiratory illness, diarrhoea and conjunctival dryness as long term complications of measles. A case-fatality rate of 1.33% was recorded. Association between length of diarrhoea and conjunctival dryness was noted. No difference was observed in the anthropometric data between cases and controls. This establishes a link between measles, diarrhoea and clinical evidence of vitamin A deficiency but fails to show an effect on growth in this small series.

INTRODUCTION

Death rate due to measles have varied widely in reports from developing countries but all these exceed substantially the figures from technically advanced societies. In Africa 5.3% of affected children died, while in South India, a case fatality rate of 1.5% has been observed. In Bangladesh during a period of severe food shortage 3.9% of the affected children died. Apart from deaths during measles, complications principally diarrhoea and malnutrition have been attributed to an attack of measles.

In the study, rural Bangladesh children who had measles 5 months earlier wre matched for age and location with children who did not have measles. This paper analyzes the distribution and significance of the episodes of illness reported in the period May-July 1980 for both groups.

The objectives of this study were to:

1. Investigate the epidemiology of measles in Bangladesh;

2. Identify the long term complication of measles in the wider-two population with special attention to diarrhoea;

3. Correlate eye-changes with length of diarrhoea.

MATERIAL AND METHODS

In July 1980, we sampled 10% of the children (under two years of age) who had an episode of measles during March-April 1980 in Matlab. ICDDR,B field assistants had identified the cases, in the under 2 population during the investigation of an epidemic of measles and a computer printout had already been made.

Seventy-five cases were found in 7 sampled villages. Four villages were situated within the 2 mile radius of the Matlab field hospital and had 45 cases. Three villages were situated further than 2 miles from the hospital and had 30 cases.

Controls were selected from same villages as the study children did not have measles and were matched from the same village and within the same age group. The groups studied were comparable with respect to socio-economic status, sex and level of mother's education.

Each case and control was visited by a single observer/investigator and the child's mother was asked a standard series of questions including the duration and type of diarrhoea during and after the rash. Clinical and anthropometric measurement were done at the time of the interview by a physician.

Table 1 shows that complications following the clearance of rash were most common in the age group 7-12 months.

Table 2 shows the association between duration of diarrhoea in days and conjunctival dryness which were significantly more common after the measles rash than amongst the controls. Table 3 shows that mucoid and bloody diarrhoea was more common after measles but no significant difference was observed among the cases and controls for watery diarrhoea. There was more conjunctival dryness amongst the cases than amongst their controls.

Table 1. Distribution of Complication by Age of Cases

Age in Months	Complication +	Complication −
< 6 months	17	14
7 – 12 "	16	3*
13 – 18 "	6	10
19 – 24 "	6	4

* Significance for this group

$x^2 = 6.56$ df = 1 $p < .01$

Table 2. Association between length of Diarrhoea (in days following clearance of rash) and Conjunctival Dryness

Conjunctival Dryness (on examination)	Length of Diarrhoea (in days) 0	≤7	>7
+	3	4	12
−	27	10	12

$x^2 = 8.4167$ df = 1 $p < .05$

Table 3. Frequency of Distribution of Symptoms on Measles Cases (after clearance of rash) and in Controls (after Marcy 1980)

VARIABLES		CASES +	CASES −	CONTROLS +	CONTROLS −	P VALUE
1. WATERY DIARRHOEA	(1)	36	40	43	34	NS
2. MUCOID "	(1)	32	44	15	62	P < .01
3. BLOODY "	(1)	16	59	7	70	P < .05
4. DIARRHOEA (ANY TYPE)	(1)	69	6	19	56	P < .01
5. CONJUNCTIVAL DRYNESS	(2)	16	58	5	68	P < .01

(1) FROM HISTORY TAKING
(2) ON CLINICAL EXAMINATION

These findings are closely associated with the duration of diarrhoeal attacks following measles. Children having conjunctival dryness had an increased length of diarrhoea. This association between duration of diarrhoea and conjunctival dryness may be due to the malabsorption of vitamins secondary to diarrhoea. There are many causes of diarrhoea in rural Bangladesh. All lead to malabsorption and nutrient wastage with varying degree of severity and duration. Measles virus itself has been reported in the stool of children 25 days after the clearance of rash. The virus enters the lymphatic channels in the fut producing prolonged diarrhoea which may also lead to malabsorption of vitamin A. Such involvement of lymphatics by measles virus might also increase susceptibility to some microbes which cause diarrhoea.

The following are the recommendations:

1. Early implementation of oral rehydration therapy.

2. Measles vaccine is not cost effective in the Bangladesh setup.

3. Introduction of vitamin A in the ORS therapy.

4. Nutrition education to the primary health worker.

The study suggests that the malabsorption of vitamin A is related to diarrhoea following measles. Further study is needed to establish the link and ways to prevent complications.

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*List of previous publications, such as, annual reports, working papers, scientific reports, special publications and thesis and dissertations, can be obtained on request. For further information, write to Head, Library and Publications Branch, International Centre for Diarrhoeal Disease Research, Bangladesh, G.P.O. Box 128, Dacca-2, Bangladesh.