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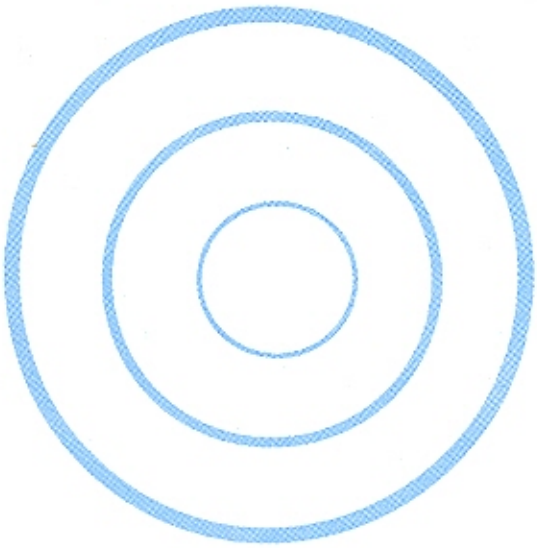

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CONTENTS

Erythromycin and Trimethoprim-sulphamethoxazole in the Treatment of Cholera in Children

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ABSTRACT

To evaluate the efficacy of erythromycin and trimethoprim-sulphamethoxazole (TMP-SMX) in the treatment of cholera in children aged 1-8 years, a randomised clinical trial was conducted at a diarrhoea treatment centre in Bangladesh from December 1991 to June 1992. Fifteen children received erythromycin, 50 mg/kg per day, in four equally divided doses, 18 children received 10 mg/kg per day of trimethoprim and 50 mg/kg per day of sulphamethoxazole in two equally divided doses (12 hourly) for five days, and 15 children received no antibiotic; children in all three groups received intravenous cholera saline for severe dehydration and for mild to moderate dehydration, a rice-based oral rehydration solution. The mean stool volumes in mL/kg body weight in the two treatment groups were less than that of the control group, and there were no significant differences in stool volume among the two treatment groups. However, 67% of the children in the erythromycin group and 82% in the TMP-SMX group recovered within 72 hours compared to 33% in the control group ($p<0.01$). Similarly, the bacteriological cures were 80% in the erythromycin group and 83% in the TMP-SMX group compared to only 27% in the control group ($p<0.001$). These results confirm that both erythromycin and trimethoprim-sulphamethoxazole are effective antimicrobials in the treatment of cholera. These drugs are of value specially in younger children in whom tetracycline is contraindicated or when the infecting *Vibrio cholerae* are resistant to tetracycline.

***Key words*: Cholera;** *Vibrio cholerae;* Erythromycin; Trimethoprim; Sulphamethoxazole; Drug-resistance, Microbial

INTRODUCTION

Cholera, the most severe of all infectious diarrhoeal diseases, is an important cause of morbidity and mortality in many developing countries. The disease is endemic in some parts of Asia, Africa, and South America (1). It is characterised by acute onset of vomiting, profuse watery diarrhoea, and development of dehydration that might lead to death if not timely treated (2,3). The advent of oral rehydration solution (ORS), however, has simplified the management of cholera and substantially reduced the mortality due to dehydration. Appropriate antibiotics, including tetracycline, doxycycline, furazolidone, and chloramphenicol, given concurrently with fluid and electrolyte replacement, reduce the volume of stools, the duration of diarrhoea, and the excretion of *Vibrio cholerae* in faeces (4-7).

However, tetracycline can not be prescribed to pregnant women and younger children due to its adverse effects on teeth and growing bone. Moreover, there have been frequent reports of multiple drug-resistant *V. cholerae,* including tetracycline and furazolidone (8-11). It is, therefore, important to identify effective alternative antimicrobials for the treatment of cholera in children and pregnant mothers and also for large-scale epidemic due to tetracycline and furazolidone-resistant strains of *V. cholerae*. Previous studies have shown good clinical and bacteriological cures of erythromycin and trimethoprim-sulphamethoxazole in cholera, but those studies were done nearly 20 years back, and by this time, antimicrobial susceptibility

to *V. cholerae* O1 might have changed. The present pilot study was undertaken to evaluate the clinical efficacy of erythromycin and trimethoprim-sulphamethoxazole in the treatment of cholera in children.

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PATIENTS AND METHODS

Patients

The study was carried out at the Clinical Research Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Patients enrolled in this study were only boys, aged 1-8 years, having a history of watery diarrhoea for less than 48 hours, and their stool containing motile *V. cholerae* as seen under a dark-field microscope. Patients who had taken antibiotics before hospital admission, and patients with systemic infections were excluded from the study.

Clinical management

On admission, body weight was obtained, and the patients were nursed on a cholera cot. A thorough clinical examination was performed, and the hydration status was assessed clinically by a physician according to World Health Organization (WHO) criteria. A stool specimen was sent to the laboratory for the dark-field microscopy to detect motile *V. cholerae* and another specimen for bacteriological culture. For patients with mild to moderate dehydration, a rice-based oral rehydration solution (R-ORS) was started (12). Children with severe dehydration were rehydrated with an intravenous solution, containing 133 mmol of sodium, 13 mmol of potassium, 98 mmol of chloride, and 48 mmol of acetate in one litre of water. Complete rehydration was achieved within 4-6 hours of admission. All stools, urine, and vomit were collected separately. The volumes of intravenous fluid, amount of R-ORS intake, volumes of stools and urine were recorded every 8-hour period, beginning at the time of admission and continued until the diarrhoea stopped. The patients stayed in the hospital for 5 days, or until the diarrhoea resolved.

Randomisation

After rehydration, the patients were randomly assigned to one of the two treatment groups (erythromycin or trimethoprim-sulphamethoxazole), or to the no-antibiotic control group by using sealed envelopes containing a numeric treatment code obtained from a table of random numbers. Erythromycin was given at 50 mg/kg per day in 4 equally divided doses, trimethoprim at 10 mg/kg per day and sulphamethoxazole 50 mg/kg per day in two equally divided doses for five days. The justification for including a non-antibiotic group as control is that replacement of fluid and electrolytes is the cornerstone therapy for the prevention of death due to dehydration. However, antibiotic therapy has been shown to reduce the duration of diarrhoea and accelerate bacteriological cure.

Bacteriology

Rectal swab specimens were obtained from the patients before starting the treatment and subsequently daily for five days. Faecal specimens were immediately plated onto MacConkey's Salmonella-Shigella and Monsur's (taurocholate-tellurite gelatine agar) media plates. Bacteria from colonies typical of *V. cholerae* were tested for agglutination with a polyvalent O group 1 antiserum.

Measurements of outcome variables

The outcome variables include volume of stool in mL which was measured every 8 hours, ORS and intravenous fluid intake, the duration of diarrhoea in hours, and the duration of *V. cholerae* excretion in stools. Duration of diarrhoea was defined as the end of the last 8-hour period in which a liquid stool was passed. Clinical success was defined as the end of diarrhoea occurring on or before 72 hours without subsequent relapse, and clinical failure was defined if diarrhoea continued beyond 72 hours. A period of 72 hours was selected because in most patients treated with appropriate antibiotics, diarrhoea usually resolves within this period (6). Similarly, bacteriological success was defined if the stool culture became negative on day 3 or before, and remained negative afterwards.

Statistical analysis

The significance of differences in means was tested by the Student's *t* test. Analysis of ariance (ANOVA) or Krushkal-Wallis test was done when appropriate, and the proportion of success was tested by the chi-squire or Fisher's exact test. A value of $p<0.05$ was considered significant.

RESULTS

Initially, 54 patients were positive for *V. cholerae* by dark-field microscopic examination of their stools. Six patients were excluded, because stool culture failed to grow *V. cholerae*. Of the remaining 48 children, 15 received erythromycin, 18 trimethoprim-sulphamethoxazole, and 15 no antibiotics. The admission characteristics, including the age, duration of diarrhoea, hydration status, and stool pathogens, are shown in Table I. There were no significant differences in these variables among three treatment groups of children on admission.

Table II shows the clinical outcome of three groups after treatment with erythromycin, trimethoprim-sulphamethoxazole or without any antibiotic. The means of total faecal output, expressed as mL/kg, were not significantly different from the two treatment groups when compared with the control group. However, the mean duration of diarrhoea was significantly less in both the erythromycin and TMP-SMX groups compared to the control group ($p<0.03$). The number of children who had clinical cure was 67% and 83% for the erythromycin and the TMP-SMX groups respectively. Both proportions are significantly different from the only 33% in the control group. Similarly, the bacteriological cure was 80% for the erythromycin and 83% in the TMP-SMX groups compared to only 27% in the control group ($p<0.001$) (Table II).

Table I. Admission characteristics of children with cholera in the three treatment groups

	Treatment groups		
Characteristic	Erythromycin	TMP-SMX*	Control
Number of patients	15	18	15
Age (months)	57±34	57±29	61±35
Body weight, kg	11.3±4.0	12.7±3.7	13.3±3.9
Preadmission hours of diarrhoea	12±6	11±7	13±4
Dehydration status (No. of children)			
Mild to moderate	4	8	6
Severe	11	10	9
Serotypes of *V. cholerae* O1 El Tor			
Ogawa	14	16	13
Inaba	1	2	2

Values are mean ±SD.
*Trimethoprim-sulphamethoxazole

Antibiotic sensitivity of *V. cholerae* was determined in 30 isolates. It showed 100% sensitivity to tetracycline (30/30; confidence limits [CL] 88% to 100%), 87% to both erythromycin and trimethoprim-sulphamethoxazole (26/30; CL 69% to 96%), and only 13% to furazolidone (4/30; CL 4% to 31%). The overlapping confidence limits indicate the absence of statistically significant differences between the antimicrobial agents used in our two study groups.

Table II. Clinical and bacteriological outcome of children with cholera in the three treatment groups

	Treatment groups					
	Erythromycin (n=15)		TMP-SMX (n=18)		Control (n=15)	
Stool volume (mL/kg body wt.)						
0-24 h (95% CI)	178±96	(125 to 231)	166±177	(78 to 254)	228±160	(139 to 317)
25-48 h	130±100	(75 to 185)	68±65	(36 to 100)	102±90	(52 to 152)
Total stool volume (5 days)	389±249	(251 to 527)	358±279	(219 to 497)	403±314	(229 to 577)
Total ORS intake, mL/kg (mean ±SD)	418±319		379±261		435±537	
Duration of diarrhoea, h	54±26*		53±21*		80±35	
Clinical cure, no. (%)	10 (67)**		15 (82)**		5 (33)	
Bacteriological cure, no. (%)	12 (80)**		15 (83)**		4 (27)	

Erythromycin vs. no-antibiotic *p<0.01 and TMP-SMX vs. no-antibiotic **p<0.001.
Clinical cure was defined as the end of diarrhoea (passage of soft stool) occurring on or before 72 hours without relapse.
Bacteriological cure is defined if the stool culture became negative on day 3 or before after starting treatment and remained negative afterwards.

Of the five clinical failures in the erythromycin group, stools became again less formed from day 4 in one patient and from day 5 in the remaining four patients, and of the three clinical failures in the TMP-SMX group, two improved on day 4 and another on day 5. On the other hand, 10 patients who failed in the control group improved on day 6.

The proportion of patients with *V. cholerae* isolated from stool culture declined from 60% on day 1 post-treatment to 0% on day 5 in the erythromycin group (9/15 and 0/15; CL 32% to 84% and 0% to 22% respectively) compared to 91% on day 1 to 53% on day 5 in the control group (14/15 and 8/15; CL 68% to 100% and 27% to 79% respectively) ($p<0.01$). Similarly, the proportion of patients with *V. cholerae* isolated from stool culture in the TMP-SMX group declined from 94% (17/18; CL 73% to 100%) on day 1 to only 16.7% (3/18; CL 36% to 41%) on day 5 ($p<0.01$) (Fig.). However, these differences in bacteriological response between the erythromycin and TMP-SMX groups were not statistically significant on any day during the treatment.

DISCUSSION

In the present study, patients receiving erythromycin or trimethoprim-sulphamethoxazole showed good clinical and bacteriological cures when compared with a no-antibiotic control. This is in agreement with other clinical studies done previously (6,13-16). However, there was no differences either in clinical or bacteriological cure between the two antibiotic treatment groups. Similarly, the duration of diarrhoea of two antibiotic treatment groups was significantly shorter than the no-antibiotic group. That agrees well with nearly two and half days required in a previous study (13).

Until recently, in this region, the El Tor biotype of *V cholerae* O1 was responsible for epidemics and outbreaks (17). However, the cholera outbreaks that took place during 1987-1989 in southern Bangladesh were mostly caused by *V. cholerae* classical biotype that were tetracycline-resistant (18). *V. cholerae* O1 El Tor biotype strains resistant to tetracycline have since long been reported from other parts of the world. However, only recently similar El Tor biotype of *V. cholerae* O1 has been found to be responsible for epidemics in this region (18,19). In 1988, a cholera epidemic due to *V. cholerae* O1 El Tor biotype was reported from Maharastra, India; 43% of those strains were tetracycline-resistant (20).

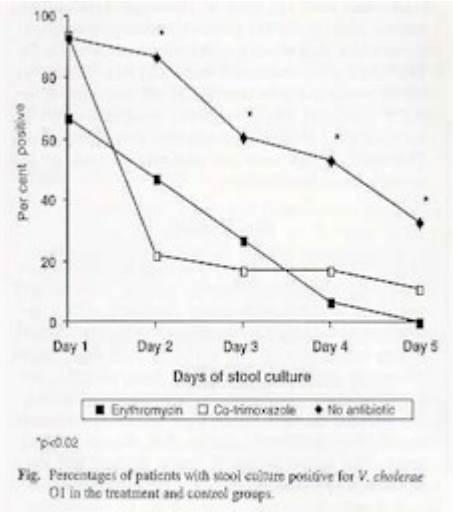

Fig. Percentages of patients with stool culture positive for *V. cholerae* O1 in the treatment and control groups.

In our study, although both the clinical and bacteriological successes were significantly better in children receiving antibiotics compared to the control patients, the volume of stool was similar in all three groups. This may be due to the small number of patients studied in each group.

Nevertheless, our study showed that both erythromycin and trimethoprim-sulphamethoxazole are similarly effective in achieving clinical and bacteriological cure. With the emergence of *V. cholerae* O1 strains resistant to tetracycline and furazolidone, erythromycin and trimethoprim-sulphamethoxazole may serve as effective alternative antimicrobials.

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REFERENCES

1. Khan MU, Eeckels R, Alam AN, Rahman N. Cholera, rotavirus and ETEC diarrhoea: some clinico-epidemiological features. *Trans R Soc Trop Med Hyg* 1988;82:485-8.
2. Lindenbaum J, Greenough WB, III, Islam MR. Antibiotic therapy of cholera. *Bull WHO* 1967;36:871-83.
3. De S, Choudhuri A, Dutta P, Dutta D, De SP, Pal SC. Doxycycline in the treatment of cholera. *Bull WHO* 1976;54:177-9.
4. Wallace CK, Anderson PN, Brown TC, Khanra SR, Lewis GW, Pierce NF, *et al*. Optimal antibiotic therapy in cholera. *Bull WHO* 1968;39:239-45.
5. Alam AN, Alam NH, Ahmed T, Sack DA. Randomised double blind trial of single dose doxycycline for treating cholera in adults. *Br Med J* 1990;300:1619-21.

6. Rabbani GH, Islam MR, Butler T, Shahrier M, Alam K. Single-dose treatment of cholera with furazolidone or tetracycline in a double-blind randomized trial. *Atimicrob Agents Chemother* 1989;33:1447-50.

7. Candau MG. The seventh cholera pandemic. *WHO Chron* 1971;25:155-60.

8. Glass RI, Huq I, Alim ARMA, Yunus M. Emergence of multiply antibiotic-resistance *Vibrio cholerae* El Tor in Bangladesh. *J Infect Dis* 1980;142:939-42.

9. Towner KJ, Pearson NJ, Mhalu FS, O'Grady F. Resistance to antimicrobial agents of *Vibrio cholerae* El Tor strains isolated during the fourth cholera epidemic in the United Republic of Tanzania. *Bull WHO* 1980;58:747-51.

10. Maimone F, Coppo A, Pazzani C, Ismail SO, Guerra R, Procacci P, *et al*. Clonal spread of multiply resistant strains of *Vibrio cholerae* O1 in Somalia. *J Infect Dis* 1986;153:802-3.

11. Finch MJ, Moriss G, Jr., Kaviti J, Kagwanja W, Levine MM. Epidemiology of antimicrobial resistant cholera in Kenya and East Africa. *J Trop Med Hyg* 1988;39:484-90.

12. Molla AM, Sarker SA, Hossain M, Molla A, Greenough WB, III. Rice-powder electrolyte solution as oral therapy in diarrhoea due to *Vibrio cholerae* and *Escherichia coli*. *Lancet* 1982;1:1317-9.

13. Cash RA, Northrup RS, Rahman ASMM. Trimethoprim and sulphamethoxazole in clinical cholera: comparison with tetracycline. *J Infect Dis* 1973;128(suppl):749-53.

14. Gharagozloo RA, Naficy K, Mouin M, Nassirzadeh MH, Yalda R. Comparative trial of tetracycline, chloramphenicol, and trimethoprim sulphamethoxazole in eradication of *Vibrio cholerae* El Tor. *Br Med J* 1970;4:281-2.

15. Uylangco C, Santiago L, Pescante M, Menday P, Christensen O. Pivmecillinam, co-trimoxazole and oral mecillinam in gastroenteritis due to *Vibrio* spp. *J Antimicrob Chemother* 1984;13:171-5.

16. Burans JP, Podgore J, Mansour MM, Farah AH, Abbas S, Abu-Elyazeed R, *et al*. Comparative trial of erythromycin and sulphatrimethoprim in the treatment of tetracycline-resistant *V. cholerae* O1. *Trans R Soc Trop Med Hyg* 1989;83:836-8.

17. Siddique AK, Baqui AH, Eusof A, Haider K, Hossain MA, Bashir I, *et al*. Survival of classic cholera in Bangladesh. *Lancet* 1991;337:1125-7.

18. Siddique AK, Zaman K, Majumder Y, Islam Q, Bashir I, Mutsuddy P, *et al*. Simultaneous outbreaks of contrasting drug resistant classic and El Tor *Vibrio cholerae* O1 in Bangladesh (letter). *Lancet* 1989;2:396.

19. Thretall EJ, Rowe B, Huq I. Plasmid-encoded multiple antibiotic resistance in *Vibrio cholerae* El Tor from Bangladesh. *Lancet* 1980;1:1247-8.

20. Fule RP, Powar RM, Menon S, Basutkar SH, Saoji AM. Cholera epidemic in Solapur during July-August, 1988. *Indian J Med Res* 1990;91:24-6.

21. Bhattacharya SK, Bhattacharya MK, Dutta P, Dutta D, De SP, Sikder SN, *et al*. Double-blind, randomized, controlled clinical trial of norfloxacin for cholera. *Antimicrob Agents Chemother* 1990;34:939-40.

22. Irgham B, Brentnal DW, Dale EA, McFadzean JA. Arthropathy induced by antibacterial fused N-alkyl-4-pyridone-3-carboxylic acids. *Toxicol Lett* 1977;1:21-6.

Differentiation of *Vibrio cholerae* O1 Isolates with Biochemical Fingerprinting and Comparison with Ribotyping

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ABSTRACT

The Phene Plate (PhP) system is a commercially available typing system based on the measurements of kinetics of selected biochemical reactions of bacteria grown in liquid medium in 96-well microplates. The system uses numerical analysis to identify biochemical phenotypes among the tested strains. In the present study, a set of 16 discriminatory tests were used to differentiate 117 strains of *Vibrio cholerae* O1 from Mexico and Bangladesh. The stability of PhP types of 16 isolates under different storage temperatures and after repeated subcultures were also evaluated. The PhP system had a reproducibility of 95%. Storage either at +4 ° C or -70 ° C, did not affect the reactions of the isolates, whereas 4 strains (25%) stored at room temperature and 5 strains (31%) subjected to 30 consecutive subcultures, exhibited minor changes in their biochemical reactions. Endemic isolates of *V. cholerae* O1 from Bangladesh were more diverse (diversity index = 0.84 to 0.93) than epidemic isolates from Mexico (diversity index = 0.73). Using a collection of 33 heterogeneous isolates of classical biotype of vibrios, PhP typing and ribotyping were compared. PhP typing discriminated more types (n=23) than ribotyping (n=5), whereas a combination of both yielded 27 types. The PhP system appears to be a simple, reliable and highly discriminating method for typing of *V. cholerae*, and may prove especially useful as a first screening method in epidemiological studies of *V. cholerae*.

***Key words*:** *Vibrio cholerae*; Phenotypes

INTRODUCTION

Vibrio cholerae belonging to serogroups O1 and O139 are the causative agents of cholera (1). There are two biotypes of *V. cholerae* O1, classical and El Tor, and each biotype can be further subdivided into two serotypes, Inaba and Ogawa (2). Suitable typing methods with a wide appeal for further discrimination of biotypes and serotypes for epidemiological studies have recently become available. These subtyping methods include ribotyping (3), ctxA genotyping (4), pulsed field gel electrophoresis (PFGE) (5), and multilocus enzyme electrophoresis (MEE) (6). The first three are DNA typing methods, among which, in ribotyping, strains are differentiated based on restriction fragment length polymorphism (RFLP) of ribosomal RNA genes using rRNA gene probes (7). MEE is based on differences in the migration of a set of enzymes that correspond to allelic differences among isolates (6). Although these subtyping methods may be sufficiently discriminatory, they are laborious and expensive, and hence may not be suitable for a large collection of isolates.

The PhenePlate or PhP system is an automated system for biochemical fingerprinting of bacterial isolates, which is based on numerical analysis of the speed of colour changes of several biochemical reactions (8). It is a commercially available system that is simple to use, discriminatory and reproducible, and is suitable for large investigations involving hundreds of isolates. The system was previously used for epidemiological and ecological studies of different groups of bacteria (9-11,21).

In the present study, we have evaluated the PhP system for typing *V. cholerae* O1 isolates. The performance of the PhP system was compared with ribotyping.

MATERIALS AND METHODS

V. cholerae O1 isolates

The isolates studied were from epidemic and endemic cases of cholera in humans. A total of 117 isolates were studied from the following five collections (Table I):

Table 1. *V. cholerae* collections studied

Collection no.	No. of isolates	Origin of isolates	Biotype	Serotype	Diversity Index
I	24	Mexico 1991	El Tor	Inaba	0.73
II	22	Bangladesh 1992	El Tor	Ogawa	0.84
III	22	Bangladesh 1974-1992	El Tor	Ogawa/Inaba	0.93
IV	33	Bangladesh 1961-1992	Classical	Ogawa/Inaba	0.97
V	16	Bangladesh 1993	El Tor	Ogawa	0.65

Collection I: Twenty-four isolates of *V. cholerae* O1 biotype El Tor, serotype Inaba, were obtained from the outbreak of cholera in Mexico in 1991.

Collection II: Twenty-two isolates of *V. cholerae* O1 biotype El Tor, serotype Ogawa, were obtained from field cases of endemic cholera in southern Bangladesh in 1992.

Collection III: Twenty-two isolates of *V. cholerae* O1 biotype El Tor, both Ogawa and Inaba serotypes, from endemic cholera cases, who attended the hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), located in Dhaka during 1974- 1992.

Collection IV: Thirty-three isolates of *V. cholerae* O1 belonging to classical biotype and both serotypes isolated from cholera patients who attended the ICDDR,B hospital in Dhaka, and from field cholera cases in southern Bangladesh, between 1961 and 1992. All of these isolates have been included in a ribotyping study previously (7).

Collections I and II were stored at -70° C in Trypticase soy broth (Gibco, NY, USA) with 15% glycerol, and collections III and IV were stored as lyophilised cultures until studied.

Collection V: Sixteen fresh isolates of *V. cholerae* O1 biotype El Tor, serotype Ogawa, from primary culture plates of taurocholate-tellurite-gelatin agar (TTGA) (12) inoculated with stool from endemic cholera cases, who attended the ICDDR,B hospital in Dhaka during January 1993. These isolates were included for stability test.

All isolates were reconfirmed as *V. cholerae* O1 by standard procedures (13). The procedures included biochemical reactions in motility-indole-urease medium and Kligler's iron agar, slide agglutination with polyvalent and serotype-specific antisera (ICDDR,B), and biotyping by chicken cell agglutination, polymyxin B susceptibility, and susceptibility to Mukherjee's classical and El Tor phages.

Biochemical fingerprinting with the PhP system

The PhP-VC plate (commercial supplier is BioSys inova, S-11351 Stockholm, Sweden) consists of 96-well microtitre plates with six sets of 15 substrates each. These 15 substrates were carefully selected from a larger set of 96 substrates for being those showing the highest discrimination and reproducibility among 80 unrelated *V. cholerae* isolates (all El Tor isolates; these were obtained from Africa in 1991, Peru in 1992 and Bangladesh during 1961-1992. The identity level (see below) obtained with these isolates was used for evaluating other strain collections used in the present study).

The 15 substrates used were: 1: D-galactose; 2: maltose; 3: D-trehalose; 4: D-lactose; 5: inositol; 6: glycerol; 7: L-rhamnose; 8: ß-methyl-D-glucoside; 9: D-gluconate; 10: D-mannitol; 11: potato starch; 12: glycogen; 13: fumarate; 14: pyruvate; 15: succinate; and the 16th well contained medium control with pH 8.2.

The stock culture was subcultured on a nutrient agar plate, and incubated at 37 ° C for 18-24 hours. One colony measuring approximately 2 mm was inoculated into 10 ml medium containing 0.1% (w/v) Bactopeptone (Difco, Detroit, MI, USA), 1.0% (w/v) sodium chloride, and 0.01% (w/v) bromothymol blue (pH 8.5). The bacterial suspension was added to a pre-prepared PhP-VC plate containing dehydrated substrates with the aid of a multi-channel pipette delivering 0.15 ml of broth culture into each well (a 96-well microtitre plate containing six sets of reagents could be used to test six different isolates.) To allow for proper rehydration of substrates, plates were stored at 4 ° C overnight, and then incubated at 37 ° C the following morning. On use of the substrate in a well, the colour of the bromothymol indicator changed. The absorbance of each reaction was measured at a wavelength of 620 nm in a microplate reader (Dynatech MR5000, Chantilly, VA, USA), at 7, 24, and 48 hours. The absorbance values were electronically transferred to a personal computer, multiplied by a factor of 10, and stored as integer values. After the last measurement at 48 hours, the mean value of all three readings was calculated for each reaction (Table IIa for an example). The biochemical fingerprint of an isolate thus consists of 16 quantitative numbers, each one ranging from 0 (acidic reaction = yellow colour on all measurements) to 30 (alkaline reaction = blue colour on all measurements) (Table IIb for an example).

Table IIa. Test results (biochemical fingerprins) from a *V. cholerae* isolate measured at three incubation times

Incubation time (h)	Substrate number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
7	12	6	4	21	4	17	21	7	4	4	8	5	18	22	8	20
24	4	5	3	21	4	4	21	3	4	4	8	9	18	26	9	24
48	3	5	4	21	4	3	21	3	3	3	9	7	18	26	24	26
Mean	6	5	4	21	4	8	21	4	4	4	8	7	18	25	14	23

Table IIb. Biochemical fingerprints of five *V. cholerae* isolates

Isolate no.	Biochemical fingerprint: Substrate number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1*	6	5	4	21	4	8	21	4	4	4	8	7	18	25	14	23
2	7	5	13	15	5	11	22	9	4	5	14	8	22	23	12	24
3	9	6	11	14	6	10	22	9	5	8	8	7	20	22	6	20
4	14	6	6	23	12	18	24	8	15	10	20	14	23	29	26	28
5	12	5	5	21	11	16	21	8	14	11	20	13	21	27	24	27

*The data for isolate 1 are extracted from Table IIa

Table IIc. Similarity matrix obtained from pair-wise comparisons of the biochemical fingerprints in Table IIb. The similarities were calculated as correlation-coefficients

Isolate no.	Correlation-coefficient compared to isolate no. 1	2	3	4	5
1	-				
2	0.90	-			
3	0.87	0.93	-		
4	0.89	0.78	0.67	-	
5	0.87	0.77	0.64	0.99	-

PhP types found: Isolates 1, 2 and 3 are single (S) types; isolates 4 and 5 belong to the same common (C) type if identity level is at correlation-coefficient >0.965

Table III. Comparison of stabilities of PhP types of 16 *V. cholerae* O1 isolates when stored for 30 days at different temperatures and after repeated subculture

Comparison between Sets	Mean similarity	No. of isolates identical
1 and 2	0.980	16
1 and 3	0.980	15
1 and 4	0.976	15
2 and 3	0.974	12
2 and 4	0.972	11
3 and 4	0.975	13

Set1: Isolates stored at +4 °C
Set2: Isolates stored at -70 °C
Set3: Isolates stored at room temperature, 25 °C
Set4: Isolates subcultured 30 times

The biochemical fingerprints of N isolates within a collection were compared pair-wise, and the similarity between each pair was calculated as the correlation- coefficient. This yielded a similarity matrix consisting of N x (N-1)/2 correlation coefficients (Table IIc for an example). The similarity matrix (Table IIc) was clustered according to the unweighted-pair group method with arithmetic averages (UPGMA) to yield a dendrogram (for an example, see Fig. 1) (14). The reproducibility of the PhP-VC plate was evaluated by assaying 24 of the above isolates in duplicate in the same assay. The level of identity (the ID level) between isolates was defined as the mean of the correlation-coefficients obtained between these duplicate assays minus two standard deviations (SD) of this mean (11) . PhP types consisting of more than one isolate were named C (common) types, whereas those consisting of only one isolate were named S (single) types.

All data handling, including optical readings, calculations of correlation-coefficients, diversity indices, as well as clustering and printing of dendrograms were performed using the PhP software (BioSys inova, Stockholm, Sweden), which is commercially available.

Stability of fingerprints upon storage at different temperatures and after subculture

The 16 fresh isolates of *V. cholerae* (collection V) were used for these tests. After confirmation of the colonies as *V. cholerae* O1 by biochemical reactions and slide agglutination test with specific antiserum, single isolated colony from each strain was subcultured onto nutrient agar (NA) plates and incubated at 37 ° C. From this first subcultured NA plate, three different sets of stock cultures were prepared. Set 1 was stored at 4 ° C on NA plates; set 2 was stored at -70 ° C in Luria broth containing 15% (v/v) glycerol; set 3 was stored at room temperature (25 ° C) in T1N1 soft agar. Finally, 30 consecutive subcultures were made from the first subcultured NA plate by transferring a single colony to a new agar plate every day. The 30th subculture was marked as set 4. Finally, from all four sets, subcultures were made once on nutrient agar, and after 20 hours incubation, the growth was used for biochemical fingerprinting, which was done in duplicate and blindly.

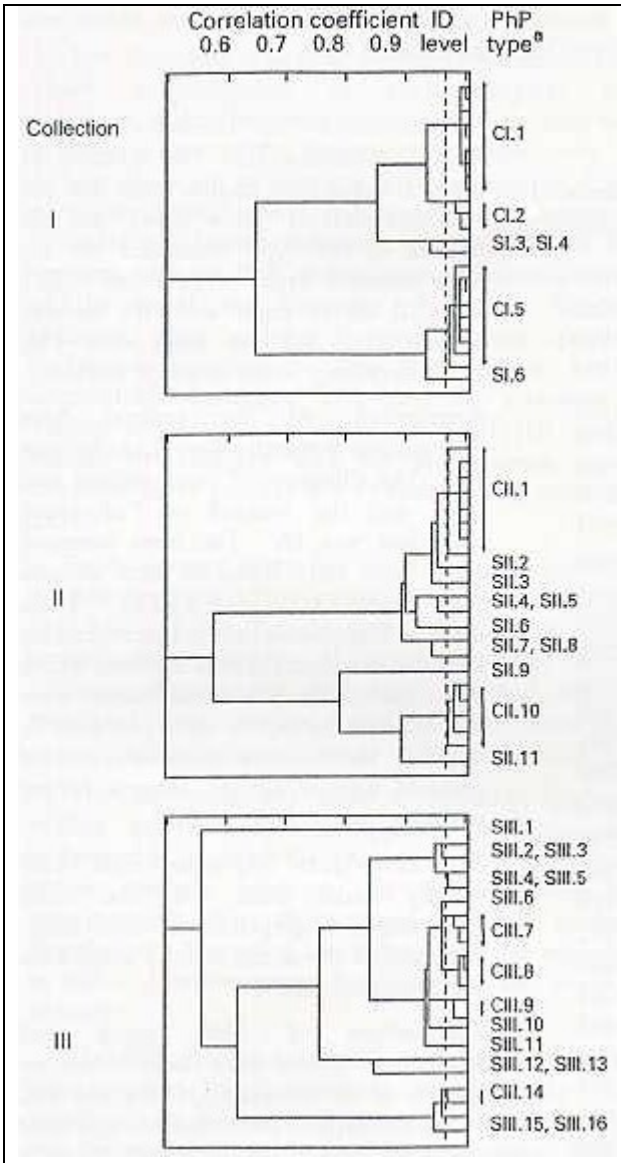

Fig. 1. Three dendrograms showing the relationship among the PhP type of V cholerae 01 isolates from three different collections. Dotted lines indicate the level of identity (correlation-coefficient >0.965).

Collection I: Twenty-four E1 Tor V.cholerae 01 isolates obtained from the epidemic cholera in 1991 in Mexico.

Collection I: Twenty-four E1 Tor V.cholerae 01 isolates obtained from the epidemic cholera cases in southern Bangladesh in 1992.

Collection I: Twenty-four E1 Tor V.cholerae 01 isolates obtained from the epidemic cholera cases seen at the Dhaka hospital,Bangladesh,during 1974-1992.

Ribotyping

The rRNA gene probe used for ribotyping was a *Bam*HI fragment of a cloned *Escherichia coli* rRNA operon obtained from the recombinant plasmid pKK3535 (15). The recombinant plasmid was purified and digested with *Bam*HI, and the insert was purified by electroelution from agarose gel as described by Maniatis *et al*. (16). The insert (probe DNA) was labelled by random priming (17) with [a -^{32}P]deoxycytidine triphosphate (3000 Ci/mmol, Amersham International plc., Aylesbury, United Kingdom) and a random primer DNA labelling system (Bethesda Research Laboratories, Gaithersburg, MD, USA). Southern blots of purified bacterial chromosomal DNA after digestion with restriction enzymes *Bg*lI and *Hind*III (Bethesda Research Laboratories) were hybridised with labelled rRNA probe, washed under stringent conditions, and autoradiographed as described previously (18). The ribotyping data on 33 classical biotype of *V. cholerae* O1 (listed as collection IV in the section Materials and Methods) were extracted from a previous publication (7).

Comparison between PhP and ribotyping

The performances of PhP typing and ribotyping were compared using the 33 isolates in collection IV.

The isolates were typed with both methods, and assigned into types. The discriminatory power of each typing method was calculated as Simpson's diversity index (Di) according to the formula: Di = 1 -S [ni x (ni - 1)/(N x (N - 1)] (19), where ni is the number of isolates in the ith type, and N is the total number of isolates studied (in this case 33). The value of this index depends both on the number of different types identified and on how even the distribution of isolates into different types is. It is high (maximum value 1.0) if most isolates belong to different types, and is low (minimum value 0) if one type is dominating.

RESULTS

Reproducibility of the PhP system

The intra-assay reproducibility was estimated by duplicate assays of the 33 isolates obtained from collection IV. The mean similarity among the isolates was 0.987, the standard deviation was 0.011, and thus, the identity level was set at a correlation-coefficient of 0.965. This yields a reproducibility of >95% (16), i.e. of 100 comparisons between identical strains, more than 95 appear as identical.

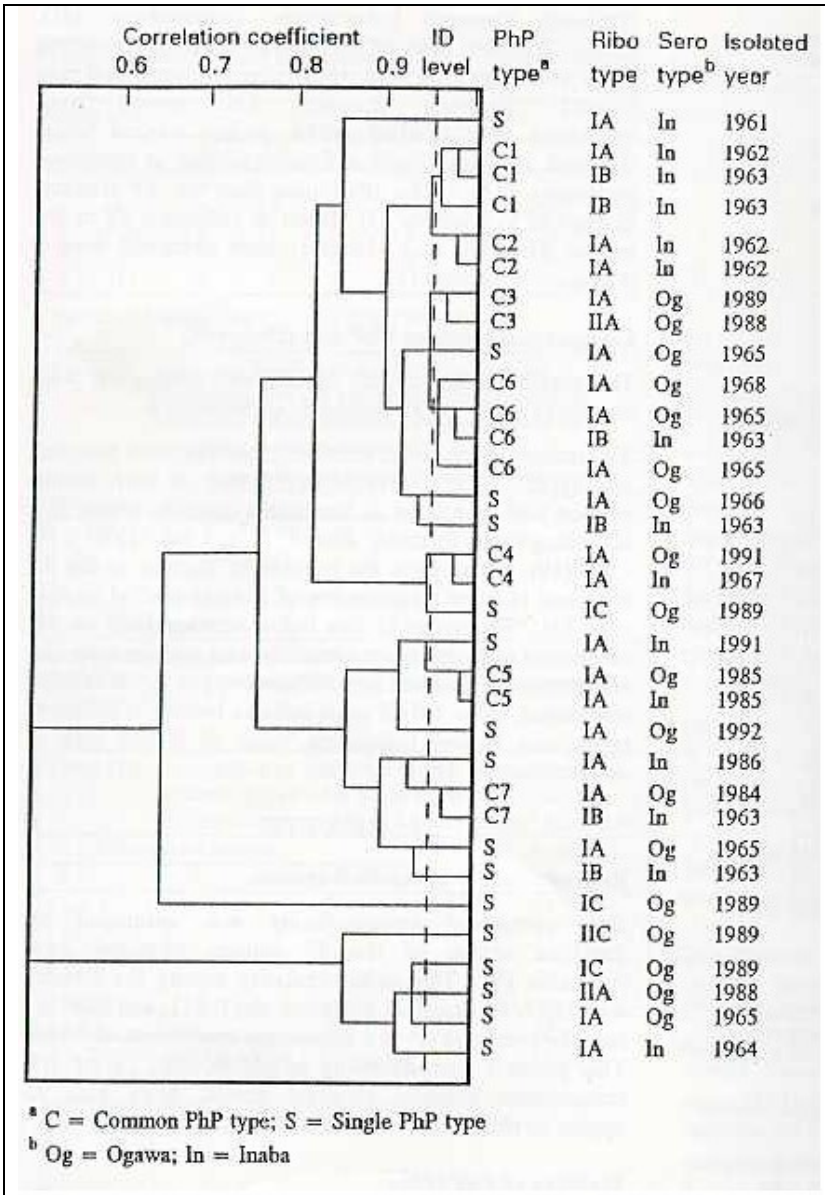

Fig. 2. Dendrogram showing the relationship among the PhP type of 33 classical biotype V. cholerae 01 isolates from a heterogeneous collection obtained during 1961-1992 (collection IV) in Bangladesh. Dotted line indicates the level of identity. The figures also show the ribotype and serotype of each isolate.

Stability of PhP types

The stability of PhP types of 16 freshly isolated *V. cholerae* O1 strains (collection V) under different temperatures of storage, and after repeated subculturing, was studied. The results are shown in Table III. When isolates of the same strain stored at 4 ^{o}C and -70 ^{o}C were compared, all yielded identical biochemical fingerprints and correlation-coefficients above the identity level. The lowest similarity was obtained when subcultured isolates were compared to those stored at -70 ^{o}C (only 11 of 16 identical). In total, 82 of 96 (84%) comparisons between the same isolates yielded the same PhP types, and in all the other 14 cases, the PhP types were still similar, showing a similarity of >0.95.

Typing of *V. cholerae* O1 by PhP system

Mexican isolates (Fig. 1, collection I): As expected of an epidemic, these isolates showed a low diversity (Di = 0.73), and only six different PhP types were found. Eighteen of the isolates consisted of two dominant PhP types (CI.1 and CI.5), three isolates belonged to a PhP type similar to CI.1 (type CI.2) and another isolate was similar to PhP type CI.5 (SI.6).

*Bangladeshi El Tor isolates from field (*Fig. 1*,* collection II): The diversity of these isolates was higher than for the Mexican isolates (Di = 0.84), and the number of PhP types identified was 11. Two common types were found (CII.1 and CII.10) in eight and five isolates respectively, and the other nine PhP types were only found in single isolates.

Bangladeshi El Tor isolates from hospitalised patients (Fig. 1, collection III): The diversity of these isolates was 0.93, and the number of PhP types identified was 16. The most common types were only found in three isolates (PhP types CIII.7 and CIII.8). Thus, these isolates were more heterogeneous than the Bangladeshi field isolates, which was expected since the field isolates were all collected during the same year (1992), whereas the isolates from hospitalised patients were collected over a longer period of time (1974 to 1992).

The above three sets were tested twice blindly under code, and the same biochemical fingerprints and correlation- coefficients above the identity level were obtained both times.

Comparison of PhP typing and ribotyping: Thirty-three classical biotype strains of *V. cholerae* O1 (Ogawa and Inaba serotypes) were studied, and their ribotype data were extracted from a previous publication (4). A comparison of PhP types, ribotypes and serotypes is shown in Fig. 2. The isolates fell into five different ribotypes, and 23 different PhP types. A combination of PhP typing and ribotyping yielded 27 different types. If serotyping was also included, 29 different types were found, and only three types containing more than one isolates were found (two isolates of PhP type C1, both isolates of PhP type C2, and three isolates of PhP type C6). In two of these types the isolates were also from the same year, which indicated that they might be of the same clone. The discriminatory power as defined by Di was 0.51 for serotyping, 0.58 for ribotyping, 0.97 for PhP typing and 1.0 for the combination of all three methods.

DISCUSSION

Earlier, biotyping was used as one of the techniques for strain differentiation in epidemiological studies. However, this technique had limitations, because too few substrates were used. Moreover, reactions were scored only qualitatively as either positive or negative at the end of the incubation period, and there was no accurate way of assessing intermediate results. Biochemical fingerprinting with the PhP system uses quantitative measures of the speed and intensity of several biochemical reactions, thus making it more discriminatory than traditional biotyping. The PhP system has been successfully developed and used for subtyping of a variety of bacteria for epidemiological and ecological studies (9-11,20,21), and the performance has been shown to be as good as that of DNA typing methods (20, 22, 23).

In the present study, we developed and evaluated the performance of the PhP system for typing *V. cholerae* O1. Our data indicate that the system is discriminatory and reproducible. Stability of fingerprints after storage at different temperatures and after repeated subcultures suggested that optimal results were achieved by preserving the isolates either at +4 ^{o}C or -70 ^{o}C.

The PhP system was further evaluated by applying the system to type epidemiologically well-characterised isolates of *V. cholerae* O1. As expected, isolates from the recent cholera epidemic in Mexico were more homogeneous than those from endemic cholera in Bangladesh. Moreover, the PhP system was reproducible as identical results were obtained when the assays were repeated.

The PhP system was compared with an established typing system for *V. cholerae*, i.e. ribotyping. It was found that the discrimination achieved by the PhP system was higher than that obtained by ribotyping. However, some degree of correlation between the two typing systems was observed since, when the isolates belonged to the same PhP type, they usually also belonged to the same ribotype. The main advantages of PhP typing, as compared to DNA typing are its simple performance and automatic data evaluation and presentation. It is, thus, a very convenient system for studies of large numbers of bacteria.

Other advantages of the PhP system are that numerical data of high precision are generated enabling cluster analysis, and new and old data out of the computer memory can also be easily compared. In contrast, molecular typing methods based on electrophoresis may result in reproducibility problems and difficulties in comparing data generated on different gels. These methods are, thus, mostly restricted to investigations where only a few strains need to be compared. For more careful epidemiological investigations of many isolates, a combination of methods should be useful, where PhP typing is used as a first screening method and suitable DNA typing methods are applied to a selected number of isolates based on their biochemical fingerprints.

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REFERENCES

1. Albert MJ, Ansaruzzaman M, Bardhan PK, et al. Large epidemic of cholera-like disease in Bangladesh caused by *V. cholerae* O139 synonym Bengal. *Lancet* 1993;342:389-90.
2. Janda JM, Powers C, Bryant RG, Abbott SL. Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clin Microbiol Rev* 1988;1:245-67.
3. Popovic T, Bopp CA, Olsvik O, Wachsmuth K. Epidemiologic application of a standardized ribotype scheme for *Vibrio cholerae* O1. *J Clin Microbiol* 1993;31:2474-82.
4. Wachsmuth IK, Bopp CA, Fields PI. Difference between toxigenic *Vibrio cholerae* O1 from South America and the U.S. Gulf coast. *Lancet* 1991;1:1097-8.
5. Cameron DN, Khambaty FM, Wachsmuth IK, Tauxe RV, Barrett TJ. Molecular characterization of *Vibrio cholerae* O1 strains by pulsed-field gel electrophoresis. *J Clin Microbiol* 1994;32:1685-90.
6. Salles CA, Momen H. Identification of *Vibrio cholerae* by enzyme electrophoresis. *Trans R Soc Trop Med Hyg* 1991;85: 544-7.
7. Faruque SM, Alim ARMA, Rahman MM, Siddique AK, Sack RB, Albert MJ. Clonal relationships among classical *Vibrio cholerae* O1 strains isolated between 1961 and 1992 in Bangladesh. *J Clin Microbiol* 1993;31:2513-6.

8. Möllby R, Kühn I, Katouli M. Computerised biochemical fingerprinting - a new tool for typing of bacteria. *Rev Med Microbiol* 1993;4:231-41.

9. Katouli M, Kühn I, Lund A, Wallgren P, Söderlind O, Möllby R. Phenotypic characterization of the intestinal *Escherichia coli* of pigs during suckling, post-weaning and fattening periods. *Appl Environ Microbiol* 1995;61:778-83.

10. Kühn I, Allestam G, Stenström TA, Möllby R. Biochemical fingerprinting of water coliform bacteria, a new method for measuring the phenotypic diversity and for comparing different bacterial populations. *Appl Environ Microbiol* 1991;57:3137-77.

11. Kühn I, Brauner A, Möllby R. Evaluation of numerical typing systems for *Escherichia coli* using the API50CH and the PhP-EC systems as models. *Epidemiol Infect* 1990;105:521-31.

12. Monsur KA. A highly selective gelatin-taurocholate-tellurite medium for the isolation of *Vibrio cholerae*. *Trans R Soc Trop Med Hyg* 1961;55:440-2.

13. Kay BA, Bopp CA, Wells JG. Isolation and identification of *Vibrio cholerae* O1 from fecal specimens. *In:* Wachsmuth IK, Blake PA, Olsvik O, editors. *V. cholerae* and cholera: molecular to global perspectives. Washington DC: American Society for Microbiology, 1994: 3-25.

14. Sneath PHA, Sokal RR. Numerical taxonomy. New York: Freeman, 1973.

15. Brosius J, Ullrich A, Raker MA, *et al.* Construction and fine mapping of recombinant plasmids containing the rrnB ribosomal RNA operon of *Escherichia coli*. *Plasmid* 1981;6:112-8.

16. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982.

17. Feinberg A, Vogelstein B. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 1984;137:266-7.

18. Stull TL, LiPuma JJ, Edlind TD. A broad spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. *J Infect Dis* 1988;157:280-6.

19. Hunter PR, Gaston MA. Numeric index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J Clin Microbiol* 1988;26:2465-6.

20. Kühn I, Huys G, Jansen P and Kersters, K. Diiversity and stability of coliforms and *Aeromonas* in water from a well, studied over four years of time. *Canadian J Microbiol* 1997 (in press).

21. Kühn I, M. Albert J, Ansaruzzaman M, Bhuiyan NA, Alabi SA, Huys G, *et al.* Hybridization groups, genotypes and virulence factors among Aeromonas strains isolated from patients with diarrhoea, from healthy controls and from environmental sources in Bangladesh. *J Clin Microbiol* 1997 (in press).

22. Kühn I, Burman L.G, Haeggman S, Tullus K, Murray B.E. Biochemical fingerprinting for epidemiological typing of enterococci compared with ribotyping and pulsed field gel electrophoresis of DNA. *J Clin Microbiol* 1995;33:2812-2817.

23. Kühn I, Austin DA, Austin B, Blanch AR, Grimont PAD, Jofre J, *et al.* Diversity of *Vibrio anguillarum* isolates from different geographical and biological habitats, determined by the use of a combination of eight different typing methods. *System Appl Microbiol* 1996;19:442-450.

Adherence, Invasion and Cytotoxin Assay of *Campylobacter jejuni* in HeLa and HEp-2 Cells

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ABSTRACT

Campylobacter jejuni is an important human enteropathogen worldwide. Chickens are the major reservoir and source of campylobacter infection. Ten clinical isolates from human and five chicken strains were tested for the adherence, invasion and cytotoxin assay in HeLa and HEp-2 cells. All human strains adhered to both the HeLa (10^3 to $3x10^4$ bacteria/mL of cell lysate) and HEp-2 cells ($2x10^3$ to $4x10^4$ bacteria/mL of lysate). All chicken strains also adhered to the HEp-2 cells (10^2 to 10^3 bacteria/mL), but only two strains adhered to the HeLa cells. Six clinical and none of the chicken strains invaded the mammalian cells. Both the adherence and invasion were better observed in HEp-2 than in HeLa cell lines. All three isolates from patients having invasive diarrhoea and only one strain from a patient having watery diarrhoea produced cytotoxin. All three invasive strains also adhered to polystyrene surface after the localised destruction of the HEp-2 cells, a phenomenon not reported earlier. Adherence was markedly inhibited by the whole cell lysate and the acid glycine extracts, and the results were comparable. This study indicates that the clinical isolates of *C. jejuni* are more virulent than the chicken strains, HEp-2 is better for the adherence/ invasion assay and HeLa is better for cytotoxin assay. The acid glycine extracts probably contain the key adhesins for *C. jejuni.*

Key words: *Campylobacter jejuni*; Enteritis; Virulence; Bacterial adhesions; Cytotoxins; Tissue culture

INTRODUCTION

Campylobacter jejuni is an important human enteropathogen worldwide (1,2). The mechanism of pathogenesis of *C. jejuni* infection is not yet clear though the adhesion, invasion, production of enterotoxin and cytotoxin have been reported to be the possible virulence factors (3-5). The least understood aspect of Campylobacter virulence is the interaction between the organism and the intestinal epithelial cells. The in vitro adhesion to epithelial cells, and both the adhesion and invasion of HEp-2 cells by surface-related antigens of *C. jejuni* have also been reported (6,7), but the identification of surface component responsible for the adhesion and invasion remains inconclusive .

Cell culture is a useful tool to study the attributes of bacterial virulence, since a uniform population of cells can be infected under defined conditions. The present study was carried out to assay the adherence, invasion and production of cytotoxin by *C. jejuni* strains in HeLa and HEp-2 cells with clinical correlation and inhibition of adhesion using different cell components to locate the key adhesin or adhesins.

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MATERIALS AND METHODS

Bacterial strains

Fifteen strains (10 human and 5 chicken) were included in the study. The human strains were isolated as the sole pathogens from patients having either watery diarrhoea (n=7) or a dysentery-like syndrome (n=3), and the chicken strains were isolated from intestinal contents of healthy chickens. *Salmonella typhimurium* ATCC-13311 and *Escherichia coli* K12 were used as the positive and negative controls respectively.

Bacterial growth

The strains were grown on Columbia agar (HiMedia), containing 7% sheep blood at 37 ^{0}C in a candle jar for 40 hours. For the preparation of cytotoxin, 5-6 colonies were inoculated in Columbia broth as described earlier, and the cell-free culture filtrate was prepared following the standard protocol (4,5).

Cell lines

HeLa and HEp-2 cell lines were used for assaying the adherence, invasion and cytotoxin production by the *C. jejuni* strains. The cell lines were maintained in Eagle's minimal essential medium (MEM) with 10% foetal calf serum (FCS). For this study, 24-well tissue culture plates (Nunc, Denmark) were seeded with $5x10^4$ cells per mL. The plates were incubated at 37 ^{0}C in a humidified 5% CO_2 incubator (Sanyo, Japan) till the semi-confluent monolayers were obtained. Prior to the experiment, the cells were washed and incubated with MEM, containing 1% FCS.

Adherence and invasion assay

Bacterial suspension was prepared in phosphate-buffered saline (PBS; pH 7.0) from the plates. The suspension was centrifuged at 10,000 x g for 10 minutes at 4 ^{0}C. The bacterial pellet was resuspended in MEM with 1% FCS, and the inoculum was adjusted to 10^7-10^8 bacteria per mL by measuring the optical density. The adherence and invasion assays were done as per the method of Konkel *et al.* (8).

(i) *Monolayers in 24 wells:* The inoculum (0.5 mL) was added to the HeLa and HEp-2 monolayers in duplicate. The plates were incubated at 37 ^{0}C in 5% CO_2 atmosphere for three hours. The monolayers were washed five times with MEM, containing 1% FCS. In one of the duplicate wells, gentamicin (250 m g/mL) was added and incubated for one hour. *C. jejuni* being sensitive to gentamicin, the antibiotic killed the bacteria that adhered to the surface of the tissue culture, while the bacteria that had already invaded and internalised the model remained unaffected. In the second well, the medium without the antibiotic was added to enumerate the number of bacteria that had adhered and invaded the cell lines. The monolayers were lysed using 0.01% Triton X-100. The lysed monolayer suspensions were diluted (10^{-1} to 10^{-4}) in PBS, and 100 m L of each dilution was uniformly plated on blood agar (Columbia agar base with 7% sheep blood). The number of viable bacteria was determined by counting the colony-forming units (CFU) on the plates, multiplied by the dilution factor. Viable bacteria recovered from the first well (with gentamicin) and from the second well (without gentamicin) were the intracellular (invasion) and the extracellular (adherence) + intracellular counts respectively. The adherence was calculated by the formula: [(CFU/mL from the second well at particular dilution - CFU/mL from the first well at the same dilution) x dilution factor].

(ii) *Monolayers in Petri dishes:* Confluent to semi-confluent monolayers were prepared in plastic 60x15-mm Petri dishes (Nunc, Denmark). The inoculum (1 mL) was added to the monolayers in duplicate. One set each of the Petri dishes was incubated at 37 ^{0}C for three hours and 24 hours. The monolayers were washed and treated with Giemsa stain.

Preparation of Campylobacter surface proteins

(I) *Whole cell lysate (WCL):* Bacterial strains were grown under standard conditions as described earlier. Suspensions were prepared in PBS, washed thrice, and turbidity was matched with MacFarland tube 5 ($1.5x10^9$ CFU per mL). The cells were sonicated in Branson's probe sonicator: 8 bursts, one minute each with rests on ice of 30 seconds in between bursts. The sonicated material was centrifuged at 12,000 x g for 15 minutes to remove intact bacteria, if any.

(ii) *Outer-membrane proteins:* Outer-membrane proteins (OMPs) were prepared as described by Blaser *et al.* (9). In brief, the bacterial growth was harvested and washed in 20 mM Tris hydrochloride buffer (pH 7.5) at 4 ^{0}C. Finally, the cells were suspended in the same buffer, and the suspension was sonicated by Branson's sonicator four times, 30 seconds each with rest on ice. Cell debris was removed by centrifugation at 10,000xg for 15 minutes. Supernatant was centrifuged at 100,000xg for 40 minutes at 4 ^{0}C to get the crude membrane as pellet. The crude membrane was then suspended in 2% sodium lauryl sarcosine (pH 7.5) for 20 minutes at 37 ^{0}C before it was centrifuged at 100,000xg for 60 minutes at 4 ^{0}C. The extracted membrane pellet was washed twice in 20 mM Tris hydrochloride buffer, and it was finally suspended in distilled water.

(iii) *Acid glycine extracts:*_C. jejuni_ was grown on Columbia blood agar as described earlier. Acid glycine extract (AGE) was prepared following the standard protocol (6). Briefly, the growth was harvested in PBS. After centrifugation, the bacterial pellet was collected and washed twice in PBS. It was then suspended in 0.2 M glycine hydrochloride (pH 2.2). After being stirred for 30 minutes at room temperature, the whole cells were removed by centrifugation at 12,000xg for 15 minutes. The supernatant designated as the glycine acid extract was neutralised with NaOH and dialysed against PBS.

Study on inhibition of adherence by WCL, OMPs and AGE

Tissue cultures in wells were incubated in duplicate with WCL (250 m g/mL), OMPs, and AGE (100 m g/mL) for one hour at 37 ^{0}C in a CO_2 atmosphere. After washing three times with MEM, containing 1% FCS, the cells were challenged with live bacteria for a standard period of 3 hours, followed by gentamicin treatment in one of the duplicate monolayers as described earlier. Finally, the counts of the adhering bacteria were made by the dilution technique following the formula mentioned earlier.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE)

Both the whole cell lysate and glycine acid extracts from invasive and adherent strains were subjected to SDS PAGE following the method of Laemmli (10). The bands were stained with Coomasie brilliant blue.

Cytotoxin assay

The cytotoxin assay was done as per the standard methods (11,12). The cell-free culture filtrate was concentrated five-fold using polyethylene glycol 600 (SRL, India). The concentrated culture filtrate was diluted to its original volume in MEM with 1% FCS when testing for the cytotoxin activity. The monolayers containing medium without culture filtrate served as the control. The monolayers in 24-well plates were incubated with 0.5 ml culture filtrate for 24 hours at 37 ^{0}C in 5% CO_2 environment. The monolayers were then fixed with 1% glutaraldehyde for 15 minutes and stained with 0.1% crystal violet for 30 minutes. The plates were then submerged in water, and the stain was removed with continuous, slow flow of water for 15 minutes. The plates were then air-dried. The stain was eluted with 0.2% Triton X, and the absorbance was measured at 590 nm. Percentage of cell destruction was calculated according to the formula: {(A-B)/A}x100, where A= OD in control and B= OD in test at 590 nm.

RESULTS

All the *C. jejuni* strains isolated from humans adhered to both the HeLa and HEp-2 cells with a range of 10^3 to $4x10^4$ CFU/mL of cell lysate. All the chicken strains also adhered to the HEp-2 cells (range 10^2 to 10^3 CFU/mL of lysate), but only two strains adhered to the HeLa cells. Six (60%) human strains were found to invade (internalise) both the HeLa and HEp-2 cells. Both adherence and invasion were more marked in the HEp-2 cells than in the HeLa cells (adherence: HeLa vs. HEp-2 = 10^3-$3x10^4$ vs. $2x10^3$-$4x10^4$; invasion: 2x10-$2x10^2$ vs. 2x10-10^3 CFU/mL of cell lysate). *C. jejuni* strains isolated from patients having pus cells and RBCs in their stools were found to be more invasive (10-30 fold) compared to *E. coli* K12 in the HEp-2 cells. None of the chicken strains invaded either the HeLa or HEp-2 cell line. The details of adherence and invasion assays are shown in Table I.

Table I: Virulence assays of *C. jejuni* (n=15) isolated from humans (H) and chicken (C)

	Adherence		Invasion		Cell destruction (%)	
Strain	HeLa	HEp-2	HeLa	HEp-2	HeLa	HEp-2
S. typhimurium (ATCC-13311)	$5.0x10^4$	$1.0x10^5$	$2.0x10^3$	$1.0x10^4$	>50	25-50
H10	$2.0x10^3$	$1.0x10^4$	0.0	0.0	<25	<25
H11	$1.0x10^3$	$2.0x10^3$	0.0	0.0	<25	<25
H24	$2.0x10^3$	$3.0x10^3$	0.0	0.0	<25	<25
H25*	$5.0x10^3$	$1.0x10^4$	$1.0x10^2$	$1.0x10^3$	>50	25-50
H26	$1.0x10^3$	$1.0x10^4$	2.0x10	2.0x10	>50	25-50
H27*	$2.0x10^3$	$2.0x10^4$	$1.0x10^2$	$2.0x10^3$	>50	25-50
H28	$1.0x10^3$	$1.0x10^4$	0.0	0.0	<25	<25
H42	$2.0x10^3$	$3.0x10^3$	2.0x10	$2.0x10^2$	<25	<25
H44*	$3.0x10^4$	$4.0x10^4$	$2.0x10^2$	$3.0x10^3$	>50	25-50
H45	$1.5x10^3$	$2.0x10^4$	3.0x10	$2.0x10^2$	<25	<25
C10	0.0	$2.0x10^2$	0.0	0.0	<25	<25
C11	3.0x10	$1.0x10^3$	0.0	0.0	<25	<25
C20	0.0	$1.5x10^2$	0.0	0.0	<25	<25
C21	$1.0x10^2$	$2.0x10^2$	0.0	0.0	<25	<25
C22	0.0	$1.0x10^2$	0.0	0.0	<25	<25
E. coli K12	$1.0x10^3$	$2.0x10^3$	$0.5x10^2$	$1.0x10^2$	<25	<25

* Invasive strains also adhered to polystyrene surface

Following three hours incubation with live bacteria, no cell destruction was observed by Giemsa staining of monolayers after washing five times with buffer. However, after 24 hours of incubation of monolayers with live bacteria, localised cell destruction with adhered bacteria to Petri dish (polystyrene) surface was observed in all three invasive strains, a phenomenon not described earlier. None of the other *C. jejuni* strains expressed this property.

All the three invasive strains expressed cytotoxic activity both in HeLa and HEp-2 cells. The cytotoxic activity was better observed in the HeLa cells (cell destruction >50%) than in the HEp-2 cells (cell destruction 25-50%). All other strains expressed a low level of cell destruction, comparable to the negative control *E. coli* K 12. The details of cytotoxin activity are shown in Table I. An inhibition of adherence to the HEp-2 cells by all three invasive strains was studied using WCL, OMPs, and AGE. A better and similar inhibition was observed with WCL and AGE (Table II).

Table II: Effects of various cell components on adherence to HEp-2 cells by *C. jejuni* strains isolated from patients having invasive diarrhoea

		Adherence after one hour incubation with		
Strain	Actual adherence	WCL (250 µg/mL)	OMPs (100µg/mL)	AGE (100µg/mL)
H25	$1.0x10^4$	$1.5x10^2$	$3.0x10^2$	$2.0x10^2$
H27	$2.0x10^4$	$1.0x10^2$	$3.0x10^3$	$1.2x10^2$
H44	$4.0x10^4$	$1.8x10^2$	$2.0x10^3$	$2.1x10^2$

WCL: whole cell lysate, OMPs: outer-membrane proteins and AGE: acid glycine extracts of *C. jejuni*.

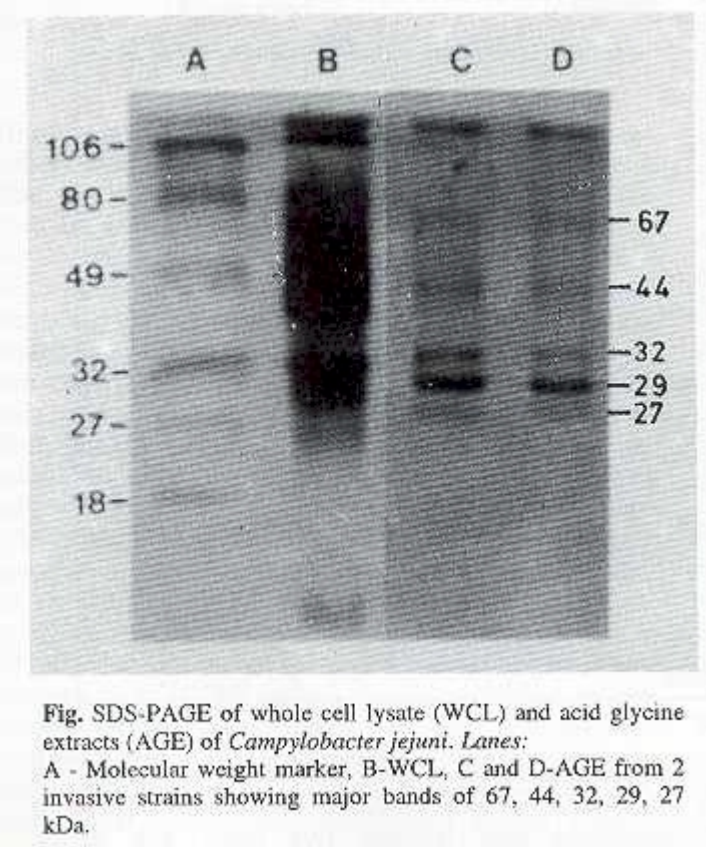

Fig. SDS-PAGE of whole cell lysate (WCL) and acid glycine extracts (AGE) of *Campylobacter jejuni*. *Lanes:*
A - Molecular weight marker, B-WCL, C and D-AGE from 2 invasive strains showing major bands of 67, 44, 32, 29, 27 kDa.

On SD-PAGE, AGE showed multiple major protein bands of various molecular sizes: 67, 44, 32, 29 and 27 kDa. All these bands were present in WCL along with many more protein bands (Fig.).

DISCUSSION

The clinical and experimental studies showing adhesion to and invasion of human epithelial cells suggest that such phenomena are important in the pathogenesis of campylobacter infection (13,14). Adhesion is considered to be the first step of bacterial-intestinal cell interaction. In the present study, all the clinical isolates adhered to both the HeLa and HEp-2 cells, corroborating the earlier studies (3,15). All the chicken strains adhered to the HEp-2 cells, but at least three of the five strains did not adhere to the HeLa cells. So far, no study on the adherence and invasive properties of chicken strains of *C. jejuni* had been carried out, though this poultry population had been reported to be a major reservoir of *Campylobacter* spp. (16,17). Bacterial adherence to HEp-2 cells was much higher than to HeLa cells (Table I). Only six of the 10 human strains and none of the chicken strains invaded both HeLa and HEp-2 cells. Like adherence, invasion was also better observed in the HEp-2 cells. This study clearly indicates that the HEp-2 cell line is a better model than HeLa cells for studying the adherence and invasive properties of this organism. While both the cell lines have been used for detecting the virulence attributes of *Campylobacter* spp. (6,7,15), no comparative evaluation had been reported so far. The strains isolated from patients having a dysentery-like syndrome invaded the HEp-2 cells at least 5 to 15-fold and 10 to 30-fold more than strains isolated from patients with watery diarrhoea and *E. coli* K12 respectively, thus indicating that the *C. jejuni* strains isolated from patients with invasive diarrhoea are potent invaders of mammalian cells in vitro. The invasive strains also adhered to the polystyrene surface after the localised cell culture destruction, a phenomenon not described earlier. However, the adherence to the polystyrene surface as reported in other enteric bacteria is related to cell surface hydrophobicity (18). Konkel and Joens (3) reported adherence to be multifactorial, attributed to a variety of surface components. In the present study, the inhibition of adhesion was studied using different cell components, like WCL, OMPs,

and AGE. Though WCL was found to be the best inhibitor at a higher concentration (250 m g/mL), the result with AGE was equally promising even at a lower concentration (100 m g/mL), indicating that AGE possibly contains the key adhesins. A similar observation had been reported by Kervella *et al.* (15) who identified two acid glycine-extracted proteins of 27 and 29 kDa as the key adhesins. The outer-membrane proteins of 26-30 kDa have been suggested as adhesins by Fauchere *et al.* (14), while Konkel *et al.* (7) identified antigens of 38-42 kDa only in invasive strains of *C. jejuni*. On SDS PAGE, the acid glycine extracts of all three invasive as well as the adherent strains showed almost all antigens (27, 29, 32, 36, 44 kDa) reported in the literature as adhesins and invasins. This highlights the role of acid glycine-extracted proteins in the pathogenesis of *C. jejuni* infection. The minor differences in size of the antigens may be related to different concentrations of gel used in the studies. Several studies on the production of enterotoxin by *C. jejuni* isolated in our country are available (5,19). So far, little is known about the cytotoxigenic status of *C. jejuni* in India. In the present study, all three invasive strains produced cytotoxin, while only one of the seven strains isolated from patients having watery diarrhoea and none of the five chicken strains produced cytotoxin. It indicates that the *C. jejuni*-infected patients having a dysentery-like syndrome harbour the cytotoxigenic strains. The prevalence rate of cytotoxigenic strains of *C. jejuni* had been reported to vary in different countries. Johnson and Lior (4) reported that 85% of the human isolates produced cytotoxins or cytotonic factors or both. Recently, it has been reported from Turkey that 93.5% of clinical isolates produced cytotoxin (20). Possibly the high prevalence rate of cytotoxigenic strains might be related to epidemiological characteristics of *C. jejuni* infections. Indeed, it has been reported that 20-80% of patients with *C. jejuni* infection had dysentery-like syndrome (21). Various tissue cultures (HeLa, MRC-5, HEp-2, Vero) have been used for the detection of cytotoxin. In the present study, HeLa cells were more sensitive to *C. jejuni* cytotoxin than HEp-2 cells.

We conclude that clinical isolates of *C. jejuni* are more virulent to tissue cultures than chicken strains. Patients having invasive diarrhoea due to *C. jejuni* harbour cytotoxigenic strains in this northern part of our country. The HEp-2 cells are better for adhesion/invasion study, while the HeLa cells are better for the cytotoxin assay. Probably, acid glycine extracts contain the key adhesins/ invasins. Further study with purified components is needed to prove this.

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REFERENCES

1. *Blaser MJ, Reller LB. Campylobacter enteritis. N Engl J Med 1981;305:1442-52.*
2. *Pal SC, Nair GB. Epidemiology of Campylobacter in developing countries. ICMR Bull 1984;14:79-84.*
3. *Konkel ME, Joens LA. Adhesion and invasion of HEp-2 cells by Campylobacter spp. Infect Immun 1989;57:2984-90.*
4. *Johnson WM, Lior H. Cytotoxic and cytotonic factors produced by Campylobacter jejuni, Campylobacter coli and Campylobacter laridis. J Clin Microbiol 1986;24:275-81.*
5. *Prasad KN, Anupurba S, Dhole TN. Enterotoxigenic Campylobacter jejuni and C. coli in the aetiology of diarrhoea in northern India. Indian J Med Res 1991;93:81-6.*
6. *de Melo MA, Pechere JC. Identification of Campylobacter jejuni surface proteins that bind to eukaryotic cells in vitro. Infect Immun 1990;58:1749-56.*

7. *Konkel ME, Babakhani F, Joens LA. Invasion related antigens of Campylobacter jejuni. J Infect Dis 1990;162:888-95.*
8. *Konkel ME, Corwin MD, Joens LA, Cieplak W. Factors that influence the interaction of Campylobacter jejuni with cultured mammalian cells. J Med Microbiol 1992;37:30-7.*
9. *Blaser MJ, Hopkins JA, Berk RM, Vasil ML, Wang WLL. Identification and characterisation of Campylobacter jejuni outer membrane proteins. Infect Immun 1983;42:276-84.*
10. *Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-5.*
11. *Daikoku T, Suzuki S, Oka S, Takama K. Profiles of enterotoxin and cytotoxin production in Campylobacter jejuni and C. coli. FEMS Microbiol Lett 1989;58:33-6.*
12. *Gillies RJ, Dider N, Denton M. Determination of cell number in monolayer cultures. Anal Biochem 1986;159:109-13.*
13. *de Melo MA, Gabbiani G and Pechere JC. Cellular events and intracellular survival of Campylobacter jejuni during infection of HEp-2 cells. Infect Immun 1989;57:2214-22.*
14. *Fauchere JL, Rosenau A, Veron M, Moyen EN, Richard S, Pfister A. Association with HeLa cells of Campylobacter jejuni and C. coli isolated from human feces. Infect Immun 1986;54:283-7.*
15. *Kervella M, Pages JM, Pei Z, Grollier G, Blaser MJ, Fauchere JL. Isolation and characterization of two Campylobacter glycine extracted proteins that bind to HeLa cells membrane. Infect Immun 1993;61:3440-8.*
16. *Prasad KN, Narang P, Dhone PM. Isolation of Campylobacter spp from chicken and their enterotoxigenicity in rat ileal loops. Indian Vet Med J 1990;14:221-5.*
17. *Harris NV, Weiss NS, Nolan CM. The role of poultry and meat in the etiology of Campylobacter jejuni/ coli enteritis. Am J Pub Hlth 1986;76:407-11.*
18. *Rosenberg M. Bacterial adherence to polystyrene: a replica method of screening for bacterial hydrophobicity. Appl Environ Microbiol 1981;42:375-7.*
19. *Saha SK, Sanyal SC. Production and characterization of Campylobacter jejuni enterotoxin in a semisynthetic medium and its assay in rat ileal loops. FEMS Microbiol Lett 1990;67:333-8.*
20. *Durdal US, Hascelik G, Diker S, Semsettin U. Cytotoxin production by Campylobacter jejuni isolated from Turkish patients with diarrhoea. J Diarrhoeal Dis Res 1995;13:130-1.*
21. *Lindblom G, Kaijser B, Sjogren E. Enterotoxin production and serogroups of Campylobacter jejuni and Campylobacter coli from patients with diarrhoea and healthy laying eggs. J Clin Microbiol 1989;27:1272-6.*

Mothers' Health-seeking Behaviour in Acute Diarrhoea in Tlaxcala, Mexico

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ABSTRACT

This study, a cross-sectional survey, was conducted to assess how mothers take care of their children with diarrhoea and to develop a model of health-care seeking behaviour. Multistage sampling was used. Mothers whose children aged less than five years had suffered from diarrhoea in the last fortnight were included. Nurses interviewed the mothers to collect data. Variables included in the interview were: mothers' characteristics, children's characteristics, clinical data, treatment given by the mother, maternal health-seeking behaviour and mothers' information about diarrhoea and dehydration. Variables corresponding to the clinical data were grouped to identify dehydration signs and the need for medical care. Dehydration was defined as the presence of two or more of the following reported signs: thirst, sunken eyes, sunken fontanelle, or scanty urine. The need for medical care was defined as the presence of one or more of the following characteristics: illness lasting more than three days, vomiting, fever, bloody diarrhoea or dehydration. A sample of 747 mothers was obtained. Household treatments consisted of herbal teas to stop diarrhoea (52.3%), liquids to prevent dehydration (92.2%), symptomatic drugs (35.2%) and changes in feeding patterns (36.3%), which consisted in suppressing milk and dairy products and interrupting breast feeding (12.2%). Mothers sought medical assistance when they perceived a worsening of clinical conditions. Clinical signs statistically associated with their decision were: bloody diarrhoea, vomiting, illness longer than three days, weight loss, and fever. The signs of dehydration were not associated with health care-seeking because the mother did not recognise them. It is concluded that maternal educational programmes should emphasise, besides the proper use of oral rehydration therapy, teaching mothers to identify signs of dehydration as an indication to seek timely medical care.

***Key words*:** Diarrhoea, Infantile; Diarrhoea, Acute; Dehydration; KAP; Maternal behaviour

INTRODUCTION

Despite progress made to treat acute respiratory infections (ARI) and acute diarrhoea, these common childhood illnesses are still taking a heavy toll. Diarrhoea and ARI are among the five leading causes of infant mortality in the developing countries (1). In the past, provision of health care was primarily considered the domain of health providers (2). However, the current trend has recognised the importance of mothers and the family in identifying, caring for and preventing children's illnesses (3).

Maternal practices regarding health care have been recognised as important social and anthropological factors, explaining high mortality rates among children aged less than five years. Maternal literacy and health education (4), parental age, family's socioeconomic status (5,6) and access to health care (7) are among the factors mentioned. Consequently, efforts to better understand mothers' beliefs, attitudes, and health practices have been carried out (8,9). Regarding acute diarrhoea, the reported risk factors for an adverse outcome include the following: lack of information to identify complications, such as dehydration; limited use of oral rehydration solutions; inadequate maternal health-seeking behaviour and dietary modifications, such as restricting certain foods or breastfeeding (10). These factors have prompted decision-makers and researchers to involve the family and particularly mothers in community-based health programmes to reduce the burden of this disease (11).

In 1991, the Mexican Ministry of Health launched a state-wide inter-institutional programme in the state of Tlaxcala to lower mortality rates due to acute diarrhoea (AD) and acute respiratory infections in children aged less than five years (12). Key to this programme was an educational intervention aimed at improving mothers' knowledge and practices regarding these two illnesses. The first stage of this intervention was a research designed to gain insight into why mothers behave as they do in the treatment of AD and ARI. Diversity of mothers' treatments in both AD and ARI encouraged us to carry out a separate analysis of each disease.

This study, a cross-sectional survey, was conducted to assess how mothers take care of their children with diarrhoea and to develop a model of health-care seeking behaviour. Three issues are addressed here: (a) the treatments given by the mothers; (b) maternal decision-making regarding the use of drugs, home remedies, restriction of food and liquids, and (c) an explanatory model of maternal health-seeking behaviour. The importance of maternal education and of involvement of mothers in the design of community-based interventions is also discussed.

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MATERIALS AND METHODS

Study site

Tlaxcala, one of the smallest states of the Mexican Highlands, has a population of 761,277, of which approximately 13% are children aged less than five years. 64.8% of the people inhabit rural communities. Regarding the characteristics of the households, 70% have piped water supply, and almost 50% have sewerage connection.

In the state, government institutions and private physicians provide health care. The public health care system has 124 primary care clinics, staffed by 165 physicians. Also, about 300 private physicians are working in the state. Geographical distribution of the clinics, either public or private, and the ease of transportation by paved and country roads allow people to reach a clinic or health post in, on an average, about fifteen minutes. Government institutions provide medical services at an affordable cost. Furthermore, most people are enrolled in the social security health-care systems which constitute another way to provide health care. Thus, almost 100% of the population have access to professional health services. Nevertheless, provision of health care in the state has an insufficient impact on childhood mortality. During 1993, the mortality rate due to acute diarrhoea among infants was 2.18/1000, far above the national average of 1.66/1000 (13).

Study design

In a cross-sectional survey, we interviewed mothers with children aged less than five years who had had diarrhoea in the last fortnight and were healthy at the time of the interview. In households with more than one ill child, mothers were interviewed regarding the most recent case. Those whose children had diarrhoea at the time of interview or had concomitantly acute diarrhoea and acute respiratory infections or any other infectious diseases, were not included in the study.

Sampling design

A multistage sampling approach was used for obtaining the study population. State communities were divided into three strata according to the number of inhabitants: communities of less than 500, communities of 500 to 5,000, and communities of more than 5,000. In each stratum, communities were randomly selected. In every selected community, the households were also randomly selected from an existing census of households with children aged less than five years. This census was previously carried out by the Ministry of Health to obtain a better control of the immunisation campaigns. Sample size was calculated, using the formula to figure out the proportion in one population (14). It was calculated in each stratum under the assumptions that the proportion of mothers appropriately treating their children with acute diarrhoea was 60% ($p=0.60$), with an expected 6% of the tolerable level of imprecision ($D=0.06$). Therefore, a sample of 256 mothers was needed in each stratum, resulting in 768 mothers to be interviewed.

Data collection

In selected households, a mother expected to meet the selection criteria was searched for; if she was not found, adjacent households were searched until a mother who fulfilled the criteria was found. Trained nurses and social workers collected data by applying a semi-structured written questionnaire during a single personal interview. No mother objected to answer the questionnaire after the interviewers had explained the purpose of the study. This

type of interview was chosen since we decided to apply the questionnaire only once, because the interviewers had to travel up to two and a half hours to reach the households. We included thirty-three specific questions and provided a guide to the interviewer to apply eight open questions regarding the mother's decision-making process. All questions were formulated, using the colloquial language of the community.

The following variables were explored: (a) maternal characteristics, such as age, literacy, and occupation; (b) children's characteristics: age, sex, duration of illness, and signs, such as presence of bloody diarrhoea, weight loss, vomiting, fever, increased thirst, sunken eyes, scarce urine, and sunken fontanelle; (c) treatment given at the household, such as home remedies, liquids thought either to stop diarrhoea or to prevent dehydration, usage of drugs and changes of feeding patterns, particularly suppressing milk or breast feeding for some days.

Maternal health-seeking behaviour was explored by means of three open questions. The mother was asked about the place where the children have received care and her reasons to look for medical assistance or to give home treatment rather than request assistance from a physician. Her information about diarrhoea and dehydration was explored through five open questions. The first question was related to her concepts of diarrhoea and the way she recognises it; the second question asked about its causes. The third question asked about her concepts of dehydration. The fourth and fifth questions were about the mothers' knowledge as to how to prevent diarrhoea and dehydration. The aim of these questions was to let mothers express themselves in their own terms, and this was registered as such in the questionnaire.

Definition of variables

Variables corresponding to the clinical picture were grouped to identify dehydration and the need for medical care. Dehydration was defined as the presence of two or more of the following conditions: thirst, sunken fontanelle, sunken eyes, and scarce urine. The need for medical care was defined as the presence of one or more of the following conditions: illness lasting more than three days, vomiting, fever, bloody diarrhoea, or dehydration. Definitions were based on the World Health Organization (WHO) criteria (15).

Maternal information was evaluated, using the answers to the five questions. A mother was considered well-informed if she correctly answered three of the five questions. Mothers' concepts were also evaluated based on the WHO guidelines (16).

Liquids given by the mothers to prevent dehydration were classified in two types: liquids recommended by WHO, thus considered oral rehydration therapy (ORT), such as herbal teas, rice beverages, milk, water, home-based solutions, and oral rehydration solution (ORS), and liquids not recommended by WHO, such as carbonated beverages, commercial solutions, or wrongly combined solutions.

Data analysis

First, we conducted an exploratory analysis, using descriptive statistics. Population characteristics and household treatments, such as provision of home remedies, liquids to prevent dehydration, symptomatic drugs, and changes of feeding patterns were analysed in a descriptive way. Second, we explored whether there were any relationships among clinical data (isolated and combined), and the mother's decision to provide a specific liquid. Analysis was carried out by comparing children having or not having the clinical features with the specific liquid provided. We used chi-square tests to assess statistical significance.

Third, an ethnographic decision model (17) was created to establish further reasons for maternal health-seeking behaviour and to try to predict which type of service mothers will choose under the specific conditions of the child. We decided to build the model upon the need for medical care because we wanted to explore whether actual need prompted mothers to seek medical assistance and in what proportion. The model looked at their reasons for such decision. The first part of the model was drawn upon the actual need for medical care. The second part was based on the mothers' decision to go to a health facility. The third part was based on the classification of their reasons to seek or not to seek medical care.

Household treatment (provision of drugs, food restriction, and home remedies) and the mothers' health-seeking behaviour were both analysed regarding three variables: the need for medical care, dehydration, and maternal level of information. The chi square test was used for evaluating statistical significance.

The choice whether to provide care at home or at the health facility and its relation to the clinical signs was analysed in two steps: first, through univariate analysis, crude odds ratios were calculated to establish the probability of seeking medical care, then, a multiple logistic regression procedure was modelled based on the significant covariates from the univariate analysis (18).

RESULTS

Seven hundred and forty-seven mothers whose children had diarrhoea in the last fortnight were interviewed (Table I). Regarding the children's characteristics, 25.3% were infants, while the rest were pre-school children. Gender was evenly distributed in the sample. In relation to the maternal characteristics, the mothers' median age was twenty-seven years. Regarding literacy, 23.3 % were illiterate, and 41.6% had finished elementary school. Most mothers were homemakers, less than 5% had paid work (data not included in the table). As mentioned above, the sample was divided in three strata, according to the number of inhabitants. In the stratum of communities with less than 500 inhabitants, we did not complete the expected sample due to logistic difficulties. The sample of this stratum had been calculated to be 33% of the total sample; only 22.2% was achieved. On the other hand, there was an over-sampling of the second stratum, being 46.3%.

Table I. Population characteristics (n=747)

Children's characteristics	Percentage
Age	
0-6 months	6.6
7-11 months	18.7
1-5 years	81.3
Sex	
male	50.5
female	49.5
Mothers' characteristics	
Mean age, years (interval)	27 (15-43)
Mothers' literacy	
Illiterate	23.3
Primary school	41.6
Secondary school	23.6
High school	11.5
Community size (inhabitants)	
less than 500	22.2
501 to 5,000	46.3
more than 5,000	31.5

The analysis of household treatment (Table II) reflected an amalgam between modern medicine and traditional remedies for diarrhoea. It was observed that 52.3% of the children had received some home remedies thought to stop diarrhoea. The remedies were mostly herbal teas, of which camomile was the most commonly used (27.7%). Most children received liquids to prevent dehydration (92.2%). In relation to the type of liquids, mothers provided some form of oral rehydration therapy (71.8%). Mothers gave tea, rice beverages, milk, or water (53.0%), while 14.1% of the children were treated with ORS, and 4.7% took home-based solutions. On the other hand, 20.0% of the children received some type of non-recommended liquids. Carbonated drinks, commercial solutions, or wrongly combined ones were the most commonly used. Only 3.0% of the children did not receive any liquids at all, neither for treating diarrhoea nor for preventing dehydration.

Table II. Treatment given at home

Types of treatment	n=747	%
Home remedies	**391**	**52.3**
Camomile (*Matricaria recutita*)	207	27.7
Other teas (alone or combined)	184	24.6
Liquids to prevent dehydration	**689**	**92.2**
Oral rehydration therapy	536	71.8
Tea, rice beverages, milk, water	396	53.0
Oral rehydration solution	105	14.1
Home-based solutions	35	4.7
Not recommended liquids, such as carbonated beverages, commercial solutions, or wrongly combined solutions	153	20.4
Symptomatic drugs	**263**	**35.2**
Changes of feeding patterns	**271**	**36.3**
Withholding of milk	203	27.2
Restriction of other types of food	68	9.1
Withholding of breast feeding	10	12.2

Regarding the use of drugs, 35.2% of the children received some medication, mainly antidiarrhoeal suspensions or symptomatic drugs. As to changes in feeding patterns, 36.3% had some dietary restrictions. Milk was the most commonly restricted food (27.2%). Among the breast-fed infants, breast feeding was interrupted in 12.2%.

To look further at the relationship between clinical features and the mother's decision to provide a specific type of liquid, both variables were analysed. Results are shown in Table III. Children who had been ill for three or fewer days, those who had diarrhoea as the single manifestation of disease and those who had diarrhoea plus vomiting and/or fever, were treated with recommended liquids (chi square test, $p<0.01$). On the other hand, children who had dehydration signs, alone or combined, were primarily treated with standard ORS (chi square test, $p<0.01$). Children with bloody diarrhoea also received ORS. Not recommended liquids were not significantly associated with any clinical characteristic.

Household treatment and maternal health-seeking behaviour were analysed in relation to three variables: children's need for medical care, presence of dehydration signs, and maternal level of information (Table IV). Among children who needed medical care, the mothers restricted food (41.9%), provided some home remedies (56.5%), and went to see a physician (66.0%). Among children who had dehydration signs, the mothers restricted food (44.9%), provided home remedies (60.6%), and sought medical care (67.9%). On the other hand, the mothers' decisions to use drugs, to restrict food or to provide home remedies or to go to a health care facility, were not based upon their level of health information. We did not observe any significant differences between well-informed and not well-informed mothers. Further, other maternal variables, such as age, literacy and size of their community, were not significantly associated with their decision-making regarding treatment or health-seeking behaviour (data not included in the table).

Table III. Type of liquids provided at the households in relation to the clinical condition

Symptoms	n=747	None	Oral rehydration therapy		
			Recommended liquids	Oral rehydration solution	Not recommended liquids
		%	%	%	%
Duration of illness					
1-3 days	456	13.2	63.6*	9.6	13.6
≥4 days	291	1.4	46.3	21.0	31.3
Only diarrhoea	336	14.9	73.5*	0.0	11.6
Fever	244	1.6	38.5*	29.9	30.0
Vomiting	201	2.0	41.3*	28.4	28.3
Bloody diarrhoea	32	0.0	18.8	46.9*	34.3
Sunken eyes	132	2.3	6.1	74.2*	17.4
Thirst	233	2.6	27.9	43.3*	26.2
Sunken fontanelle	25	4.0	0.0	96.0*	0.0
Low urine output	55	7.3	9.1	45.5*	38.1
Dehydration signs**	274	2.2	28.5	38.3*	31.0
Need for medical care §	494	1.2	46.6*	21.3	31.0

* Chi square test, p<0.01 comparing between children with and without the clinical data.
** Presence of two or more of the following dehydration signs: sunken eyes, thirst, sunken fontanelle, or low urine output.
§ Presence of at least one following data: dehydration, fever, illness lasting four or more days, bloody diarrhoea, or vomiting.

Table IV. Household treatment and health-seeking behaviour according to the severity of diarrhoea and mothers' information

Characteristics	n=747	Drugs	Food restriction	Home remedy	Health-seeking behaviour	
					Home-based care	Medical care
		%	%	%	%	%
Need for medical care						
Yes	494	37.4	41.9*	56.5*	34.0	66.0*
No	253	30.8	25.3	44.3	55.3	44.7
Dehydration signs						
Yes	274	39.4	44.9*	60.6*	32.1	67.9*
No	473	32.8	31.3	47.6	46.5	53.5
Mothers informed	599	37.1	37.2	52.6	40.7	59.3
Mothers not informed	148	27.7	32.4	51.4	43.2	56.8

* Chi square test, $p < 0.01$

Table V. Mothers' health care-seeking behaviour (univariate analysis in relation to the clinical conditions)

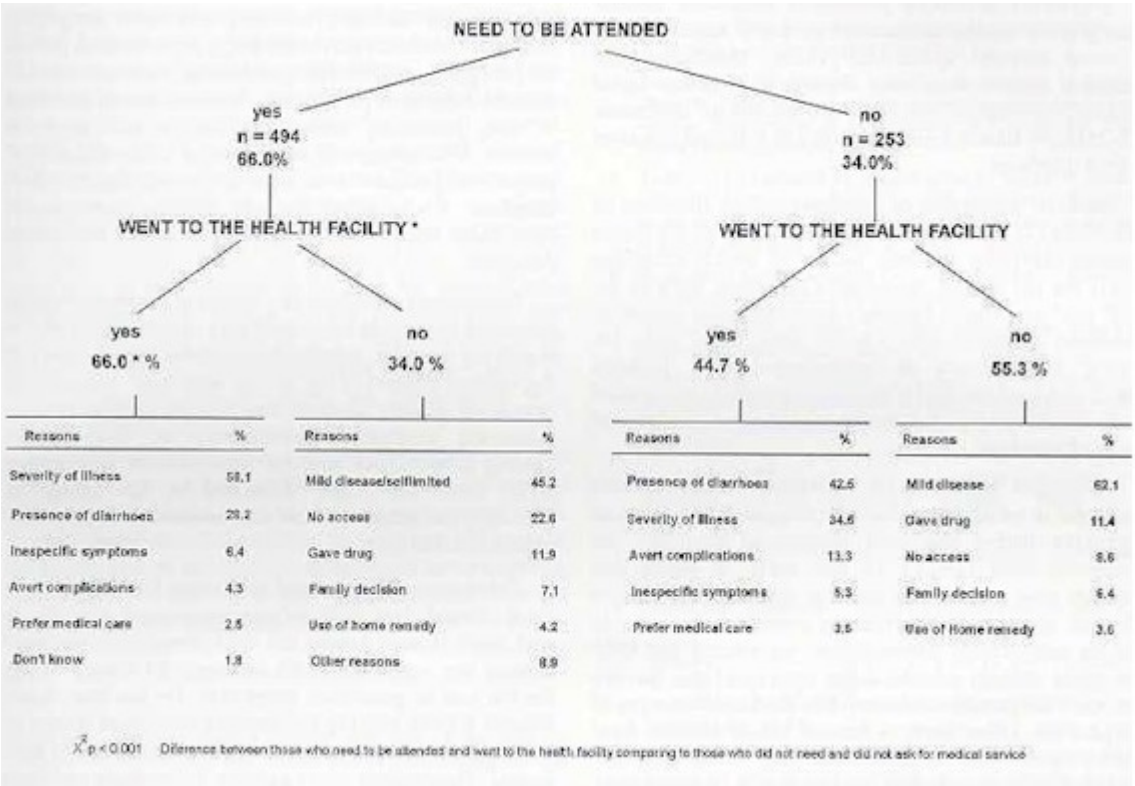
Signs	n=747	Place of attention (%)		Odds ratio	95% CI
		Clinic	Home		
Bloody diarrhoea	32	90.6	9.4	7.2	2.17-23.82
Vomiting	201	76.6	23.4	3.0	2.07-4.33
Weight loss	167	76.0	24.0	2.7	1.84-4.03
Sunken Eyes	132	75.0	25.0	2.4	1.58-3.71
Fever	244	72.1	27.9	2.3	1.69-3.28
Length: 4 or more days	291	69.4	30.6	2.1	1.53-2.85
Thirst	233	68.2	31.8	1.8	1.29-2.48
Dehydration signs	274	67.9	32.1	1.8	1.34-2.50
Need to receive medical care	494	66.0	34.0	2.4	1.76-3.27

Table VI. Mothers' health care-seeking logistic regression model according to clinical data

Symptoms	Odds ratio	95% CI	p value
Bloody diarrhoea	4.6	1.38-16.03	0.013
Vomiting	2.3	1.49-3.53	0.000
Length: 4 or more days	1.7	1.22-2.35	0.002
Weight loss	1.6	1.04-2.55	0.031
Fever	1.5	1.00-2.17	0.050

The ethnographic decision model (Figure) reveals that 66% of the children required medical care and 34% did not. Among children needing medical attention, 66% were taken to some health care facility. Maternal reasons for this decision deal with clinical data of which the most important was the perceived severity of the illness (56%) along with the presence of diarrhoea as such (28.2%). Mothers who did not take their children to a health care facility, even when required, considered diarrhoea a mild and self-limited disease (45.2%). Another significant reason was limited access to medical care (22.6%) either for geographic or economic reasons, or because the physician was absent at the clinic that day.

Of the children not requiring medical assistance, 44.7% were taken to the health care facility, while the others were cared for at home. Arguments for seeking medical attention were similar to those given by mothers whose children required medical care: presence of diarrhoea as such (42.5%) and perceived severity of illness (34.5%). On the other hand, mothers who decided to care for their children at home explained that the illness was mild (62.1%) or that they had decided to administer some drugs themselves (11.4%). Limited access was a less frequently mentioned reason when compared with the other group.



Analyses of the relationship between mothers' health-seeking behaviour and clinical data in which isolated symptoms, need for medical care and dehydration (Table V) were included, revealed that the most important sign that induced a mother to take her child to a health care facility was bloody diarrhoea (odds ratio 7.2, 95% CI 2.17-23.82). In decreasing order, vomiting, weight loss, sunkenz eyes, fever, illness for longer than three days, and thirst were also closely related to the mothers' decision to look for health care. Isolated signs of dehydration had higher odds ratios than the presence of actual dehydration. The need for medical care was also significant (odds ratio 2.4, 95% CI 1.76-3.27).

A backward stepwise variable selection procedure was used for building logistic regression models. Table VI shows the final model. To build the model, all variables, found to be significant in the univariate analysis, were included. Covariates remaining in the model were: bloody diarrhoea, vomiting, illness for more than four days, weight loss, and fever. We did not find interaction terms during the analysis. All isolated signs of dehydration were eliminated

from the model.

DISCUSSION

To understand the social context of diarrhoeal disease, ethnographic studies carried out in Latin America have followed different approaches (19,20). Descriptive and analytical studies, examining changes in food and liquid intake (21), weaning practices, and household treatments (22-25) have shown a great diversity in maternal practices to treat diarrhoea.

Mothers' perception of diarrhoea differs from that of physicians (2). To the lay population, causes of diarrhoeal diseases comprise different factors in which infectious agents are not always identified. Conditions, such as the "evil eye" or "fright disease", are considered causes of childhood diarrhoea (26). On the other hand, there are several factors, such as contaminated food, polluted water, and unclean habits that are commonly recognised by both the public and health providers as a potential cause of diarrhoea.

Household treatment for diarrhoea includes a vast repertoire in which home-made liquids, such as rice-based beverages, herbal teas, and commercial solutions, are commonly used (18,21). In this study, it seems that mothers gave liquids not only to stop diarrhoea or to alleviate signs, such as fever or vomiting, but also to prevent dehydration. Nevertheless, we noticed that ORS was given only to 14.1% of the cases, and this therapy was more frequently used when the child showed signs of dehydration. Other surveys carried out in Mexico have shown up to 45% of ORS use (22), while the rates observed in other countries are lower than in our sample. For instance, Brazil has an ORS use rate of 6.8% (27). Being a key element to prevent dehydration, ORS has been widely promoted in developing countries where consistent campaigns have been carried out (28). Thus, mothers have certain knowledge of the existence of ORS, although their information regarding its purpose and correct way of administration is incomplete. Some studies have reported maternal misconceptions about the properties of ORS. In Zimbabwe, for instance, mothers thought that ORS was a sort of medicine to stop diarrhoea (29). Nicaraguan mothers attributed nutritional benefits to ORS instead of the properties it actually has (30). These misconceptions are also related to improper information given by health providers. Furthermore, it has been reported that mothers misuse the solution either by mistakes in administration or by administering it for shorter periods than required (31). Concerning preparing ORS, the rate of mistakes in Brazil is 62% (27), while in Mexico it is 40% (23).

In our study, we found that prevailing household treatment for diarrhoea is a combination of folk practices and cosmopolitan medicine. This amalgam could be due to the influence that physicians and health providers have on the mothers. The usual physicians' prescribing behaviour, hardly ever adequate to treat diarrhoea (32), misleads people and motivates them to use potentially dangerous drugs, such as antibiotics that are usually not indicated. In Mexico, we may say that analgesics, antibiotics, and antidiarrhoeal drugs have become part of the folk practices (33) This problem has increased and has reached enormous proportions, because almost any kind of drug (including antibiotics) can be sold over the counter. This, along with the influence of the physicians' prescribing patterns, is reflected in the way mothers treat diarrhoea. We observed through the way they provide care, how they are trying to simulate biomedical practices.

Explanatory models of maternal decision-making processes to provide household care or seek medical care reveal the complex relationship between this disease and the cultural context (7,10,24). In our study, the primary reason for seeking medical care was the perception of a worsening condition. Furthermore, we found, after running a univariate analysis, that isolated dehydration signs, while not clearly identified by the mother as indicating dehydration, were also important determinants to seek medical care.

Other reports have noted that other factors, different from clinical data, still influence mothers' decision to seek medical care. Among the most commonly mentioned reasons are: access to health services and money to pay for the visit or prescribed drugs (19). On the other hand, reasons for not looking for medical care were related to the mothers' perception that the illness was mild or self-limited. Nonetheless, most patients in our study were not taken to a medical care facility because of limited access or the maternal decision to give treatment at home.

Health-seeking behaviour is also influenced by cultural motivations. Previous studies in Mexico (34) have revealed that children with a sunken fontanelle were taken to traditional healers instead of physicians, because a sunken fontanelle was not considered by the mother a sign of the severity or a complication of diarrhoea.

Maternal health-seeking behaviour and decision-making processes deserve a more in-depth analysis. Verbal autopsies carried out among mothers of infants who died from acute diarrhoea and acute respiratory infections have stressed the importance of the maternal decision-making processes in obtaining timely medical assistance (6). In our study, 34.1% of the mothers did not seek medical care, even when their children needed attention. This finding stresses that mothers do not have enough knowledge to recognise the need to seek medical care and act accordingly. On the other hand, many mothers took their children to the health facility even when this was not necessary. These findings highlight the importance of educating mothers on when to seek medical care for their children..

Previous studies have shown the importance of understanding the mothers' environment before initiating health programmes (35). A key factor to obtain sound results is to improve maternal practices through education (36). Interventions aimed at improving maternal behaviour in which the sociocultural context was neglected have not been successful. A main flaw is to focus the interventions at narrow clinical criteria, overlooking the beliefs, attitudes, local norms, cultural meaning and interpretation of illness (37). Mothers must be taught to identify early dehydration. A significant weakness of the programmes is that these are not intended to teach mothers how to recognise the signs and symptoms of dehydration, or to look for medical care when needed. The findings of our research stress that mothers have been taught to give liquids as part of the treatment, but they were not able to recognise signs of dehydration and did not know that dehydration is a complication of diarrhoea.

Future programmes should focus on teaching mothers not only how to use ORS or ORT but also how to identify signs of dehydration, and to seek timely medical care. Lastly, more detailed studies to untangle household treatment and its relationship to maternal motivations in seeking medical services should be carried out to improve community-based programmes.

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REFERENCES

1. The World Bank. World development report. Washington, DC: The World Bank, 1993.
2. Kleinman A, Eisenberg L. Good B. Culture, illness, and Care. Clinical lessons from anthropologic and cross cultural research. *Ann Intern Med* 1978;88:251-8.
3. Pelto PJ, Pelto PH. Developing applied medical anthropology in Third World countries. *Soc Sci Med* 1992;35:1389-95.
4. Mull D, Mull D. Mothers' concepts of childhood diarrhoea in rural Pakistan: what ORT program planners should know. *Soc Sci Med* 1988;27:53-67.
5. Scrimshaw S, Hurtado E. Anthropological involvement in the Central American Diarrhoeal Disease Control Project. *Soc Sci Med* 1988;27:97-105.
6. Reyes H, Tome P, Guiscafre H, Martínez H, Romero G, Gutiérrez G, *et al*. Autopsia verbal en niños con infección respiratoria y diarrea aguda. Análisis del proceso enfermedad-atención-muerte. *Bol Med Hosp Infant Mex* 1993;50:7-16.
7. Weiss M. Cultural models of diarrhoeal illness. Conceptual framework and review. *Soc Sci Med* 1988;27:5-16.
8. Kendal C. The implementation of a diarrhoeal disease control program in Honduras: is it 'selective primary health care' or "integrated primary health care"? *Soc Sci Med* 1988;27:17-23.
9. Nichter M. Use of social science research to improve epidemiologic studies of and interventions for diarrhea and dysentery. *Rev Infect Dis* 1991;13(4 Suppl):s265-71.
10. Bentley M. The household management of childhood diarrhoea in rural north India. *Soc Sci Med* 1988;27:75-85.

11. Gutiérrez G, Guiscafré H, Reyes H, Prez-Cuevas R, Vega R, Tome P. Reducción de la mortalidad por enfermedades diarreicas agudas. Experiencias de un programa de investigación acción. *Salud Pub Méx* 1994;36:168-79.

12. Gutierrez G, Guiscafré H, Pérez-Cuevas R, Reyes H, Tome P, Vega R, Nazar A. Impact of a statewide program (DAIRA) to lower mortality rates due to acute respiratory infection and acute diarrhoea. *Arch Med Res* 1992;23:218.

13. Secretaría de Salud, Dirección General de Estadística, Informática y Evaluación: Mortalidad 1993, México 1993.

14. Reinke W. Health planning for effective management. New York: Oxford University Press, 1988:215-24.

15. World Health Organisation. A manual for the treatment of diarrhoea for use by physicians and other senior health workers. Geneva: World Health Organization, 1990. (WHO/CDD/SER/ 80.2 Rev 2).

16. World Health Organisation. The management and prevention of diarrhoea. practical guidelines. Geneva: World Health Organization, 1993.

17. Bernard HR. Research methods in anthropology. 2d. ed. Sage, 1994:373-7.

18. Hosmer DW, Lemeshow S. Applied logistic regression. New York: Wiley, 1989.

19. Martínez H, Calva J, Meneses L, Viais H. Uso de bebidas y alimentos en el hogar durante la diarrea aguda del niño. Estudio etnográfico en una zona rural mexicana. *Bol Med Hosp Infant Mex* 1991;48:235-41.

20. Bentley M, Stallings R, Fukumoto M, Elder J. Maternal feeding behaviour and child acceptance of food during diarrhoea, convalescence, and health in the Central Sierra of Peru. *Am J Public Health* 1991;81:43-7.

21. Martínez H. Alimentos de uso común en la comunidad para el tratamiento de diarreas. *In:* Mota F. Prevención y Control de las Diarreas. SSA/OPS/UNICEF. México. 1990.

22. Mota F, Tapia R, Welti C, Franco A, Gomez J, Teresa G. Manejo de la enfermedad diarreica en el hogar en algunas regiones de México. *Bol Med Hosp Infant Mex* 1993;50:367-75.

23. Consejo Nacional para el Control de las Enfermedades Diarreicas. Encuesta sobre el Manejo Efectivo de Casos de Diarrea en el Hogar. Dirección General de Epidemiología, Secretaría de Salud México 1994.

24. Martínez H, Saucedo G. Mothers perceptions about childhood diarrhoea in rural Mexico. *J Diarrhoeal Dis Res* 1991;9:235-43.

25. Reyes H, Guiscafré H, Sarti E, Montoya Y, Tapia R, Gutiérrez G. Variaciones urbano rurales en la atención del niño con enfermedad diarreica. *Salud Pub Mex* 1996;38;157-66.

26. Paredes-Solari P, De la Peña M, Flores E, Yeager B, García T. Study of the determinants of health seeking behaviour during childhood diarrhoea in a peri-urban slum of Lima, Peru. Lima: Instituto de Investigación Nutricional, 1992.

27. Barros FC, Victora CG, Forsber B, Maranhao A, Stegeman A, González R. Management of childhood diarrhoea at the household level: a population-based survey in North East Brazil. *Bull WHO* 1991;69:59-65.

28. McDivitt J, Hornik R, Carr C. Quality of home use of oral rehydration solutions: results from seven healthcom sites. *Soc Sci Med* 1994;38:1221-34.

29. Nyatoti V, Nyati Z, Mtero S. Knowledge, attitudes and practices of mothers and health workers in relation to the use of sugar and salt solution in Masvingo Province, Zimbabwe. *Centr Afr J Med* 1993;39:95-102.

30. Hudelson P. ORS and the treatment of childhood diarrhoea in Managua, Nicaragua. *Soc Sci Med* 1993;37:97-103.

31. Chopraprowon C, Chunsutiwat S, Kachondham Y, Weiss M. Cultural study of diarrhoeal illnesses in Central Thailand and its practical implications. *J Diarrhoeal Dis* 1991;9:204-12.

32. Pérez-Cuevas R, Muñoz O, Guiscafré H, Reyes H, Gutiérrez G. Patrones de prescripción teraputica en infección respiratoria aguda y diarrea aguda en dos instituciones de salud (IMSS-SSA) IV. Características de la Prescripción Médica. *Gac Med Mex* 1992;128:31-542.

33. Guiscafré H, Muñoz O, Padilla G, Reyes R. Estrategias para mejorar los patrones terapéuticos utilizados en diarrea aguda en unidades de atención médica primaria. I. Metodología de estudio y características de las unidades médicas y de la población estudiada. *Arch Invest Med* 1988;19:335-50.

34. Larrauri S, Larrauri C, Jufresa J. Learning to prevent dehydration in distant Mexican communities and markets. *Soc Sci Med* 1994;38:1499-507.

35. Bentley M, Pelto G, Straus W, Shumann D, Adegbola C. De la Pena E, *et al*. Rapid ethnographic assessment: Applications in a diarrhoea management program. *Soc Sci Med* 1988;27:107-16.

36. Ueli B. Oral Rehydration Therapy. Qualitative studies of balance between pragmatism and scientific rigour in managing diarrhoea. *Soc Sci Med* 1993;36:525-31.

37. López R, Kroeger A. Intervenciones educativas populares contra el uso inadecuado de medicamentos. *Bol Sanit Panam* 1994;116: 135-45.

Interaction Between Acute Diarrhoea and Falciparum Malaria in Nigerian Children

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ABSTRACT

Although both malaria and diarrhoea are major public health problems in developing countries, and separately each has been the subject of intense research, few studies have investigated the interaction between these two conditions. The interaction between diarrhoea and malaria among children aged 4 months to 12 years in two tertiary health-care facilities, University College Hospital, Ibadan, and Lagos University Teaching Hospital, Lagos, Nigeria was studied. In Ibadan, the prevalence of diarrhoea among the cerebral malaria patients on admission was 11.7% (7/60) compared to 9.3% (215/2312) among other admissions in 1990 (chi square=0.16; p=0.6913). Similarly, no significant difference in the prevalence of diarrhoea was found between the cerebral malaria patients (14.3%) and other patients (16.1%) seen in Lagos in 1992 (chi square=0.06, p=0.81). Thus, cerebral malaria does not seem to be associated with an increased or decreased prevalence of diarrhoea when compared with other conditions. The prevalence of malarial parasitaemia among the 554 diarrhoea patients studied in Ibadan during 1993-1994 was 13.6% compared with 17.9% among the 347 controls (chi square=3.75, p=0.053). However, of the children with diarrhoea, malarial parasitaemia was more common among the dehydrated patients (25.4%) than among the well-hydrated patients (11.6%) (chi square = 8.11, p=0.004). These data suggest that diarrhoea is merely coincidental in severe malaria and conversely, malarial parasitaemia is similarly coincidental in children with acute diarrhoea, although it may be more frequent among dehydrated diarrhoea patients than well-hydrated ones.

***Keywords*:** Malaria; Diarrhoea, Infantile; Diarrhoea, Acute; Dehydration; Prospective studies

INTRODUCTION

Both malaria and diarrhoea are the major public health problems in children in the tropics, and both conditions are major causes of mortality in children aged less than 5 years (1,2). Although both conditions are the subjects of intense research and health policy, little information on the interaction between the two diseases is available. In one study, Greenwood *et al.* (3) did not find any interactions between acute gastroenteritis, acute respiratory infections, and malaria. By contrast, the interaction between acute respiratory infections and malaria has more frequently been studied (4-6).

Malaria, long recognized as a major cause of morbidity and mortality in African children, does not have any specific clinical features (7). In *Plasmodium falciparum*-associated infections, which account for 97% of malaria in Nigeria, fever and convulsions are common, while vomiting and diarrhoea also occur (8). Therefore, it is not surprising that, according to some studies, diarrhoea is a prominent symptom of malaria (9,10), while other studies assert that malaria can seldom be incriminated as the primary cause of significant diarrhoea (11). In recognition of the overlap that may exist between the two conditions and the possibility that both conditions may coexist in the same child, the Control of Diarrhoeal Diseases (CDD) Programme of the World Health Organization (WHO), recommends that in areas of endemic malaria, a child with diarrhoea aged 2 months or older and whose temperature is >38 ° C, should be treated for

malaria (2). Unfortunately, this may result in unnecessary treatment, since fever or elevated temperature has been shown to be a poor predictor of malaria parasitaemia (12-14). These issues highlight some of the difficulties currently being met in dealing with the two conditions.

Given the clinical and public health importance of the two conditions, it is obviously important to attempt to unravel the interaction between them. We decided to study this interaction in Nigerian children in two cities (Ibadan and Lagos), using cerebral malaria (the most severe form of malaria) as one starting point and children with acute diarrhoea as another starting point. We hypothesized that if there is an interaction between malaria and diarrhoea, the prevalence of diarrhoea among children with cerebral malaria should be different (higher or lower) from that in the general population of otherwise ill children and that, conversely, the prevalence of malaria parasitaemia among children with acute diarrhoea should be different from that among other sick children without diarrhoea in the same setting. Thus, we studied the relationship between cerebral malaria and diarrhoea on the one hand and the relationship between acute diarrhoea and uncomplicated malaria on the other.

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MATERIALS AND METHODS

The study was actually a series of three interrelated prospective studies of children aged 4 months to 12 years carried out from 1990 to 1994. First, the prevalence of diarrhoea among children with cerebral malaria (CM) was compared with that among other acutely ill children admitted to the children's emergency ward, University College Hospital, Ibadan, during 1990. Second, the prevalence of diarrhoea among children with cerebral malaria was compared with that among other acutely ill children admitted to the Lagos University Teaching Hospital, Lagos during 1992. Finally, the prevalence of *P. falciparum* parasitaemia among children with diarrhoea at the Diarrhoea Training Unit (DTU) of the University College Hospital (UCH), Ibadan, was compared with that among non-diarrhoea controls from July 1993 to April 1994. Ethical approval was obtained from the ethical committees of the hospitals involved in the study. In addition, ethical approval for the acute diarrhoea study was obtained from the ethical committee of Harvard University.

Studies of diarrhoea in cerebral malaria

The diagnosis of cerebral malaria was based on the WHO definition of the disease, and the treatment given was according to the WHO recommendations (15). A history of diarrhoea during the illness with which the child presented in hospital was asked for in all children presenting at the children’s emergency wards at the two sites (Ibadan and Lagos) during the study periods. The prevalence of diarrhoea among cerebral malaria patients was recorded and compared with that among all the other patients (i.e. not cerebral malaria) admitted to the same children's emergency ward during the same period. The other patients served as control groups for the relevant groups of cerebral malaria patients.

Study of acute diarrhoea and uncomplicated malaria

All children presenting with acute diarrhoea at the DTU of the UCH, during July 1993-April 1994 were studied. The control group comprised children in the same age range presenting within 48 hours of diarrhoea patients in the same hospital but without diarrhoea. Diarrhoea was defined as three or more loose bowel motions in the preceding 24 hours. The presence of dehydration was evaluated according to the standard WHO guidelines (2), and according to the same guidelines, all diarrhoea and dehydrated control patients received oral rehydration therapy. All the children studied (diarrhoea patients and controls) were screened for malaria parasitaemia on admission by means of Giemsa- stained thick and

thin blood films. Uncomplicated malaria was defined as the presence of asexual forms of *P. falciparum* in a blood smear of a child with symptoms. All parasitaemic patients (diarrhoea and controls) received chloroquine orally, according to the WHO recommendations (15), but those who were still parasitaemic four days after starting the treatment had sulphadoxine-pyrimethamine administered.

Laboratory data

For all groups of patients, haematocrit, total leukocyte count (TLC), haemoglobin electrophoresis, fluorescent spot (screening) test for glucose-6-phosphate dehydrogenase (G6PD) deficiency as well as thick and thin blood films for malaria parasites were done, using standard methods (16). Absolute parasite counts in those with malaria parasitaemia were done by counting the number of parasites (P) among 200 white blood cells on the thick film, and using the equation:

Absolute parasite count = P x TLC/200

Where TLC = patient's own total leukocyte count.

The chi-square test with Yates correction was used for comparing the prevalences between groups. The Student's *t* test and Kruskal-Wallis test were used as appropriate. A probability value (p) of <0.05 was considered significant.

RESULTS

Diarrhoea among children with cerebral malaria

In Ibadan, the prevalence of diarrhoea among the cerebral malaria patients on admission was 11.7% (7/60) compared to 9.3% (215/2312) among other admissions in 1990 (chi square=0.16; p=0.6913). Similarly, no significant difference in the prevalence of diarrhoea was found between the cerebral malaria patients (14.4%) and other patients (16.1%) seen in Lagos during 1992 (chi square=0.06, p=0.81) (Table I). Thus, cerebral malaria does not seem to be associated with an increased or decreased prevalence of diarrhoea when compared with other conditions in either of the two study sites. In Lagos, where the pre-admission duration of fever was specifically and accurately assessed routinely, the 10 cerebral malaria patients with diarrhoea had been febrile for a median duration of 77 hours, compared with a median of 72 hours among the 60 cerebral malaria patients without diarrhoea (Kruskal-Wallis test H=38.399; p=0.091). Thus, diarrhoea did not appear to be related to the duration of fever before hospitalization.

Table I. Prevalence of diarrhoea among cerebral malaria patients and other paediatric admissions at the two study sites

Site	Cerebral malaria	Other admissions	χ^2	p value
UCH, Ibadan (1990)				
Patients seen	11.7% (7/60)	9.3% (215/2312)	0.16	0.69
Age (yrs) (Mean ± SD)	5.24±2.88	5.18±3.01	0.07*	0.79
LUTH, Lagos (1992)				
Patients seen	14.3% (10/70)	16.2% (352/2178)	0.06	0.81
Age (yrs) (Mean ± SD)	5.26±3.05	5.07±3.66	0.6*	0.41

Malarial parasitaemia among children with acute diarrhoea

As shown in Table II, the 554 diarrhoea patients and the 347 controls were similar in terms of potentially confounding factors for malaria parasitaemia, such as the previous use of antimalarial drugs and the presence of malaria-related genetic factors: haemoglobin types and G6PD deficiency.

Table II. Characteristics which may affect malaria parasitaemia among diarrhoea patients and controls at the University College Hospital, Ibadan, Nigeria

Characteristic	Diarrhoea (n=554) %	Controls (n=347) %	χ^2	p value
Age < 2 years	82.0	69.0	19.56	<0.001
Haemoglobin type				
AA	78.8	73.9		
AS	14.4	20.3		
Others	6.8	5.8	5.86	0.0535
G6PD deficiency	13.9	13.5	0.00	0.940
Prior treatment with antimalarial drug	31.9	36.5	1.79	0.181

The prevalence of malaria parasitaemia among the 554 diarrhoea patients studied in 1993 was 13.2% compared with 18.2% among the 347 controls (chi square=3.75, p = 0.053) (Table III). Although the difference was not significant statistically at the $p<0.05$ level, the prevalence of malaria parasitaemia was higher among the controls, the opposite of what should be the case if malaria were associated with diarrhoea. This finding is further accentuated by the fact that the diarrhoea patients were statistically, significantly younger (Table II), and therefore, should have had higher frequencies of malaria parasitaemia than the controls. However, of the children with diarrhoea, malaria parasitaemia was more common among the dehydrated patients (25.4%) than among the well-hydrated patients (11.6%; chi square=8.11, p=0.004). The small number (6) of dehydrated patients among the non-diarrhoeal controls precluded a similar comparison between the dehydrated and well-hydrated patients among them.

Malarial parasite species

All the parasitaemic patients had *P. falciparum*. Mixed infections with *P. malariae* were present in four children in each of the (diarrhoea and control) groups studied. Thus, the contribution of non-falciparum malaria to these data was negligible.

DISCUSSION

Malaria and diarrhoea remain the important public health problems in south-western Nigeria where this study was carried out. Thus, a study of their interaction is of clinical and public health significance. In conducting this study, we have avoided a limitation common to some reports on the relationship between diarrhoea and malaria which use only clinical suspicion and/or microscopical proof of malaria as the primary basis for the selection of patients (3,17,18). In addition, we have looked at the interaction from two ends: starting from the most severe form of malaria (cerebral malaria) on one hand and starting from children with acute diarrhoea on the other. The data obtained in this study are probably unique in the sense that, as far as we are aware, these are the first set of such data that began with diarrhoea patients. Also, the specific study of diarrhoea as a presenting symptom among the cerebral malaria patients that we have reported has not been commonly documented previously.

Table III. Prevalence of malaria parasitaemia among diarrhoea patients and controls at the University College Hospital, Ibadan, Nigeria

Group	All patients	Dehydrated patients	Well-hydrated patients
Diarrhoea patients	13.2% (73/554)†	*25.4% (16/63)	*11.6% (57/491)
Controls	18.2% (63/347)†	16.7% (1/6)	18.2% (62/341)

†χ^2=3.75, p=0.053 *χ^2=8.11, p=0.004

Using appropriate control groups, we have shown that the prevalence of diarrhoea among children with cerebral malaria is no different from that among other paediatric emergency admissions in two different sites in south west Nigeria. This was surprising since the pathological changes in patients infected with malaria are complex and involve many organs, including the small bowel (19). Given the fact that cerebral malaria is the most severe form of malaria, it should be expected that the increased production of tumour necrosis factor and of free oxygen radicals, the postulated mechanisms by which malaria may cause diarrhoea (19), should be present to the maximum degree possible in cerebral malaria and lead to a higher prevalence of diarrhoea. That this was not found argues against diarrhoea as a prominent feature of malaria, even of severe malaria. The possibility, however, exists that the mechanisms by which malaria may cause diarrhoea (increased production of tumour necrosis factor, free oxygen radicals, prostaglandins, and cyclic AMP) are a feature of most acute paediatric infections and are not specific for malaria. Under such conditions, one would find no difference between diarrhoea in malaria and other acute illnesses, as was found in this study. It is also possible that diarrhoea is an early rather than late symptom of malaria, occurring early in the malaria disease process before the child comes to hospital or clinic. But this is very unlikely, because the question asked was about diarrhoea at any time during the current illness. In any case, there was no significant difference in the duration of illness (fever) between the non-diarrhoea and diarrhoea subsets of cerebral malaria patients.

The overall malaria frequency of 15.1% obtained for both the acute diarrhoea patients and controls combined is similar to the figure obtained by Familusi and Sinnette (20) but lower than those of Hendrickse *et al.* (8), some 20 years ago in the same children's emergency ward. Our figure is probably explained by the fact that Hendrickse excluded children who had received antimalarial drugs in the preceding two weeks, which we did not do in our study.

Among the diarrhoea patients, the significantly high frequency of parasitaemia among the dehydrated patients may be a concentration effect. Clinically detectable dehydration implies at least a 5% loss of body water, mainly at the expense of the interstitial compartment from which much of the capillary blood (obtained by finger prick and used for making the blood films) is derived. Thus, in the presence of dehydration, there may be an enhanced chance of detecting low parasitaemia that would otherwise have been missed. However, it should be noted that malaria itself might increase the risk of dehydration through pyrexia, increased metabolism, increased respiratory rate and, subsequently, increased insensible water loss. Where anorexia is severe and there is vomiting, the risk of dehydration in malaria is further increased. Thus, malaria and diarrhoea may interact significantly in causing dehydration. In this study, it was impossible to decide if the association of malaria parasitaemia with dehydration is due to dehydration itself (leading to haemoconcentration) or due to the fact that dehydration is more likely in a diarrhoea patient who has malaria. This was due to the absence of the sufficient number of non-diarrhoea patients with dehydration to make a valid comparison.

In conclusion, we have found that the prevalence of malaria parasitaemia is significantly higher in patients with diarrhoea and dehydration than in well-hydrated diarrhoea patients. Still, overall there is no evidence for diarrhoea being a major feature of malaria. This conclusion is further strengthened by the fact that parasitaemia was, in fact, more common among the controls than among the diarrhoea patients, contrary to what would have been consistent with malaria causing diarrhoea.

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REFERENCES

1. *Struchler D. Malaria: how much is there worldwide? Parasitol Today 1989;5:39-40.*
2. *World Health Organization. Diarrhoea Diseases Control Programme. A manual for the treatment of diarrhoea. Geneva: World Health Organization, 1990 (WHO/CDD/SER/80.2 Rev.2 1990).*
3. *Greenwood BM, Byass P, Greenwood AM, Hayes RJ, Menon A, Shenton FC, Stephens J, Snow RW. Lack of an association between acute gastroenteritis, acute respiratory infections and malaria in young Gambian children. Trans R Soc Trop Med Hyg 1989;83:595-8.*
4. *Redd SC, Bloland PB, Kazembe PN, Patrick E, Tembenu R, Campbell CC. Usefulness of clinical case-definitions in guiding therapy for African children with malaria or pneumonia. Lancet 1992;340:1140-3.*
5. *O'Dempsey TJD, McArdle TF, Laurence BE, Lemont AC, Todd JE and Greenwood BM. Overlap in the clinical features of pneumonia and malaria in African children. Trans Roy Soc Trop Med Hyg 1993;87:662-5.*
6. *World Health Organization. Malaria Action Programme. Severe and complicated malaria. Trans Roy Soc Trop Med Hyg 1986;80 (suppl):13.*
7. *Bassett MT, Taylor P, Bvirakare J, Chiteka F, Govere E. Clinical diagnosis of malaria: can we improve? J Trop Med Hyg 1991;94:65-9.*
8. *Hendrickse RG, Hasan AH, Olumide LO, Akinkunmi A. Malaria in early childhood. Ann Trop Med Parasitol 1971;65:1-20.*
9. *Maegraith B. Malaria. In: Clinical tropical diseases. 9th ed. Oxford: Blackwell, 1989:201-46.*
10. *Karney WW, Tung J. Malabsorption in Plasmodium falciparum malaria. Am J Trop Med Hyg 1972;21:1-5*
11. *Hendrickse RG. Parasitic disease. In: Paediatrics in the tropics. Oxford: Blackwell, 1991:695-710.*
12. *Rougemont A, Breslow N, Brenner E, Moret AC, Dumbo A, Dolo A, Sonla G, Perrin L. Epidemiological basis for clinical diagnosis of childhood malaria in endemic zone in West Africa. Lancet 1991;338:1292-5.*
13. *Ejezie GC, Ezedinachi EN, Usang EA, Gemade EI, Ikpatt NW, Alaribe AA. Malaria and its treatment in rural villages of Aboh Mbaise, Imo State, Nigeria. Acta Trop Basel 1990;48:17-24.*
14. *Gbadegesin RA, Sodeinde O, Adeyemo AA, Ademowo OG. Body temperature is a poor predictor of malaria parasitaemia in childhood acute diarrhoea. Ann Trop Paediatr 1997 (In press).*
15. *World Health Organization. Severe and complicated malaria. Trans R Soc Trop Med Hyg 1990:84(2 Suppl):1-65.*
16. *Dacie JV, Lewis SM. Practical haematology. 6th ed. London: Blackwell, 1986.*
17. *Nalin DR, Hassan R, Haque S, Boltansky. Association between malaria, immunosuppression, and high relative risk for diarrhoea attacks. In: Acute infections in children - new prospects for treatment and prevention. London: Elsevier, 1981:256-9.*

18. *Rooth I, Bjorkman A. Fever episodes in a holoendemic malaria area of Tanzania: parasitological and clinical findings and diagnostic aspects related to malaria. Trans R Soc Trop Med Hyg 1992;86:479-82.*
19. *Prasad RN, Virk KJ. Malaria as a cause of diarrhoea - a review. Papua New Guinea Med J 1993;36:337-41.*
20. *Familusi JB, Sinette CH. Febrile convulsions in Ibadan children. Afr J Med Sci 1971;2:135-49.*

Production of Haemolysin and Enterotoxin by Aeromonas jandaei and Aeromonas trota Strains after Animal Passage

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ABSTRACT

Five *Aeromonas jandaei* and 12 *Aeromonas trota* isolates were tested for the production of haemolysin and enterotoxin, and the correlation between these two properties. The majority (10 isolates) of the strains produced b -haemolysis. The titres of haemolytic activity for both species were 8-64 HU/mL. In the initial ileal loop test, only two (*A. trota*) of the 17 isolates produced enterotoxin. One each of these 2 *A. trota* strains was b -haemolytic and non-haemolytic. The remaining isolates of *A. trota* and *A. jandaei* included a -, b -and non-haemolytic strains, and failed to cause any fluid accumulation in the initial tests, but did so after one-to-five sequential passages through the rabbit ileal loops. Three a - and 4 non-haemolytic strains switched over to the production of b -haemolysis when they showed the positive ileal loop reaction. However, on repeated subcultures or on storage in the laboratory, all of them reverted back to their original a - or non-haemolytic character and no longer produced enterotoxin.

Key words**:** *Aeromonas;* Haemolysin; Enterotoxins; Disease models, Animal

INTRODUCTION

Aeromonas spp. have been reported as aetiologic agents of diarrhoea in man (1-6). The production of heat-labile enterotoxin by *Aeromonas* strains was first demonstrated in an adult rabbit ileal loop (RIL) model (2,3,7,8), and subsequently, in other animal and tissue culture assays (9-13). *Aeromonas hydrophila* and *A. sobria* produce extra-cellular products, such as haemolysin (12-14), aerolysin (15), cytotoxin (4), and various enzymes (16). Enterotoxigenic strains of *Aeromonas* spp. have been reported to be b -haemolytic (17,18). Most of these haemolytic strains were either *A. hydrophila* or *A. sobria,* but rarely *A. caviae* (17-20). Earlier studies indicated that enterotoxic and haemolytic properties of *Aeromonas* spp. are different entities (11), determined by separate genes located on different segments of the chromosome (21). b - or non-haemolytic strains of *A. hydrophila*, *A. sobria,* and *A. caviae* that caused little or no fluid accumulation in the initial test switched over to the production of haemolysin and enterotoxin after sequential passage through the rabbit ileal loops (22). Although the recently recognised species of *A. jandaei* and *A. trota* have been demonstrated to produce an enterotoxic substance, such effect of passage on the haemolytic character of these two species has not yet been reported. The present study was, therefore, undertaken to test the production of haemolysis and enterotoxin by *A. jandaei* and *A. trota*, to examine correlation, if any, between haemolytic and enterotoxic activity, and to see the changes in haemolytic character of strains showing enterotoxin production after animal passage.

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MATERIALS AND METHODS

Bacterial strains

Seventeen strains, isolated from children and adults with diarrhoea (12), blood (2), wound (1), calf (1), and infected fish (1), were included in the study. The organisms were identified as *A. jandaei* (5) and *A. trota* (12) according to their ability to hydrolyse aesculin, ferment salicin, and sucrose and to produce gas, acetoin and H_2S, and resistance to ampicillin following the criteria of Carnahan *et al.* (23,24). Strains were maintained in peptone agar stab cultures at room temperature, and did not undergo more than three subcultures prior to the experiments.

Detection of haemolysis

The production of haemolysin by *Aeromonas* strains was tested initially on sheep blood agar (5%) inoculated with a 4-5-hour culture of each organism in Brain Heart Infusion Broth (BHIB, Difco). After the overnight incubation at 37 ° C, the plates were examined under the microscope for the presence of a - (incomplete) or b - (complete) haemolysis around the colonies (14).

Titration of haemolysin

The production of haemolysin(s) by each strain was confirmed by the method of Smith (25) as modified by Rennie and Arbuthnott (26). Briefly, the sheep erythrocytes (SRBC) were washed three times in isotonic saline, and a 2% suspension was prepared in 0.04 M phosphate-buffered saline (PBS, pH 7.4) and used within five days of collection. The haemolytic activity was determined by mixing 0.5 mL of two-fold serial dilutions of the culture filtrates of each strain with an equal volume of SRBC 2% suspension, incubated at 37 ° C in a water bath for two hours and left at 4 ° C for 12 hours. The lysed portion was diluted four-fold with sterile normal saline, and the optical density at 540 nm was measured in a colorimeter. Standardisation of the erythrocyte suspension was done by lysis of 0.5 mL of SRBC 2% with a few crystals of saponin. An optical density of 0.5 at 540 nm was considered to indicate a standardised SRBC 2%. The negative control was 0.5 ml of saline and/or BHIB instead of culture filtrate mixed with SRBC 2% that did not cause release of haemoglobin under experimental conditions. One haemolytic unit (HU) was defined as the amount of culture filtrates that caused 50% haemolysis.

Preparation of culture filtrates CF for haemolysin and enterotoxin production

Culture filtrates of the isolates that gave positive ileal loop reactions, either in the initial test or after the passage with the whole cells were prepared by the method of Annapurna and Sanyal (7). In brief, 10 ml of BHIB in a 50-mL conical flask was inoculated with five or six smooth colonies grown overnight on nutrient agar. The flasks were incubated at 37 ° C in a water bath with shaking for 16-18 hours with 80-120 oscillations per minute. The cultures were centrifuged at 22,000xg for 20 minutes at 4 ° C, and the supernatants were filtered through membrane filters (Millipore, 0.22 µm) and stored at 4 ° C. These culture filtrates were used for haemolysin and enterotoxin assays. a -haemolytic strains were grown at 25 ° C for 48 hours for the preparation of culture filtrates, because maximum production of this haemolysin takes place at this temperature (14).

Ileal loop test

The whole cells and culture filtrates of the 17 strains of *A. jandaei* and *A. trota* were tested in RILs by the method of De and Chatterje (27) as adapted by Annapurna and Sanyal (7) for the detection of enterotoxin production. Briefly, bacteria grown in BHIB for three hours were diluted 10-fold in the same medium and inoculated into RILs in one-mL doses containing 10^5-10^6 cfu. A BHIB culture of toxigenic strain 569B of *Vibrio cholerae* was used as a positive control and a BHIB culture of non-toxigenic strain of *Escherichia coli* 265 served as a negative control in each animal. Culture filtrates in one mL amounts were tested in

the same way. Each test was done in two rabbits, 8-10 loops were ligated in each animal, and they were sacrificed after 18 hours.

Passage in RIL

The strains of *A. jandaei* and *A. trota* that caused little or no accumulation of fluid in the initial tests were passaged through RILs according to the method of Sanyal *et al.* (28,29). Briefly, each strain was cultured aseptically from a RIL on nutrient agar and incubated overnight, five or six colonies were inoculated into BHIB and incubated for three hours, and one mL of a 10-fold diluted culture was inoculated again into a RIL. The process was continued until a positive response was obtained.

Statistical analysis

The enterotoxic activity in terms of fluid accumulation of *A. jandaei* and *A. trota* in relation to their haemolytic activity was analysed using the student '*t'* test.

RESULTS

Ten of the 17 isolates (3 *A. jandaei* and 7 *A. trota*) produced b -haemolysis on sheep blood agar (Table I). a -haemolysis was shown by four strains comprising one *A. jandaei* and three *A. trota*. Only three strains were non-haemolytic, one *A. jandaei* and two *A. trota*.

When culture filtrates of the strains were tested for haemolytic activity, only those causing b -haemolysis on sheep blood agar plates showed lysis of 2% SRBC. The titres of haemolytic activity produced by *A. jandaei* and *A. trota* isolates were 8-64 HU/mL (Table I).

The whole cells and culture filtrates of only two (*A. trota*) of the 17 strains tested caused fluid accumulation in RIL in the initial sets of experiments. One of these two *A. trota* strains was b -haemolytic, the other one non-haemolytic (Table II).

Table I. Haemolytic activity of culture filtrates of *A. jandaei* and *A. trota* before and after animal passage

Species and haemolytic character (No. of isolates)	No. (%) tested before passage	Haemolysin production (HU/mL)	Number tested after passage*	Haemolysin production (HU/mL)
A. jandaei				
α-haemolytic	1 (20.0)	---	1	64
β-haemolytic	3 (60.0)	8-64	0	---
non-haemolytic	1 (20.0)	---	1	16
A. trota (12)				
α-haemolytic	3 (25.0)	---	3	6-64
β-haemolytic	7 (58.4)	8-64	0	---
non-haemolytic	2 (16.6)	---	2	8-16

* α- or non-haemolytic strains tested only after one-to-five sequential passages through RILs
--- : Not tested

Table II. Correlation between haemolytic and enterotoxic activities of *A. jandaei* and *A. trota*

Species and haemolytic character	No. of strains tested	No. showing fluid accumulation before passage	Fluid accumulation (mL/cm of RIL)	No. showing fluid accumulation after passage*	Fluid accumulation (mL/cm of RIL)
A. jandaei					
α-haemolytic	1	0	---	1	0.40-0.55
β-haemolytic	3	0	---	3	0.52-0.84
non-haemolytic	1	0	---	1	0.45-0.58
A. trota					
α-haemolytic	3	0	---	3	0.38-0.62
β-haemolytic	7	1	0.80-0.99	6	0.56-0.86
non-haemolytic	2	1	0.45-0.52	1	0.51-0.68
Positive control†	1	1	0.90-1.20	1	1.10-1.50
Negative control‡	---	---	---	---	-

*One to five sequential passages through RILs
+BHIB culture of V. cholerae strain 569B
++BHIB culture of E. coli strain 265
---:Not tested

Table III. Influence of animal passage on fluid outpouring capability and changes in haemolytic character of *A. jandaei* and *A. trota*

Species and strain no.	Haemolytic type	Fluid accumulation (mL/cm) after passage no. 0	1	2	3	4
A. jandaei						
AER-10	None	ND	0.45*	0.50	0.57	0.80
AER-41		ND	ND	ND	0.40*	0.56
AER-235		ND*	ND	0.60	0.72	0.85
A. trota						
AT-9	None	0.45	0.54*	0.71	-	-
AT-5654	None	ND	ND	ND*	0.50	0.69
AT-5		ND	ND	0.44*	0.61	-
AT-2625		ND	0.42*	0.54	0.65	-
AT-5688		ND	ND	ND	0.43*	0.58
AT-5504		0.80*	0.84	0.95	1.2	-

ND: Not detected
*: Enterotoxin produced

After one to five sequential passages through RILs, all of the *A. jandaei* and the majority (10 of 12) isolates of *A. trota* that caused little or no fluid accumulation in the initial tests did cause fluid accumulation, regardless of the type of haemolysis produced.

Three non-haemolytic, four a -haemolytic and two b -haemolytic strains showed a marked increase in fluid accumulation after each passage through RILs. The non-haemolytic and a -haemolytic strains switched over to the production of b -haemolysis when they showed a positive ileal loop reaction (Table III), and the titres of haemolytic activity also increased after each passage (Table IV). However, on storage for 2-4 weeks in peptone agar stab cultures at room temperature, or on repeated subcultures, all these strains reverted back to their original haemolytic character (either a - or non-haemolytic) and no longer produced enterotoxic activity.

Table IV. Enhancement of titres of haemolysin of *A. jandaei* and *A. trota* after animal passage

Species and strain no.	Haemolytic type	Haemolysin titres (HU/mL) after passage no. 0	1	2	3	4
A. jandaei						
AER-10	Non	ND	16*	32	64	---
AER-41		ND	ND	ND	64*	64
AER-235		16	32	64*	---	---
A. trota						
AT-9	Non	ND*	8	16	32	---
AT-5654	Non	ND	ND	16	32*	
128						
AT-5		ND	ND	8*	32	64
AT-2625		ND	32*	64	64	---
AT-5688		ND	ND	ND	64*	64
AT-5504		32*	64	128	---	---

ND: Not detected (lower limit for detection 0.3 mL/cm)
*: Change to haemolysis detected

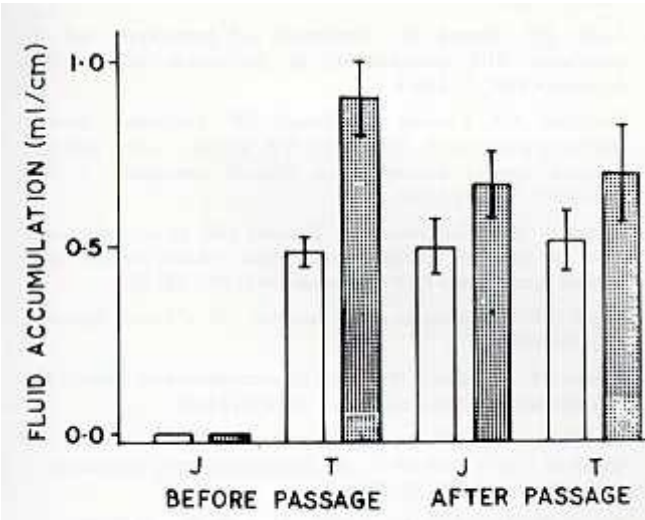

Fig. Enterotoxicity of haemolytic or non-haemolytic strains of *A. jandaei* (J) and *A. trota* (T) before (2 strains) and after (15 strains) passage.

DISCUSSION

The results of the present study showed that a majority of the strains produced b -haemolysis, indicating that this property is also extended to *A. jandaei* and *A. trota* as reported earlier for *A. hydrophila* and *A. sobria* (17,18). This phenomenon may be explained by the genetic evidence that sequences homologous to the *A. hydrophila* b -haemolysin gene are also present in *A. jandaei* and *A. trota* (30). Furthermore, b -haemolysin in *Aeromonas* spp. has been suggested to be a cytotoxin. The observation of this study, thus, suggests that *A. jandaei* and *A. trota* indeed possess this property.

Several workers have reported that enterotoxin is produced by most of the b -haemolytic strains of *Aeromonas* spp. (17,18,20). However, the results of the present study with *A. jandaei* and *A. trota* indicated that the majority of b -haemolytic strains (90%) did not show any enterotoxic activity when first tested ($p<0.05$). Only one non-haemolytic isolate of *A. trota* produced enterotoxin in the initial test. However, the b -haemolytic strains caused significantly more enterotoxic activity than the a - or non-haemolytic strains either before or after the animal passage (Fig.). This study clearly indicates that the capacity for enterotoxin production of *A. jandaei* and *A. trota* is not confined only to the b -haemolytic strains, but the a - and non-haemolytic strains also possess this property, although to a lesser extent like those observed with other *Aeromonas* spp. (22). These observations, thus, suggest that there is no correlation between enterotoxicity and haemolytic activity. However, b -haemolysin may play some role in enhancing the virulence in terms of enterotoxicity.

The observation that all the strains of *A. jandaei* and *A. trota* that failed to produce enterotoxin in the initial tests did produce an enterotoxic effect after sequential passages through RILs suggests that all the strains are potentially enterotoxigenic, regardless of the type of haemolysin produced. Similar observations on the effect of passage through the gut of a susceptible host were also made in our earlier studies with *Aeromonas* spp. (2,3,22,31) and other enteropathogenic organisms (28,29,32-35). Such a change may result from the existence of a repression-derepression phenomenon depending on a particular micro-environment and controlling the expression of a toxin gene, as observed in certain strains of *V. cholerae* (36) and *Aeromonas* spp. (27).

That a - and non-haemolytic strains of *A. jandaei* and *A. trota* switched over to the production of b -haemolysis after one-to-five serial passages through RILs, along with the initiation of fluid secretion,

indicates that this process may influence the control of b -haemolysin and toxin production. These observations, thus, indicate that a repression and derepression phenomenon may also be operative in the case of the b -haemolysin gene and that the rabbit gut provides a micro-environment conducive to its expression; this is confirmed by reversion of these strains to their original a - or non-haemolytic character either on prolonged storage or repeated subcultures. Similar observations on switching over to haemolysin production by a - and non-haemolytic strains, once their live cells gave a positive ileal loop reaction, were also made in our laboratory with other *Aeromonas* spp. (22,37). It was also demonstrated that *E. coli* strains produced a - and b - haemolysin. However, the release of a -haemolysin was brought about by complexing of as yet unidentified large molecular weight factors in the medium with cell-associated b -haemolysin (38-40).

The data of this study indicate that strains of *A. jandaei* and *A. trota* may also elicit a secretory response in RIL, irrespective of expression of their haemolysin gene. Passage through the gut of a susceptible host probably controls the expression of b -haemolysin and enterotoxin production.

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REFERENCES

1. Von Graevenitz A, Mensch AH. The genus Aeromonas in human bacteriology; reports of 30 cases and review of the literature. New Eng J Med 1968;278:245-9.
2. Sanyal SC, Singh SJ, Sen PC. Enteropathogenicity of Aeromonas hydrophila and Plesiomonas shigelloides. J Med Microbiol 1975;88:195-9.
3. Annapurna E, Sanyal SC. Studies on enteropathogenicity of Aeromonas hydrophila in an experimental model. Indian J Prev Soc Med 1975;6:234-7.
4. Cumberbatch N, Gurwith MJ, Langston C, Sack RB, Brunton JL. Cytotoxic enterotoxin produced by Aeromonas hydrophila: relationship of toxigenic isolates to diarrhoeal diseases. Infect Immun 1979;23:829-37.
5. Gracey M, Burke V, Robinson J. Aeromonas associated gastroenteritis. Lancet 1982;2:1304-6.
6. Holmberg SD, Farmer JJ. Aeromonas hydrophila and Plesiomonas shigelloides as cases of intestinal infections. Rev Infect Dis 1984;6:633-9.
7. Annapurna E, Sanyal SC. Enterotoxigenicity of Aeromonas hydrophila. J Med Microbiol 1977;10:317-23.
8. Ljungh A, Popoff M, Wadstrom T. Aeromonas hydrophila in acute diarrhoeal disease: detection of enterotoxin and biotyping of strains. J Clin Microbiol 1977;6:96-100.
9. Dubey RS, Sanyal SC. Enterotoxicity of Aeromonas hydrophila: skin responses and in vitro neutralization. Zentralbl Bakteriol Hyg A 1979;242:487-99.
10. Burke V, Robinson J, Berry RJ, Gracey M. Detection of enterotoxins of Aeromonas hydrophila by a suckling mouse test. J Med Microbiol 1981;14:401-8.
11. Dubey RS, Sanyal SC, Malhotra OP. Purification of Aeromonas hydrophila enterotoxin and its mode of action in experimental model. In: Eaker D, Wadstrom T, editors. Natural toxins. Oxford: Pergamon Press, 1980:259-68.

12. Barer MR, Millership SE, Tabaqchali S. Relationship of toxin production to species in the genus Aeromonas. J Med Microbiol 1986;22:303-9.
13. Kuijper EJ, Steigerwalt AG, Schoenmakers BS, Peeters MF, Zanen HC, Brenner DJ. Phenotypic characterization and DNA relatedness in human fecal isolate of Aeromonas spp. J Clin Microbiol 1989;27:132-8.
14. Ljungh A, Wadstrom T. Aeromonas toxin. In: Dorner F, Drews J, editors. Pharmacology of bacterial toxins. Oxford: Pergamon Press, 1986:289-305
15. Bernheimer AW, Avigad LS. Partial characterization of aerolysin; a lytic exotoxin from Aeromonas hydrophila. Infect Immun 1974;9:1016-21.
16. Janda JM. Biochemical and exoenzymatic properties of Aeromonas species. Diagn Microbiol Infect Dis 1983;3: 223-32.
17. Burke V, Robinson J, Atkinson HM, Gracey M. Biochemical characteristics of enterotoxic Aeromonas spp. J Clin Microbiol 1982;15:48-52.
18. Burke V, Robinson J, Beaman J et al. Correlation of enterotoxicity with biotype in Aeromonas spp. J Clin Microbiol 1983;18:1196-200.
19. Majeed KN, Egan AF, MacRae IC. Incidence of aeromonads in samples from an abattoir processing lambs. J Appl Bacteriol 1989;67:597-604.
20. Eko OF, Utsalo SJ. Characterization and significance of Aeromonas spp isolated from diarrhoeic stools in Nigeria. J Trop Med Hyg 1989;92:97-101.
21. Chakraborty T, Huhle B, Bergbauer H, Goebel W. Cloning, expression and mapping of the Aeromonas hydrophila aerolysin gene determinant in Escherichia coli K-12. J Bacteriol 1986;167:368-74.
22. Singh DV, Sanyal SC. Production of haemolysis and its correlation with enterotoxicity in Aeromonas spp. J Med Microbiol 1992;37:262-7.
23. Carnahan AM, Fanning GR, Joseph SW. Aeromonas jandaei (formerly genospecies DNA group 9 A. sobria) - a new sucrose-negative species isolated from clinical specimens. J Clin Microbiol 1991;29:560-4.
24. Carnahan AM, Chakraborty T, Fanning GR, et al. Aeromonas trota sp. nov., an ampicillin susceptible species isolated from clinical specimens. J Clin Microbiol 1991; 29:1206-10.
25. Smith HW, Haemolysins of Escherichia coli. J Pathol Bacteriol 1963;85:197-211.
26. Rennie RP, Arbuthnott JP. Partial characterization of Escherichia coli haemolysin. J Med Microbiol 1974;7:174-88.
27. De SN, Chatterje DN. An experimental study of the mechanism of action of Vibrio cholerae on the intestinal mucous membrane. J Pathol Bacteriol 1953;66:559-62.
28. Sanyal SC, Huq MI, Neogi PKB, Alam K, Kabir MI, Rahman ASMH. Experimental studies on the pathogenicity of Vibrio mimicus strains isolated in Bangladesh. Aust J Exp Biol Med Sci 1984;62:515-21.
29. Sanyal SC, Neogi PKB, Alam K, Huq MI, Al-Mahmud KA. A new enterotoxin produced by Vibrio cholerae O1. J Diarrhoeal Dis Res 1984;2:3-12.
30. Husslein V, Notermans SHE, Chakraborty T. Gene probes for detection of aerolysin in Aeromonas spp. J Diarrhoeal Dis Res 1988;6:124-30.
31. Singh DV, Sanyal SC. Enterotoxicity of clinical and environmental isolates of Aeromonas spp. J Med Microbiol 1992;36:269-72.
32. Shanker P, Agarwal RK, Sanyal SC. Experimental studies on enteropathogenicity of Vibrio cholerae serotype other than 1. Zentralbl Bakteriol Hyg 1982;A 252:514-4.

33. Sanyal SC, Sarswathi B, Sharma P. Enteropathogenicity of Plesiomonas shigelloides. J Med Microbiol 1980;13:401-9.
34. Sanyal SC, Agarwal RK, Annapurna E, Lee JV. Enterotoxicity of group F vibrios. Jpn J Med Sci Biol 1980;33:217-22.
35. Raychoudhury A, Agarwal RK, Sanyal SC. Enteropathogenicity of Klebsiella pneumoniae strains isolated from stools of diarrhoeal patients and other clinical specimens: an experimental study. Zentralbl Bakteriol Hyg 1984;A 258:94-103.
36. Singh SJ, Sanyal SC. Enterotoxicity of so called NAG vibrios. Ann Soc Belge Med Trop 1978;58:133-40.
37. Singh DV, Sanyal SC. Haemolysin and enterotoxin production by Aeromonas caviae isolated from diarrhoeal patients, fish and environment. J Diarrhoeal Dis Res 1992; 10:16-20.
38. Snyder IS, Koch NA. Production and characteristics of haemolysins of Escherichia coli. J Bacteriol 1966;91: 763.
39. Snyder IS, Zwadyk P. Some factors affecting the production and assay of Escherichia coli haemolysins. J Gen Microbiol 1969;55:139.
40. Zwadyk P, Snyder IS. Purification and kinetic studies of the haemolysin from Escherichia coli. Can J Microbiol 1971;17:741.

SHORT REPORT

Bacterial Lipopolysaccharide Induces Diarrhoea in Caecectomized Mice

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ABSTRACT

Castor oil, lipopolysaccharide of *Escherichia coli,* and endotoxin of *Salmonella typhimurium* were used for inducing diarrhoea in sham operated or caecectomized mice. Copious diarrhoea was induced by lipopolysaccharide (LPS) in caecectomized mice. Characteristics of diarrhoea induced by castor oil were not different between the two groups. It is concluded that caecectomized mice may be a good model to study lipopolysaccharide-induced diarrhoea.

Key words: Diarrhoea; Mice; Castor oil; *Escherichia Coli;* Endotoxins; Disease models, Animal; Lipopolysaccharides

INTRODUCTION

In 20%-40% of the patients suffering from diarrhoea, stool culture does not reveal the presence of enteric pathogens (1). Many such patients show microvascular changes in their rectum, akin to a Shwartzman reaction (2). It is most likely evoked by the lipopolysaccharides (LPS) of the bacterial endotoxins (3). However, mice given large doses of LPS orally or through stomach tube do not develop diarrhoea, but respond readily when subsequently challenged by a sub-cutaneous injection of LPS (4). After a single intravenous injection of LPS, microvascular lesions have been found. They lead to an influx of protein and fluid into the intestine. The role of nitric oxide (5), platelet-activating factor (6), and thromboxane A_2 (7) in this process has been documented. Yet, only occasional episodes of diarrhoea have been reported in these animals.

One reason for the lack of diarrhoeal response may be the caecum. Acting as reservoir it may hold the secretions, and discharge them slowly over some period of time into the colon, which completely absorbs these fluids (8). It has been observed that removal of the caecum makes rats and mice highly responsive to the diarrhoeagenic effects of cholera toxin (8) and 16-dimethyl prostaglandin E (9). The main objective of this study is to find if caecectomy induces diarrhoea in mice after a single intravenous injection of LPS.

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MATERIALS AND METHODS

Swiss albino mice weighing 25-30 g have been used for this study. Only fed animals were used. Caecectomy was performed under light anaesthesia induced by Nembutal (50 mg/kg) injected intraperitoneally. A curved incision, 1 cm below and parallel to the left costal margin, was made on the abdominal wall. It was stretched transversely with scissors. The caecum was visualised and pulled out by holding its tail end with a pair of blunt forceps. A ligature was placed so as to occlude the caecum and its vasculature, without compromising ileo-colonic patency, and the caecum was then resected. The exposed mucosa was cleaned with cotton soaked in saline. The intestinal segment was returned to the abdominal cavity. The incised muscle and skin were sutured separately with proline (6-0) thread. Immediately following the surgical procedure, the mice were returned to their cages and allowed free access to food and water. After 3 days, the mice started passing normal stools and were used for the experiments. To serve as controls, some mice were sham-operated by pushing back the caecum into the abdominal cavity without excising it.

Diarrhoea was induced by the administration of castor oil (Hospital Pharmacy, CMC Hospital, Vellore, India), *E. coli* LPS (Sigma, USA), and endotoxin of *Salmonella typhimurium* (Sigma, USA). Groups of sham-operated and caecectomized mice were given castor oil (0.3 mL orally) or LPS of *E. coli* (0.6 mg/kg i.v.) or LPS of *S. typhimurium* (0.3 mg/kg i.v.). There were six animals in each group. Animals were kept in individual cages with false bottoms lined with filter paper. The time taken for the onset of passage of stools, in minutes, was noted in each animal. On the basis of consistency of stool, a numerical score was assigned as follows: 1 = normal stools, 2 = formed stools, 3 = watery stools. The faecal output index (FOI) is defined as the summation of the number of stools and consistency score within the observation period of 4 hours. It is expressed as the mean ± SEM of each group. Student's *t* test was used for calculating the statistical significance.

RESULTS AND DISCUSSION

Table shows that the time of onset and FOI in various groups. After administration of castor oil, the onset time did not show any difference between the sham-operated and caecectomised groups of mice. The FOI was only slightly but significantly ($p<0.05$) elevated in the caecectomised mice. On the other hand, large and significant differences ($p<0.001$) were found in both the variables between the two groups when either lipopolysaccharide of *E. coli* or endotoxin of *S. typhimurium* was employed.

Table. Secretory diarrhoea in mice induced by castor oil, lipopolysaccharide of *E. coli*, and endotoxin of *S. typhimurium*

Secretogogue	Diarrhoea: Onset of passage of stools (in minutes)		Diarrhoea: Faecal output index	
	Sham operated	Caecectomised	Sham-operated	Caecectomised
1. Castor oil (0.3 mL/mouse; oral)	43±44	48±3	14±1	18±1*
2. *E. coli* (LPS) (0.6 mg/kg; i.v.)	No stool	42±2**	No stool	19±1**
3. *S. typhimurium* (0.3 mg/kg, i.v.)	87±3	96±2**	3±1	32±3**

*Significantly different from the sham-operated controls, $p<0.05$.
** Significantly different from the sham-operated controls, $p<0.001$.

Caecectomy was not able to amplify the diarrhoeal response to castor oil, since the predominant action of this agent seems to be on the colon (10). Shimizu (9) made similar observations with respect to castor oil-induced diarrhoea in caecectomized animals.

Parenteral administration of lipopolysaccharides of Gram-negative bacteria is known to injure the mucosa and vasculature of the intestine leading to increased permeability and leakage of fluid (5-7). A variety of

interventions seem to enhance these changes. The methods of conditioning include inoculation with live enterobacteria (4), *Trypanosomes* (11), *Schistosomes,* (12), BCG (13) or administration of inhibitors of nitric oxide synthase (5). However, in all these studies occurrence of diarrhoea has been reported only infrequently. In our experiments, diarrhoea was easily elicited by administration of LPS intravenously into the caecectomized mice at a relatively small dose without any prior treatment. While this response is most likely due to the loss of the reservoir function of the caecum (8) other possibilities cannot be excluded. Perhaps in humans the breakdown of the barrier function of the intestine may lead to translocation of bacteria and their toxins and cause similar changes progressing to diarrhoea.

In conclusion, the diarrhoeagenic effects of bacterial lipopolysaccharide can be amplified and brought out by using caecectomized mice.

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REFERENCES

1. Black RE. Relative importance of enteropathogens affecting humans. In: Tzipori S, editor. Infectious diarrhoea in the young. Amsterdam: Excerpta Medica, 1985:365-70.
2. Choudari CP, Mathan M, Rajan DP, Raghavan R, Mathan VI. A correlative study of aetiology, clinical features and rectal mucosal pathology in adults with acute infectious diarrhea in southern India. Pathology 1985;17:443-50.
3. Mathan M, Mathan VI. Local Shwartzman reaction in the rectal mucosa in acute diarrhoea. J Pathol 1985;146:179-88.
4. Mathan VI, Penny GR, Mathan MM, Rowley D. Bacterial lipopolysaccharide-induced intestinal microvascular lesions leading to acute diarrhoea. J Clin Invest 1988;82:1714-21.
5. Hutcheson IR, Whittle BJR, Boughton-Smith NK. Role of nitric oxide in maintaining vascular integrity in endotoxin induced acute intestinal damage in the rat. Brit J Pharmacol 1990;101:815-20.
6. Wallace JL, Steel G, Whittle BJR, Lagente V, Wargasftig B. Evidence for platelet activating factor as a mediator of endotoxin induced gastrointestinal damage in the rat. Gastroenterology 1987;93:765-73.
7. Boughton-Smith NK, Hutcheson I, Whittle BJR. Relationship between paf-acether and thromboxane A_2 biosynthesis in endotoxin-induced intestinal damage in the rat. Prostaglandins 1989;38:319-33.
8. Fondacaro JD, Kolpak DC, Burnham DB, McCafferty GP. Caecectomized rat: a model of experimental secretory diarrhoea in conscious animals. J Pharmacol Meth 1990;24:59-71.
9. Shimizu I, Kawaashima E, Yoshida N, Ito T. Analysis of 16, 16-dimethyl prostaglandin E2-induced diarrhoea in caecectomized rats. Arch Int Pharmacodyn 1994;328:326-43.
10. Beubler R, Juan H. Effect of ricinoleic acid and other laxatives on net water flux and prostaglandin E release by the rat colon. J Pharm Pharmacol 1979;31:681-5.
11. Ferrante A, Carter RF, Ferluga J, Allison AC. Lipopolysaccharide hyper reactivity of animals infected with Trypanosoma lewisi or Trypanosoma musculi. Infect Immun 1984;46:501-6.

12. Ferluga J, Doenhoff MJ, Allison AC. Increased hepatotoxicity of bacterial lipopolysaccharide in mice infected with Schistosoma mansoni. Parasite Immunol 1979;1:289-94.
13. Suter E. Hyperreactivity to endotoxin after infection with BCG. J Immunol 1984;92:49-54.

SHORT REPORT

Antidiarrhoeal Activities of Ocimum gratissimum (Lamiaceae)

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ABSTRACT

The antidiarrhoeal activities of leaf extracts of *Ocimum gratissimum* were investigated by disc diffusion and tube dilution methods. The extracts were active against *Aeromonas sobria, Escherichia coli, Plesiomonas shigelloides, Salmonella typhi, and Shigella dysenteriae.* The leaf extracts were most active against *S. dysenteriae* and least active against *S. typhi.* The sensitivity of the organisms measured in terms of zone of inhibition ranged from 8.00 to 19.50 mm. The minimum inhibitory concentrations were from 4.00 to 50.00 mg ml-1, while the minimum bactericidal concentration ranged from 8.00 to 62 mg m1-1. The potentials of the leaf extract for the treatment of diarrhoeal diseases is discussed.

***Keywords*:** Plants, Medicinal; *Ocimum gratissimum;* Antidiarrhoeals; *Aeromonas sobria; Escherichia coli; Plesiomonas shigelloides; Shigella dysenteriae*

INTRODUCTION

Medicinal plants constitute an effective source of both traditional and modern medicine. Herbal medicine has been shown to have genuine utility, and about 80% of rural populations depend on it as their primary health care (1). In Nigeria, various plant parts are used for curing different ailments with remarkable success. Among the enormous number of these medicinal plants are members of the genus *Ocimun L. (Lamiaceae).* The genus is represented by six species in West Africa (2). However, only three species, *O. gratissimum L. O. basilicum L.* and *O. canum Sims* have been reported to have medicinal properties (3). Extracts of leaves of *O. gratissimum* or of whole plants are popular for the treatment of diarrhoea. Cold infusions of the leaves are used for the relief of stomach upset and haemorrhoids(4). The leaf is reported to be rich in thymol, which has antimicrobial properties (5).

The problems of unavailability of pharmaceutical drugs in remote and rural areas, fake drugs and increasing rate of resistance of diarrhoeagenic bacteria to orthodox drugs prompted this investigation. The activities of leaf extracts against some of the bacterial species associated with diarrhoea are reported here.

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MATERIALS AND METHODS

Plant materials

Fresh leaves of *O. gratissimum* were collected from the plants growing on the premises of Lagos State University, Ojo Campus. The plants were authenticated and a voucher sample deposited in the Departmental Herbarium.

Extraction

The dried leaves were milled into a fine powder, using a Waring blender (Mill Mx - 391 N). The ingredients of the powdered leaves (50 g) were then extracted with 200 mL of distilled water in a Soxhlet extractor apparatus. The extract was sterilised, using a membrane filtration unit (Sartorius). The resulting sterile filtrate was aseptically transferred into a labelled sterile bottle.

Microbial cultures

The following bacteria, isolated from stool samples of diarrhoea patients in Lagos University Teaching Hospital, were used for the antimicrobial property determination: *A. sobria, E. coli, P. shigelloides, A. hydrophila, S. typhi, S. dysenteriae, and Pseudomonas aeruginosa.* Three strains of each species were tested. The organisms were maintained on blood agar slopes at 4 ºC and subcultured for 24 hours before use.

Bacterial sensitivity testing

Inocula containing 1x106 cells per ml were introduced onto the surface of sterile nutrient agar plates. They were distributed evenly with a sterile glass spreader. A sterile paper disc previously soaked in the leaf extract was carefully placed at the centre of the labelled plate of each of the bacterial strains. The plates were incubated at 37 ºC and examined for zone of inhibition after 24 hours. Distilled water was used as the control.

Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts were determined by diluting the extracts to various concentrations (0.0-65.0 mg mL-1), using nutrient broth in test tubes. Each test tube was inoculated with a bacterial suspension containing $1x10^6$ cells per mL and incubated at 37 ºC for 24 hours. The MIC was regarded as the lowest concentration of the extract that did not permit any visible growth when compared with drug free broths inoculated with each of the bacterial suspensions. The MBC was determined using the method of Rotimi et al (6). Tubes that showed no visible growth were streaked on fresh nutrient agar plates, incubated at 37oC for 24h and examined for growth. The MBC was regarded as the lowest concentration of the extracts that prevents the growth of any bacterial colony on solid medium.

RESULTS

The sensitivity of the aqueous extract of *0 gratissimum* measured by the zone of inhibition varies from (means + 1 SEM) 8.0±1.8 mm in *S. typhi* to 19.5±0.6 mm in *S. dysenteriae. P. aeruginosa and A. hydrophila* were not inhibited by the extract (Table I). The antibacterial activity of the leaf extracts was measured in terms of MIC and MBC. The lowest minimum inhibitory concentration of 4.0 mg mL^{-1} was against *S. dysenteriae,* while the highest value of 50.0 mg mL^{-1} was against *S. typhi* (Table II). Also the MBC ranged from 8.0 mg mL^{-1} in *S. dysenteriae* to 62.0 mg mL^{-1} in *S. typhi* (Table II).

Table I. Sensitivity of bacteria to leaf extracts of *O. gratissimum*

Test organism	Zone of inhibition*(mm)
S. dysenteriae	19.5±0.6
P. shigelloides	15.5±0.3
A. sobria	13.6±1.3
E. coli	11.5±1.5
S. typhi	8.0±1.8
A. hydrophila	0.0
P. aeruginosa	0.0
Sterile distilled water (control)	0.0

* means ± 1 SEM of 3 values.

Table II. Antibacterial activities of leaf extracts of *O. gratissimum* measured in terms of MIC and MBC

Organisms tested	MIC (mg mL^{-1})	MBC (mg mL^{-1})
S. dysenteriae	4.0	8.0
P. shigelloides	8.0	16.0
A. sobria	16.0	31.0
E. coli	32.0	45.0
S. typhi	50.0	62.0

DISCUSSION

The aqueous extracts of the leaves of *0 gratissimum* contain substances with antibacterial properties. This agrees with the works of Olowokudejo and Pereira-Sheteolu (3) and Sofowora (5). The extracts were active against the following bacteria of medical importance. *A. sobria, P. shigelloides, E. coli, S. dysenteriae, and S. typhi.* Some of the infections by these bacteria include diarrhoea, gastrointestinal disorders, and typhoid fever. Resistant strains of these organisms to many pharmaceutical drugs have been widely reported. *Aeromonas* spp, and *Plesiomonas* spp. which have been implicated in diarrhoeal diseases, are mostly resistant to penicillin, ampicillin, and carbenicillin (7).

The extract was most active against *S. dysenteriae* and least active against *S. typhi*. Previous chemical analyses of the plant showed the presence of thymol and eugenol that might be responsible for the antibacterial properties. The MIC values of the extract were lower than the MBC values, suggesting that the plant extract is bacteriostatic at lower concentrations but bactericidal at higher concentrations. These results offer a scientific rationale for the traditional use of the aqueous extract for the treatment of diarrhoeal diseases. In Nigeria, where the plant grows wild, the leaves are plucked, squeezed in water, sieved and the aqueous extract is drunk to treat diarrhoea with remarkable successes.

Recently, there has been an increase in the number of people in Nigeria depending on herbal drugs because of decline in purchasing power and the increasing fear of purchasing fake orthodox drugs. Herbal drugs are cheap, readily available and unadulterated. Their antibacterial activity could be increased by partial purification and subsequent concentration of the active ingredients.

REFERENCES

1. World Health Organisation. The promotion and development of traditional medicine. Geneva: World Health Organization, 1978. (Technical report series no. 622).
2. Hutchinson J, Dalziel MD. Flora of West Tropical Africa. London, 1963. 148 p.
3. Olowokudejo JD, Pereira-Sheteolu O. The taxonomic value of epidermal characters in the genus Ocimum (Lamiaceae). Phytomorphology 1988;38:147-58.
4. Essien EA, Adebanjo AO, Adewunmi CO, Adebiyi OO. Anti-infective Agents of Higher Plant Origin. 3d ed. Bristol: J Wright, 1983. 170 p.
5. Sofowora EA. Medicinal Plants and Traditional Medicine in Africa. Chichester: J Wiley, 1982. 256 p.
6. Rotimi VO, Lanhon BS, Bartlet JS and Mosadomi HA. Activities of Nigerian chewing sticks extracts against Bacteroides gingivalis and Bacteroides melaninogenicus. Antibacter Agents Chemother 1988;32:598-600.

7. Fainstein V, Weaver S, Bodey GP. In vitro susceptibilities of Aeromonas hydrophila against new antibiotics. Antimicrob Agents Chemother 1982;22:513-4.

SHORT REPORT

Epithelial Adherence of Candida albicans is Enhanced by Passage Through Rat Small Intestine

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SUMMARY

Seven *Candida albicans* isolates (four from patients with diarrhoea and three from healthy persons) underwent two passages through rat ileal loop (RIL) to see the effect of consecutive passages on the adherence to rat intestinal epithelium. The isolates from patients with diarrhoea showed a significant enhancement in adherence after the first passage ($1.95x10^4$ cfu/cm^2 versus $3.67x10^4$ cfu/cm^2). There was no further increase between the first passage ($3.67x10^4$ cfu/cm^2) and the second one ($3.61x10^4$ cfu/cm^2). A similar pattern was observed with the three non-diarrhoeal isolates. Animal passage of this fungus probably leads to better interactions between the cell surfaces causing the enhanced adherence.

Key words**:** *Candida albicans;* Bacterial adhesion; Virulence; Disease models, Animal

INTRODUCTION

Several studies have reported the association of fungal overgrowth and diarrhoea (1-6). Adherence to the host surfaces is an initial and important event in colonisation and subsequent disease production for fungal as well as other enteropathogens. Many reports have indicated in vitro adherence of *Candida albicans* to acrylic surfaces, HeLa cells, and epithelial cells of oral as well as intestinal origin (7-9). Unlike for bacteria, it is the adherence, not fluid accumulation in ligated rat ileal loops (RIL), that is tested as the virulence marker of fungi. Further, several studies suggest that non-cultivable bacteria become cultivable, along with enhanced expression of their virulence and significant fluid accumulation in RIL, after repeated passages of potentially pathogenic bacteria in the intestine of experimental animals (10-13). This aspect has not been looked into so far in relation to *C. albicans*. The present study, therefore, explores the effect of the subsequent passage of *C. albicans* of diarrhoeal and non-diarrhoeal origin in the rat ileal loop on the adherence to the intestinal epithelial cells.

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MATERIALS AND METHODS

Organism

Four diarrhoeal (St 1, St 2, St 188, and St 3042) and three non-diarrhoeal (Spt 2, Spt 4, and Ga 98) isolates of C. albicans were included in this study. The diarrhoeal isolates grew predominantly from watery stool specimens, and were not associated with known enteropathogens. All the four patients responded well to antifungal therapy. The three non-diarrhoeal strains were obtained as pure growth from a gastric aspirate (Ga 98) and swabs from non-healing oral ulcers (Spt 2 and Spt 4). The strains were preserved on SDA slants and did not undergo more than two subcultures before use in the present study. The organisms were grown at 37 ° C in a culture medium (2 mL), containing yeast nitrogen base with 50 mM of glucose in an orbital shaker, operating at 150 rpm for 24 hours. Eight mL of Krebs–Henseleit (KH) buffer were added to each of the bottles to achieve an approximate concentration of 1 to $3x10^7$ cfu/mL. The concentration was checked by quantitative culture on the SDA plates.

RIL passage

Albino rats (Charles-Foster strain) weighing more than 200 g were used in this study. The animals were starved for 24 hours, but were allowed to drink water freely. For the consecutive passages, ligated ileal loops were used, following the method of Sanyal et al. (10). The peritoneum of each rat was exposed by abdominal incision under light ether anaesthesia, and the intestine was exposed up to ileocaecal junction. Then, starting from the ileocaecal junction, six ligated loops of the intestine were made, each measuring 5-6 cm. They were separated by 2-3 cm interloop to avoid leakage. To each loop 0.5 ml of the test material was inoculated. It comprised four strains of C. albicans with one made positive for fluid accumulation (by adding Vibrio cholerae 569B) and one negative control (not inoculated broth and KH buffer). The abdomen was closed, and the rats were allowed to drink water only. They were sacrificed after 18 hours, and C. albicans were recovered on SDA plates containing gentamicin. The second passage was made with the organism thus obtained in a similar manner as described for the first time.

Adherence assay

Measure of adherence of the yeast to intestinal mucosa was carried out as described by Freter and Jones (14), with slight modifications. Four slices of 1 cm^2 each of the intestine of healthy rats were removed and thoroughly washed with KH buffer to remove all debris. The four slices from three different rats were incubated in separate bottles containing 10 mL of KH buffer and broth culture of one each of the 7 C. albicans isolates at 37 ° C for one hour in shaking water bath before and after the passages. Each slice was washed thrice into three tubes containing physiological saline. After homogenisation of these tissue slices with 3 mL of KH buffer, 0.1 mL of the material was inoculated on SDA plates for quantitating the colony-forming unit by serial 10-fold dilution in KH buffer. After the incubation at 37 ° C for 48 hours, the colonies were counted, and the number of adhering units was calculated. For each strain, all the above tests were done in triplicate, and the average number of cfu/cm^2 tissue slice was calculated. The statistical analysis was done using the Kruskal–Wallis test.

RESULTS AND DISCUSSION

Adherence of the diarrhoeal isolates to the epithelial cell surfaces was observed to be enhanced (2–fold approximately) after the first passage, from $1.95x10^4/cm^2$ to $3.67x10^4/cm^2$ but without any increase after the second passage ($3.61x10^4/cm^2$). Interestingly, a similar pattern was observed with respect to the non-diarrhoeal isolates, i.e. from $1.43x10^4$ organism/cm^2 to $3.61x10^4/cm^2$ after the first passage, but without further increase after the subsequent passage ($3.69x10^4/cm^2$). Also, it was surprising to note that the strain-to-strain variation in the adherence of all the seven isolates before passage vanished after the first passage (Table). The reason of this phenomenon deserves exploration. The enhancement in adherence after the first passage was statistically significant ($p<0.001$) for both types of isolates and remained so after the second passage. It may be concluded that the influence of passage on the adherence of C. albicans was not exclusive of the diarrhoeal isolates. The enhancement may be occurring either because of better interactions between cell surfaces or a repression-derepression phenomenon controlling the expression of virulence marker (adherence) in the in vivo micro-environment (10-12). The later possibility seems to be less likely as C. albicans is part of the normal gut flora, and therefore, the role of natural passage of this fungus in the enhancement of its diarrhoeagenic potential is doubtful.

Table: Adherence of *Candida albicans* before and after passage through rat ileal loop (RIL). Number of organisms adhering per cm^2 area of intestinal mucosa

Strain	Before passage (P_0) No. x 10^4	After 1st passage (P_1) No. x 10^4	After 2nd passage (P_2) No. x 10^4
[A] DIARRHOEAL (D)			
St 1	2.44	3.79	3.75
St 2	1.49	3.80	3.54
St 188	2.65	3.62	3.66
St 3042	1.21	3.49	3.50
Mean	1.95	3.67	3.61
[B] NON-DIARRHOEAL (ND)			
Spt 2	1.94	3.54	3.50
Spt-4	1.70	3.90	3.76
Ga 98	0.67	3.40	3.82
Mean	1.43	3.61	3.69

DP_0 vs. NDP_0, DP_1 vs. NDP_1 and DP_2 vs. NDP_2 = Not significant
DP_0 vs. DP_1, DP_0 vs. DP_2 | $p<0.001$
NDP_0 vs. NDP_1, NDP_0 vs. NDP_2 |
DP_1 vs. DP_2 and NDP_1 vs. NDP_2 = Not significant

REFERENCES

1. Kane JG, Chretien JH, Garagusi VF. Diarrhoea caused by Candida. Lancet 1976;1:35-6.
2. Kumar V, Chandrasekaran R, Kumar L. Candida diarrhoea. Lancet 1976;1:752.
3. Alam SA, Tahir M, De MN. Candida as a cause of diarrhoea in children. Bangladesh Med Res Council Bull 1977;3:32-6.
4. Chretien JH, Garagusi VF. Current management of fungal enteritis. Med Clin North Am 1982;66:675-87.
5. Bolivar R, Bodey GP. Candidiasis of the gastrointestinal tract. In: Bodey GP, Faintein V, editors. Candidiasis. New York: Raven Press, 1985;1981-2001.
6. Talwar P, Chakrawarti A, Chawla A, Mehta S, Walia BNS, Kumar L, Chugh KS. Fungal diarrhoea association of different fungi and seasonal variation in their incidence. Mycopathologia 1990;110:101-5.
7. King RD, Lee JC, Morris AL. Adherence of C. albicans and other Candida spp. to mucosal epithelial cells. Infect Immun 1980;27:667-74.
8. McCourtie J, Douglas LJ. Relationship between cell surface composition, adherence and virulence of C. albicans. Infect Immun 1984;45:6-12.
9. Kennedy MJ, Johnson AM, Volz PA, Neely AN, Yancey RJ. Virulence and adhesive properties of serotype A and B of Candida albicans isolated from pediatric burn patients. J Med Microbiol 1992;36:428-36.
10. Sanyal SC, Saraswati B, Sharma P. Enteropathogenicity of Plesiomonas shigelloides. J Med Microbiol 1980;13:401-9.
11. Shanker P, Agarwal RK, Sanyal SC. Experimental studies on enteropathogenicity of Vibrio cholerae serotype other than O1. Zentral Bakteriol Mikrobiol Hyg 1982;252:514-24.
12. Singh DV, Sanyal SC. Haemolysin and enterotoxin production by Aeromonas caviae isolated from diarrhoeal patients, fish and environment. J Diarrhoeal Dis Res 1992;10:16-20.
13. Tikoo A, Singh DV, Sanyal SC. Influence of animal passage on haemolysin and enterotoxin production in Vibrio cholerae O1 biotype El Tor strain. J Med Microbiol 1994; 40:246-51.
14. Freter R, Jones GW. Model for studying the role of bacterial attachment and pathogenesis. Rev Infect Dis 1983;5:5647-58.

BIBLIOGRAPHY ON DIARRHOEAL DISEASES

295. Addiss DG, Pond RS, Remshak M, Juranek DD, Stokes S, Davis JP. Reduction of risk of watery diarrhea with point-of-use water filters during a massive outbreak of waterborne Cryptosporidium infection in Milwaukee, Wisconsin, 1993. Am J Trop Med Hyg 1996 Jun;54(6):549-53. 21 ref, Eng. Division of Parasitic Diseases, Mailstop F-22, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, Atlanta, GA 30341, USA

296. Alam NH, Bardhan PK, Haider R, Mahalanabis D. Trimethoprim-sulphamethoxazole in the treatment of persistent diarrhoea: a double blind placebo controlled clinical trial. Arch Dis Child 1995 Jun;72(6):483-6. 12 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"The efficacy of an absorbable antimicrobial agent trimethoprim-sulphamethoxazole (TMP-SMX) in the management of children with persistent diarrhoea was evaluated in a double blind, randomised, and placebo controlled trial. Of the 55 patients studied, 28 received TMP-SMX, and 27 received placebo. A trend in stool weight reduction was observed from the third day after the drug was started, and the reduction was statistically significant on day 6 and day7. However, the difference in total stool output (g/kg) up to day 7 was not significantly different between the two groups. The proportion of children whose diarrhoea resolved by day 7 (therapeutic success) was significantly more in the treatment group compared with the placebo group (23 v 15). Additionally, mean duration of diarrhoea in the group that received TMP-SMX was less compared with the placebo group (6.0 v 8.3 days); this difference, however, was not significant. Hospital infection (probably nosocomial infection) was significantly less in the TMP-SMX treated group (1 v 10). The results of our study indicate that TMP-SMX has a clinical benefit in respect of reducing the stool output, and higher recovery rate within seven days of treatment. In addition, it prevented possible hospital acquired infection."

297.Albert MJ, Bhuiyan NA, Rahman A, Ghosh AN, Hultenby K, Weintraub A, Nahar S, Kibriya AKMG, Ansaruzzaman M, Shimada T. Phage specific for *Vibrio cholerae* O139 Bengal (note). J Clin Microbiol 1996 Jul;34(7):1843-5. 13 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"From the stool of a Vibrio cholerae O139 Bengal-infected patient, a phage that specifically lysed capsulated V. cholerae O139 strains only was isolated. The phage is useful for the confirmatory diagnosis of V. cholerae O139 infection and for the differentiation of variants that lack the capsule."

298. Albert MJ. *Vibrio cholerae* O139 Bengal: probable causative agent of the eighth pandemic of cholera. Hong Kong J Paediatr 1995 Sep;6(1 Suppl):56-62. 15 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

299. Azim T, Sarker MS, Hamadani J, Khanum N, Halder RC, Salam MA, Albert MJ. Alterations in lymphocyte phenotype and function in children with shigellosis who develop complications. Clin Diagn Lab Immunol 1996 Mar;3(2):191-6. 29 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"This study was designed to see whether alterations occur in peripheral blood mononuclear cell phenotype and function in children with Shigella dysenteriae 1 infection with complications (leukemoid reaction and/or hemolytic-uremic syndrome) and whether there are any alterations prior to the development of complications. The following groups of children (ages, 12 to 60 months) were compared: children without any infection (n=51), children with uncomplicated shigellosis (n=65), children admitted with complicated shigellosis (leukemoid reaction and/or hemolytic-uremic syndrome) (n=29), and children with shigellosis who developed complications after enrollment (subsequently complicated shigellosis) (n=12). Tests for the peripheral blood mononuclear cell phenotype (CD3, CD4, CD8, CD16, CD20, and

CD25), spontaneous proliferation, and the proliferative response to phytohemagglutinin, pokeweed mitogen, and the lipopolysaccharide of S. dysenteriae 1 were performed, as were skin tests for delayed-type hypersensitivity (DTH). Children was subsequently developed complications differed from other groups of children as follows: (i) the numbers of CD3+ and CD4+ cells were lower than in uninfected children (P<0.05), (ii) the CD4/CD8 ratio was lower than in children with uncomplicated shigellosis (P<0.05) and in uninfected children (P<0.05), and (iii) the levels of spontaneous proliferation of peripheral blood mononuclear cells were higher and DTH responses were lower than those in children with uncomplicated shigellosis (P<0.05 and P<0.05 and P<0.017, respectively). Children with complications differed by having (i) increased numbers of CD3- CD16- CD20- cells (P<0.05) compared with those in other groups of children and (ii) lower CD4/CD8 ratios (P<0.05), higher levels of spontaneous proliferation (P<0.05), and lower DTH responses (P=0.005) than children with uncomplicated shigellosis. Three to five days after enrollment, the number of CD4+ cells increased in children who subsequently developed complications (P=0.025), i.e., when they developed complications and at this time their CD4+ cell number was similar to that of other groups of children. Thus, lymphocyte phenotype and function are altered prior to the development of complications in children with shigellosis, and once complications develop, the pattern of alterations changes. Whether these alterations have a role in precipitating complications or whether they reflect early events underlying the development of complications remains to be elucidated."

300. Ball HJ, Finlay D, Zafar A, Wilson T. The detection of verocytotoxins in bacterial cultures from human diarrhoeal samples with monoclonal antibody-based ELISAs. J Med Microbiol 1996 Apr;44(4):273-6. 29 ref, Eng. Veterinary Sciences Division, Stoney Road, Stormont, Belfast BT4 3SD, UK

301. Ball JM, Tian P, Zeng CQ-Y, Morris AP, Estes MK. Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein. Science 1996 Apr 5;272(5258):101-4. 28 ref, Eng. Medical Institute and Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030, USA

302. Bass D. Viral infections of the gastrointestinal tract. Curr Opin Gastroenterol 1996 Jan;12(1):76-81. 44 ref, Eng. Division of Pediatric Gastroenterology, Stanford University School of Medicine, Stanford, CA 94305-5119, USA

303. Beaugerie L, Baumer P, Chaussade S, Berard H, Rozenbaum W, Pialoux G, Quintrec YL, Schwartz J-C, Lecomte J-M. Treatment of refractory diarrhoea in AIDS with acetorphan and octreotide: a randomized crossover study. Eur J Gastroenterol Hepatol 1996 May;8(5):485-9. 35 ref, Eng. Laboratoire Bioprojet, 30 rue des France-Bourgeois, 75003 Paris, France

304. Bhardwaj A, Aggarwal V, Chakravarty A, Mittal SK. Does rota virus infection cause persistent diarrhoea in childhood? Trop Gastroenterol 1996 Jan-Mar;17(1):18-21. 10 ref, Eng. Department of Pediatrics and Microbiology, Maulana Azad Medical College, New Delhi 110002, India

305. Bik EM, Gouw RD, Mooi FR. DNA fingerprinting of *Vibrio cholerae* strains with a novel insertion sequence element: a tool to identify epidemic strains. J Clin Microbiol 1996 Jun;34(6):1453-61. 44 ref, Eng. Molecular Microbiology Unit, National Institute of Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands

306. Brydon WG, Nyhlin H, Eastwood MA, Merrick MV. Serum 7 -hydroxy-4-cholesten-3-one and selenohomocholyltaurine (SeHCAT) whole body retention in the assessment of bile acid induced diarrhoea. Eur J Gastroenterol Hepatol 1996 Feb;8(2):117-23. 19 ref, Eng. Gastrointestinal Laboratory, Western General Hospital, Edinburgh EH4 2XU, UK

307. Burnens AP, Frey A, Nicolet J. Association between clinical presentation, biogroups and virulence attributes of *Yersinia enterocolitica* strains in human diarrhoeal disease. Epidemiol Infect 1996 Feb;116(1):27-34. 35 ref, Eng. Swiss National Reference Laboratory for Foodborne Diseases, Institute for Veterinary Bacteriology, University of Berne, Langgass-Strasse 122, 3012 Berne, Switzerland

308. Burns JW, Siadat-Pajouh M, Krishnaney AA, Greenberg HB. Protective effect of rotaivrus VP6-specific IgA monoclonal antibodies that lack neutralizing activity. Science 1996 Apr 5;272(5258):104-7. 30 ref, Eng. Department of Medicine, Medical School Lab Surge Building, Room P304, Stanford University School of Medicine, Stanford, CA 94305, USA

309. Butzner JD. Impact of protein-energy malnutrition and acute enteric infection on intestinal function in the infant. Hong Kong J Paediatr 1995 Sep;6(1 suppl):89-91. 29 ref, Eng. Department of Pediatrics, University of Calgary, Clinical Investigator, Alberta Heritage Foundation for Medical Research, Room 1845, Health Science Centre 3330 Hospital Drive NW, Calgary, AB, Canada T2N 4N1

310. Buysse JM, Hartman AB, Strockbine N, Venkatesan M. Genetic polymorphism of the *ipaH* multicopy antigen gene in *Shigella spps.* and enteroinvasive *Escherichia coli*. Microbial Pathogen 1995 Nov;19(5):335-49. 46 ref, Eng. Molecular Biology Research, Upjohn Laboratories, 301 Henrietta Street, Kalamazoo, MI 49007, USA

311. Campos E, Bolanos H, Acuna MT, Diaz G, Matamoros MC, Raventos H, Sanchez LM, Sanchez O, Barquero C. *Vibrio mimicus* diarrhea following ingestion of raw turtle eggs. Appl Environ Microbiol 1996 Apr;62(4):1141-4. 23 ref, Eng. Centro Nacional de Referencia para Colera, Instituto Costarricense de Investigacion y Ensenanza en Nutricion y Salud, Apdo 4, Tres Rios, Costa Rica

312. Carbonare SB, Palmeira P, Silva MLM, Carneiro-Sampaio MMS. Effect of microwave radiation, pasteurization and lyophilization on the ability of human milk to inhibit *Escherichia coli* adherence to HEp-2 cells. J Diarrhoeal Dis Res 1996 Jun;14(2):90-4. 21 ref, Eng. Instituto Butantan, Av. Dr. Vital Brasil, 1500, 05503-900, Sao Paulo, SP, Brazil

"The effect of different physical treatments on the ability of colostrum and human milk to inhibit the adherence of enteropathogenic Escherichia coli (EPEC) to human epithelial cells was studied. Pools of colostrum and milk were submitted to microwave radiation, pasteurization or lyophilization, and then tested for the ability to inhibit the adherence of EPEC O111:H- to HEp-2 cells. The inhibitory effect of untreated colostrum and human milk on localized adherence was not significantly modified after exposure to any treatment. The total protein values of colostrum and milk were maintained, but IgA concentration and colostral anti-EPEC IgA were reduced after pasteurization. Nevertheless, the remaining IgA was sufficient to be effective in adhesion inhibition assay. Western blotting assays carried out with EPEC antigens showed that the treated and untreated pools recognize a 94-kDa outer-membrane protein which molecular weight is compatible with intimin, an EPEC adhesin related to bacterial attachment to epithelial cells. These results suggest that the protection of colostrum and milk to infantile diarrhoea due to EPEC remains unalterable after the physical treatments studied."

313. Cevallos A-M, Carnaby S, James M, Farthing MJG. Small intestinal injury in a neonatal rat model of giardiasis is strain dependent. Gastroenterology 1995 Sep;109(3):766-73. 51 ref, Eng. Department of Gastroenterology, St. Bartholomew's Hospital, London EC1A 7BE, UK

314. Chapman PA, Siddons CA. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from cases of bloody diarrhoea, non-bloody diarrhoea and asymptomatic contacts. J Med Microbiol 1996 Apr;44(4):267-71. 22 ref, Eng. Public Health Laboratory, Herries Road, Sheffield S5 7BQ, UK

"Enrichment culture in modified buffered peptone water followed by immunomagnetic separation (IMS) with magnetic beads coated with an antibody against Escherichia coli O157 was compared with direct culture on cefixime rhamnose sorbitol MacConkey agar (CR-SMAC) and cefixime tellurite sorbitol MacConkey agar (CT-SMAC) for the isolation of E. coli O157 from human faeces. In total, 690 samples were examined; E. coli O157 was isolated from 25 samples by IMS but from only 15 and 12 by direct culture on CT-SMAC and CR-SMAC, respectively. The difference in sensitivity of detection was at its most marked on screening repeat faecal samples from known cases and samples from asymptomatic contacts, when of 12 strains of E. coli O157 isolated by IMS, only five were isolated by direct culture. IMS is a sensitive and simple technique for the isolation of E. coli O157 from human faecal samples and should prove useful in elucidating further the epidemiology of this micro-organism."

315. Chaudhury A, Nath G, Shukla BN, Sanyal SC. Biochemical characterisation, enteropathogenicity and antimicrobial resistance plasmids of clinical and environmental *Aeromonas* isolates. J Med Microbiol 1996 Jun;44(6):434-7. 19 ref, Eng. Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

316. Chaudhury A, Nath G, Shukla B, Panda S, Singh TB. Diarrhoea associated with *Candida* spp.: incidence and seasonal variation (short report). J Diarrhoeal Dis Res 1996 Jun;14(2):110-2. 12 ref, Eng. Department of Medical Microbiology and Paediatrics, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

"To study the incidence and seasonal variation of diarrhoea associated with Candida, 978 diarrhoeal stool specimens from patients of all age groups were examined by microscopy and culture. Candida spp. was the sole pathogen (unassociated with other diarrhoeagenic bacteria, protozoa, or helminths) in 15.3% of the total cases. Candida albicans (94.9%) was the predominant species isolated. The incidence was highest among the infants aged 012 months (37.1%), followed by a decline in the rest of the children aged less than 5 years with a second peak in the people aged over 5 years, including adults. The paediatric age group had a significantly higher incidence in the summer season compared to the rainy ($p<0.05$) or winter ($p<0.01$) months. Thus, there was a definite age and seasonal variation in the incidence of diarrhoea caused by the overgrowth of Candida in the Varanasi region of the Indian subcontinent."

317. Concha A, Giraldo A, Castaneda E, Martinez M, de la Hoz F, Rivas F, Depetris A, Svennerholm A-M, Sack DA. Safety and immunogenicity of oral killed whole cell recombinant B subunit cholera vaccine in Barranquilla, Colombia. Bull Pan Am Health Organ 1995 Dec;29(4):312-21. 14 ref, Eng. Carrera 115 No. 6-140 C20, Cali, Colombia

"In January and February 1992, an assessment was conducted of the safety and immunogenicity of two doses of a new oral cholera vaccine prepared from the recombinant B subunit of the toxin and from killed whole cells (rBS/WC) in 1165 individuals between the age of 12 months and 64 years in Barranquilla, Colombia. This was a randomized, double-blind placebo-controlled study. Participants received two doses of either the vaccine or a placebo (killed Escherichia coli K12) over a two-week interval. Few symptoms were detected during the three days following administration of the initial dose and even fewer following the second. Sera obtained upon administration of the first dose and two weeks after administration of the second were tested for Vibrio cholerae O1 inaba vibriocidal antibodies and antitoxins. Geometric mean titers (GMT) of vibriocidal antibodies were found to increase two-fold in subjects receiving the vaccine. In the paired samples taken from vaccinated subjects, two-fold or greater increases were observed in 44% and four-fold or greater increases were observed in 34%, as compared to similar increases in 9.2% and 2.2% of the sera taken from those receiving the placebo ($P<0.05$). The GMTs of IgG and IgA antitoxins, as determined by ELISA, increased by factors of 4 and 3.2, respectively,

in those receiving the vaccine, as compared to factors of 1.1 and 1.1 in those given the placebo (P<0.001 for IgG, P<0.01 for IgA). Approximately 80% of the paired samples from the vaccinated group showed an increase of both IgG and IgA antitoxins 1.5, as compared to only about 20% of those in the placebo group (P<0.000001). Belonging to the O blood group did not significantly affect the immune response. Children under age four tended to show a weaker vibriocidal antibody response and a stronger antitoxin response than older subjects. The two doses of oral vaccine were found to be safe and without attributable side-effects. The vibriocidal antibody and antitoxin responses were similar to those obtained previously with the conventional oral killed whole cell B subunit cholera vaccine."

318. Dansinger ML, Johnson S, Jansen PC, Opstad NL, Bettin KM, Gerding DN. Protein-losing enteropathy is associated with *Clostridium difficile* diarrhea but not with asymptomatic colonization: a prospective, case-control study. Clin Infect Dis 1996 Jun;22(6):932-7. 28 ref, Eng. Department of Medicine (111), Veterans Affairs Lakeside Medical Center, 333 East Huron Street, Chicago, Illinois 60611, USA

319. Das SC, Mullick SG, Chaudhury S, Roy PK. Pattern of antimicrobial sensitivity of bacteria isolated from the genitalia of dairy cows with particular reference to *Campylobacter* species. Indian J Comp Microbiol Immunol Infect Dis 1995 Jul & Oct;16(34):172-5. 10 ref, Eng. Indian Veterinary Research Institute Regional Station, 37-Belgachia Road, Calcutta 700037 (W.B.), India

320. Davidson GP. Update on viruses. Hong Kong J Paediatr 1995 Sep;6(1 Suppl):1-13. 87 ref, Eng. Gastroenterology Unit Women's and Children's Hospital, 72, King William Road, Adelaide SA 5006, Australia

321. Davies-Adetugbo AA. Promotion of breast feeding in the community: impact of health education programme in rural communities in Nigeria. J Diarrhoeal Dis Res 1996 Mar;14(1):5-11. 29 ref, Eng. Department of Community Health, College of Health Sciences, Obafemi Awolowo University, Ile Ife, Nigeria

"Breast feeding has been recognized as a child survival strategy, while breast feeding programmmes have been increasingly implemented in many communities. This study assesses the effectiveness of a breast feeding education programme launched through the primary health care programme in the rural communities of Nigeria. Late trimester pregnant women were enrolled into the study and given a questionnaire on knowledge, attitudes, and practices (KAP) about breast feeding. Women in the study group (n=126) received breast feeding counselling before and after delivery, while those in control group (n=130) did not receive any counselling. Both groups were monitored after delivery and followed with the KAP questionnaire. The results of the study showed marked improvements in the intervention group for colostrum feeding (p=0.0000). Moreover, 31.6% of the mothers in the intervention group practised timely initiation of breast feeding compared to 5.6% of the controls, and the prevalence of exclusive breast feeding at 4 months was 39.8% in the intervention group compared to 13.9% for the controls. Multivariate analysis showed that the intervention was a powerful and the only significant predictor of the increase in breast feeding behaviours (p=0.0000), and that an early initiation of breast feeding is a strong predictor of exclusive breast feeding at 4 months of age. It is concluded that breast feeding promotion in rural communities is feasible and can lead to behavioural changes."

322. de Haan L, Holtrop M, Verweij WR, Agsteribbe E, Wilschut J. Mucosal immunogenicity of the *Escherichia coli* heat-labile enterotoxin: role of the A subunit. Vaccine 1996 Mar;14(4):260-6. 38 ref, Eng. Department of Physiological Chemistry, Groningen Institute for Drug Studies, University of Groningen, Bloemsingel 10, 9712 KZ Groningen, The Netherlands

323. Desikan P, Daniel JD, Kamalarathnam CN, Mathan MM. Molecular epidemiology of nosocomial rotavirus infection. J Diarrhoeal Dis Res 1996 Mar;14(1):12-5. 28 ref, Eng. The Wellcome Trust Research Laboratory, Department of Gastrointestinal Sciences, Christian Medical College and Hospital, Vellore 632004, India

"The molecular epidemiology of nosocomial rotavirus infection in children admitted to the paediatric ward of a large hospital in southern India was studied by transmission electron microscopy and RNA electrophoresis on stool samples. Six hundred and twenty-six samples were collected and rotaviruses were detected in 35 of them. A predominant electropherotype was found in all the samples, and there was no seasonal variation in the incidence of infection. The predominant electropherotype pattern in this study was different from the electropherotype patterns of strains isolated elsewhere in the country and also from the pattern of the strains isolated earlier from children with acute diarrhoea at the same hospital."

324. Desjeux JF, Desjeux D, Favre I, Simongiovanni J, Varga L, Cailol M-H, Taponnier S. How to explain the gap between recommendation and prescription in the treatment of diarrhoea? Hong Kong J Paediatr 1995 Sep;6(1 Suppl):134-7. 9 ref, Eng. INSERM u 290, Hopital Saint-Lazare 75010, Paris, France

325. Dutta D, Bhattacharya SK, Bhattacharya MK, Deb A, Deb M, Manna B, Moitra A, Mukhopadhyay AK, Nair GB. Efficacy of norfloxacin and doxycycline for treatment of *Vibrio cholerae* 0139 infection. J Antimicrob Chemother 1996 Mar;37(3):575-81. 15 ref, Eng. National Institute of Cholera and Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

326. Dvorak AM, Beil WJ. Recent studies on the role of tumor necrosis factor- in small intestinal diseases. Curr Opin Gastroenterol 1996 Mar;12(2):141-6. 36 ref, Eng. Department of Pathology, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215, USA

327. Eberhart-Phillips J, Besser RE, Tormey MP, Feikin D, Araneta MR, Wells J, Kilman L, Rutherford GW, Griffin PM, Baron R, Mascola L. An outbreak of cholera from food served on an international aircraft. Epidemiol Infect 1996 Feb;116(1):9-13. 12 ref, Eng. Department of Pediatrics, University of California, San Diego, 200 W. Arbor Drive (8454), San Diego, CA 92116, USA

328. Elliott EJ, Backhouse JA, Leach JW. Pre-admission management of acute gastroenteritis. J Paediatr Child Health 1996 Feb;32(1):18-21. 21 ref, Eng. Sydney University Teaching Unit, Royal Alexandra Hospital for Children, PO Box 2124, Parramatta, NSW 2050, Australia

329. Faith NG, Shere JA, Brosch R, Arnold KW, Ansay SE, Lee M-S, Luchansky JB, Kaspar CW. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. Appl Environ Microbiol 1996 May;62(5):1519-25. 28 ref, Eng. Food Research Institute, Department of Food Microbiology and Toxicology, 1925 Willow Drive, Madison, WI 53706-1187, USA

330. Falt IC, Mills D, Schweda EKH, Timmis KN, Lindberg AA. Construction of recombinant *aroA* salmonellae stably producing the *Shigella dysenteriae* serotype 1 O-antigen and structural characterization of the *Salmonella/Shigella* hybrid LPS. Microbial Pathogen 1996 Jan;20(1):11-30. 44 ref, Eng. Department of Immunology, Microbiology, Pathology and Infectious Diseases, Division of Clinical Bacteriology, Huddinge Hospital, F82, S-141 86 Huddinge, Sweden

331. Farthing MJG. Chronic diarrhoea: current concepts on mechanisms and management. Eur J Gastroenterol Hepatol 1996 Feb;8(2):157-67. 60 ref, Eng. Digestive Diseases Research Centre, St Bartholomew's, and the Royal London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6 BQ, UK

332. Faruque ASG, Mahalanabis D, Hamadani JD, Zetterstrom R. Reduced osmolarity oral rehydration salt in cholera. Scand J Infect Dis 1996; 28(1):87-90. 16 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"In a controlled clinical trial conducted in 34 adults with severe cholera diarrhea, the use of a relatively dilute oral rehydration salt (ORS) solution (sodium 67, potassium 20, chloride 66, citrate 7, glucose 89 mmol/l, osmolality 249 mOsmol/kg) caused a 29% (P=0.003) reduction in stool output over the first 24 h and a 37% (P=0.001) reduction over the first 48 h compared with 29 controls who received the hyperosmolar WHO/UNICEF ORS. No controls but 3 study-group patients had marked but asymptomatic hyponatraemia (sodium <125 mmol/l) at 24 h. Twenty-four % of controls and 12% of patients receiving the dilute ORS needed unscheduled intravenous therapy for recurrence of dehydration. The ORS intake was twice the 48 h stool volume in controls and 3 times in the study group. The test ORS with a reduced glucose and sodium concentration is more efficient than the WHO/UNICEF ORS in preserving net intestinal fluid balance in severe cholera."

333. Favre D, Struck M-M, Cryz SJ, Jr., Viret J-F. Further molecular characterization and stability of the live oral attenuated cholera vaccine strain CVD103-HgR. Vaccine 1996 Apr;14(6):526-31. 27 ref, Eng. Swiss Serum and Vaccine Institute, PO Box, CH-3001 Berne, Switzerland

334. Fleckenstein JM, Kopecko DJ, Warren RL, Elsinghorst EA. Molecular characterization of the *tia* invasion locus from enterotoxigenic *Escherichia coli*. Infect Immun 1996 Jun;64(6):2256-65. 54 ref, Eng. Infectious Disease Division, Walter Reed Army Medical Center, Washington, D.C. 20307-5001, USA

335. Gaboriau-Routhiau V, Moreau M-C. Gut flora allows recovery of oral tolerance to ovalbumin in mice after transient breakdown mediated by cholera toxin or *Escherichia coli* heat-labile enterotoxin. Pediatr Res 1996 Apr;39(4):625-9. 31 ref, Eng. Unité d'Ecologie et de Physiologie du Systéme Digestif, Bat 440, CRJ, Institut National de la Recherche Agronomique, 78352 Jouy-en-Josas, Cedex, France

336. Gardel CL, Mekalanos JJ. Alterations in *Vibrio cholerae* motility phenotypes correlate with changes in virulence factor expression. Infect Immun 1996 Jun;64(6):2246-55. 60 ref, Eng. Department of Microbiology and Molecular Genetics, Harvard Medical School, Building D1517, 200 Longwood Ave, Boston, MA 02115, USA

337. Gaunt PN, Piddock LJV. Ciprofloxacin resistant *Campylobacter* spp. in humans: an epidemiological and laboratory study. J Antimicrobial Chemother 1996 Apr;37(4):747-57. 19 ref, Eng. Public Health Laboratory, Derriford Hospital, Derriford Road, Plymouth PL6 8DH, UK

338. George O V, Jesudason MV, John TJ. Coagglutination for the rapid detection of *Vibrio cholerae* O139. Indian J Med Microbiol 1995 Oct;13(4):185-6. 11 ref, Eng. Department of Microbiology, Christian Medical College Hospital, Vellore 632004, India

339. Gerding DN. Diagnosis of *Clostridium difficile*-associated disease: patient selection and test perfection (editorial). Am J Med 1996 May;100(5):485-6. 7 ref, Eng. Medical Service, VA Lakeside Medical Center, 333 East Huron St., Chicago, Illinois 60611, USA

340. Graninger W, Zedtwitz-Liebenstein K, Laferl H, Burgmann H. Quinolones in gastrointestinal infections. Chemotherapy 1996;42(1 Suppl):43-53. 84 ref, Eng. Department of Infectious Diseases and Chemotherapy, University Clinic of Internal Medicine I, Wahringer Gurtel 18-20, A-1090 Vienna, Austria

341. Greenspan A, Levin MS. Diagnosis and management of small intestinal diseases. Curr Opin Gastroenterol 1996 Mar;12(2):164-8. 22 ref, Eng. Washington University School of Medicine, Box 8124, 660 South Euclid Avenue, St. Louis, MO 63110, USA

342. Gutierrez ADZ, Liendo AMA, Valdivia DC. Attitudes of Bolivian pharmacists in dealing with diarrhea cases. Bull Pan Am Health Organ 1995 Dec;29(4):322-7. 14 ref, Eng. Secretaria Regional de Salud de El Alto, Direccion postal no. 6971, La Paz, Bolivia

343. Gutzwiller A, Blum JW. Effects of oral lactose and xylose loads on blood glucose, galactose, xylose and insulin values in healthy calves and calves with diarrhea. Am J Vet Res 1996 Apr;57(4):560-3. 26 ref, Eng. Division of Nutrition Pathology, Institute for Animal Breeding, Veterinary Faculty, University of Berne, Bremgartenstrasse 109a 3012 Berne (Blum), Switzerland

344. Guyot A. Antibiotic resistance of *Shigella* in Monrovia. Trop Doct 1996 Apr;26(2):70-1. 10 ref, Eng. Department of Microbiology, St Joseph's Catholic Hospital, Monrovia, Liberia

345. Haider R, Islam A, Hamadani J, Amin NJ, Kabir I, Malek MA, Mahalanabis D, Habte D. Breast-feeding counselling in a diarrhoeal disease hospital. Bull WHO 1996;74(2):173-9. 18 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"Lactation counsellors were trained to advise mothers of partially breast-fed infants who were admitted to hospital because of diarrhoea, so that they could start exclusive breast-feeding during their hospital stay. Infants (n=250) up to 12 weeks of age were randomized to intervention and control groups. Mothers in the intervention group were individually advised by the counsellors while mothers in the control group received only routine group health education. During follow-up at home by the counsellors a week later, only the mothers in the intervention group were counselled. All the mothers were evaluated for infant feeding practices at home two weeks after discharge. Among the 125 mother-infant pairs in each group, 60% of mothers in the intervention group were breast-feeding exclusively at discharge compared with only 6% in the control group ($P<0.001$); two weeks later, these rates rose to 75% and 8% in the intervention and control groups, respectively ($P<0.001$). However, 49% of mothers in the control group reverted back to bottle-feeding compared with 12% in the intervention group ($P<0.001$). Thus, individual counselling had a positive impact on mothers to start exclusive breast-feeding during hospitalization and to continue the practice at home. Maternal and child health facilities should include lactation counselling as an integral part of their programme to improve infant feeding practices."

346. Haque QM, Sugiyama A, Iwade Y, Midorikawa Y, Yamauchi T. Diarrheal and environmental isolates of *Aeromonas* spp. produce a toxin similar to Shiga-like toxin 1. Curr Microbiol 1996;32(5):239-45. 44 ref, Eng. Department of Public Health, School of Medicine, Mie University, 2-174 Edobashi Tsu, Mei-Ken 514, Japan

347. Hasan KZ. The diarrhoea malnutrition cycle. Hong Kong J Paediatr 1995 Sep;6(1 Suppl):44-55. 73 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

348. Hennessy TW, Hedberg CW, Slutsker L, White KE, Besser-Wiek JM, Moen ME, Feldman J, Coleman WW, Edmonson LM, MacDonald KL, Osterholm MT. A national outbreak of *Salmonella enteritidis* infections from ice cream. N Engl J Med 1996 May 16;334(20):1281-6. 26 ref, Eng. Acute Disease Epidemiology Section, Minnesota Department of Health, 717 Delaware St., SE, PO Box 9441, Minneapolis MN 55440-9441, USA

349. Hernandez JE, Mejia CR, Cazali IL, Arathoon EG. Nosocomial infection due to *Vibrio cholerae* in two referral hospitals in Guatemala. Infect Cont Hosp Epidemiol 1996 Jun;17(6):371-2. 4 ref, Eng. Department of Medicine, Section of Infectious Diseases, Health Sciences Center, West Virginia University, Charleston Division, 3110 MacCorkle Ave SE, Charleston, WV 25304, USA

350. Hicks S, Candy DCA, Phillips AD. Adhesion of enteroaggregative *Escherichia coli* to formalin-fixed intestinal and ureteric epithelia from children. J Med Microbiol 1996 May;44(5):362-71. 29 ref, Eng. University Department of Paediatric Gastroenterology, Royal Free Hospital, Pond Street, London NW3 2QG, UK

351. Holtug K, Hansen MB, Skadhauge E. Experimental studies of intestinal ion and water transport. Scand J Gastroenterol 1996;31(216 Suppl):95-110. 176 ref, Eng. Department of Internal Medicine A 2101, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

352. Hormacche CE, Mastroeni P, Harrison JA, de Hormaeehe RD, Svenson S, Stocker BAD. Protection against oral challenge three months after i.v. immunization of BALB/c mice with live aro *Salmonella typhimurium* and *Salmonella enteritidis* vaccines is serotype (species)-dependent and only partially determined by the main LPS O antigen. Vaccine 1996 Mar;14(4):251-9. 78 ref, Eng. Department of Microbiology, The Medical School, University of Newcastle, Framlington Place, Newcastle upon Tyne NE2 4HH, UK

353. Huq A, Xu B, Chowdhury MAR, Islam MS, Montilla R, Colwell RR. A simple filtration method to remove plankton-associated *Vibrio cholerae* in raw water supplies in developing countries. Appl Environ Microbiol 1996 Jul;62(7):2508-12. 27 ref, Eng. University of Maryland Biotechnology Institute, 4321 Hartwick Road, St Suite 550, College Park, MD 20740, USA

"Plankton to which cells of Vibrio cholerae 01 and/or 0139 were attached was introduced into 0.5% instant ocean microcosms maintained at 25oC. The bulk of the plankton and associated particulates was removed with a filter constructed from either nylon net and one of several different types of sari material, the latter being very inexpensive and readily available in villages in Bangladesh, were V. cholerae is endemic. V. cholerae was enumerated before and after filtration to evaluate the efficiency of the filtration procedure. The results obtained indicate that 99% of V. cholerae, i.e., those cells attached to plankton, were removed from the water samples. Epidemic strains of V. cholerae O1 and O139 from various geographical sources, including Bangladesh, Brazil, India, and Mexico, were included in the experiments. Removal of vibrios from water by this simple filtration method was found to yield consistent results with all strains examined in this study. Thus, it is concluded that a simple filtration procedure involving the use of domestic sari material can reduce the number of cholera vibrios attached to plankton in raw water from ponds and rivers commonly used for drinking. Since untreated water from such sources serves as drinking water for millions of people living in developing countries (e.g., Bangladesh), filtration should prove effective at reducing the incidence and severity of outbreaks, especially in places that lack fuel wood for boiling water and/or municipal water treatment plants. The results of this study provide the basis for determining such reductions, which are to be carried out in the near future."

354. Husain M, Seth P, Dar L, Broor S. Classification of rotavirus into G and P types with specimens from children with acute diarrhea in New Delhi, India. J Clin Microbiol 1996 Jun;34(6):1592-4. 22 ref, Eng. Department of Microbiology, All India Institute of Medical Sciences, New Delhi 110029, India

355. Islam MS, Alam MJ, Begum A, Rahim Z, Felsenstein A, Albert MJ. Occurrence of culturable *Vibrio cholerae* O139 with *ctx* gene in various components of the aquatic environment in Bangladesh (short report). Trans R Soc Trop Med Hyg 1996 Mar-Apr;90(2):128. 8 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

356. Islam MS, Rezwan FB, Khan SI. Survival of *Shigella flexneri* in artificial aquatic environment: effects of different physicochemical stress factors. J Diarrhoeal Dis Res 1996 Mar;14(1):37-40. 20 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"Survival of bacteria in aquatic environment is influenced by the prevailing physicochemical characteristics of water. A study was carried out to assess the effects of temperature, pH, and salinity of water on the survival of *Shigella flexneri* in laboratory microcosms. Survival of *S. flexneri* was assessed by viable counts of *Shigellla* on MacConkey agar plates. At pH 7.0 and temperature of 4 C, *S. flexneri* survived longer in 0.5% salinity than in salt-free distilled water, while at pH 7.0 and at higher temperatures (25 C and 37 C), the bacteria survived longer in distilled water than in 0.5% salinity. It was also observed that 2.0% NaCl solution was detrimental to bacterial survival at pH 8.0 and temperature of 37 C. Survival times of the viable cells increased with reduction of temperatures and salinity. These findings demonstrated that survival of *S. flexneri* in the aquatic environment is greatly influenced by physicochemical factors."

357. Ives DV, Smith RN. A 48-year-old man with the acquired immunodeficiency syndrome, abdominal pain, and bloody diarrhea. N Engl J Med 1996 May 30;334(22):1461-7. 45 ref, Eng. Virology Research Clinic, Division of Infectious Diseases, Beth Israel Hospital, Israel

358. Jiang X, Chai T-J. Survival of *Vibrio parahaemolyticus* at low temperatures under starvation conditions and subsequent resuscitation of viable, nonculturable cells. Appl Environ Microbiol 1996 Apr;62(4):1300-5. 39 ref, Eng. Seafood Science Program, Horn Point Environment Laboratory, Center for Environmental and Estuarine Studies, The University of Maryland System, Cambridge, Maryland 21613, USA

359. Jianguo X, Bokun C, Yanping W, Libao H, Qingdong D, Xinhe L, Bingyang L. A new diarrhea pathogen: entero-adherent-invasive-toxigenic *Escherichia coli*. Chin Med J 1996 Jan;109(1):16-7. 5 ref, Eng. Department of Microbiology, Institute of Epidemiology and Microbiology, Chinese Academy of Preventive Medicine, Beijing 102206, People's Republic of China

360. Kaltenthaler EC, Drasar BS. Understanding of hygiene behaviour and diarrhoea in two villages in Botswana. J Diarrhoeal Dis Res 1996 Jun;14(2):75-80. 16 ref, Eng. Department of Clinical Sciences, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

"This paper describes a study which took place in two villages in north-east district of Botswana from July 1990 through July 1991. Qualitative data collection methods were used including: observations, key-informant interviews, focus groups, and in-depth interviews. The data were used for understanding and interpreting the cultural belief systems regarding specific hygiene behaviours and diarrhoea, with emphasis on hand washing. Hand washing was said to occur for three main reasons: to remove contamination, for social reasons, and for comfort reasons. Sources of dirt on the hands included human blood and faeces. Many perceived causes of diarrhoea were identified, including pogwana (dehydration associated with sunken anterior fontanelle). Traditional concepts regarding the treatment and prevention of diarrhoea were also identified. It is suggested that beliefs surrounding hygiene behaviour and diarrhoea should be incorporated into health education programmes."

361. Katz DA, Lynch ME, Littenberg B. Clinical prediction rules to optimize cytotoxin testing for *Clostridium difficile* in hospitalized patients with diarrhea. Am J Med 1996 May;100(5):487-95. 50 ref, Eng. University of Wisconsin-Madison, Department of Medicine, 2870 University Avenue, Suite 100, Madison, Wisconsin 53705, USA

362. Kelkar SD, Ray PG, Bedekar SS. Assay of neutralizing antibodies to animal rotavirus strains and human rotavirus serotype G8 by a modified method in the residents of Pune, India. J Diarrhoeal Dis Res 1996 Jun;14(2):101-6. 32 ref, Eng. National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune 411001, India

"In the western literature, four G serotypes (G1-G4) of human rotaviruses have been found to be of a major epidemiological importance. During the analysis of rotavirus serotypes from faecal samples in Pune, over 50% of specimens could not be serotyped with the available monoclonal antibodies against G1-G4 serotypes. The results prompted to look for the prevalence of neutralizing antibodies against serotypes other than the major human serotypes (G1-G4) in adults. Neutralizing antibodies against animal rotavirus serotype, viz. G3, G6 and G10, and human rotavirus serotype G8 were determined in adult sera, by a modified technique, which is ELISA-based and mechanized. The results showed that, of the 68 sera tested at 1:100 dilution, 65 (95.58%) were reactive for G3 (SA-11), 52 (76.47%) for G6 (Bovine Lincoln), 6 (8.82%) for G10 (B223), and 40 (58.82%) for G8 (M69) serotypes. It appears that the prevalence of rotaviruses in India may be quite different from that in the developed countries."

363. Kimura A, Hara Y, Kimoto T, Okuno Y, Minekawa Y, Nakabayashi T. Cloning and expression of a putative alcohol dehydrogenase gene of *Entamoeba histolytica* and its application to immunological examination. Clin Diagn Lab Immunol 1996 May;3(3):270-4. 27 ref, Eng. Laboratory of Medical Zoology, Osaka Prefectural Institute of Public Health, Nakamichi 1-3-69, Higashinari-ku, Osaka 537, Japan

364. Koo D, Aragon A, Moscoso V, Gudiel M, Biettii L, Carrillo N, Chojoj J, Gordillo B, Cano F, Cameron DN, Wells JG, Bean NH, Tauxe RV. Epidemic cholera in Guatemala, 1993: transmission of a newly introduced epidemic strain by street vendors. Epidemiol Infect 1996 Apr;116(2):121-6. 20 ref, Eng. Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Mailstop A-38, Atlanta, GA 30333, USA

"Epidemic cholera reached Guatemala in July 1991. By mid-1993, Guatemala ranked third in the hemisphere in reported cases of cholera. We conducted a case-control study with two age-, sex-, and neighbourhood-matched controls per patient in periurban Guatemala city. Twenty-six patients hospitalized for cholera and 52 controls were enrolled. Seven (47%) of 15 stool cultures obtained after admission yielded toxigenic Vibrio cholerae O1. All seven were resistant to furazolidone, sulfisoxazole, and streptomycin, and differed substantially by pulsed-field gel electrophoresis from the Latin American epidemic strain dominant in the hemisphere since 1991. In univariate analysis, illness was associated with consumption of left-over rice (odds ratio [OR]=7.0, 95% confidence interval [CI]=1.4-36), flavored ices (`helados') (OR=3.6, CI=1.1-12), and street-vended non-carbonated beverages (OR=3.8, CI=1.2-12) and food items (OR=11.0, CI=2.3-54). Street-vended food items remained significantly associated with illness in multivariate analysis (OR=6.5, CI=1.4-31). Illness was not associated with drinking municipal tap water. Maintaining water safety is important, but slowing the epidemic in Guatemala city and elsewhere may also require improvement in street vendor food handling and hygiene."

365. Kurazono H, Yamasaki S, Ratchtrachenchai O-a, Nair GB, Takeda Y. Analysis of *Vibrio cholerae* 0139 Bengal isolated from different geographical areas using macrorestriction DNA analysis. Microbiol Immunol 1996;40(4):303-5. 10 ref, Eng. Research Institute, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162, Japan

"Vibrio cholerae 0139 isolated from different countries, as well as from different locations within a country, were examined using macrorestriction DNA analysis to determine the clonality of the 0139 strains. NotI digests of genomic DNA of representative strains from Nepal, India, Bangladesh, China, Thailand, and Malaysia revealed very similar but not identical patterns. Examinations of the banding patterns generated by pulsed-field gel electrophoresis of strains isolated within countries revealed complete homogeneity. These results further reiterate the spread of an identical clone of V. cholerae 0139 although it appears that genetic polymorphism among the 0139 strains is becoming apparent."

366. Lampel KA, Keasler SP, Hanes DE. Specific detection of *Salmonella enterica* serotype enteritidis using the polymerase chain reaction. Epidemiol Infect 1996 Apr;116(2):137-45. 43 ref, Eng. US Food and Drug Administration, 200 C Street, S.W., Washington, D.C. 20204, USA

367. Lebens M, Shahabi V, Backstrom M, Houze T, Lindblad M, Holmgren J. Synthesis of hybrid molecules between heat-labile enterotoxin and cholera toxin B subunits: potential for use in a broad-spectrum vaccine. Infect Immun 1996 Jun;64(6):2144-50. 32 ref, Eng. Department of Medical Microbiology and Immunology, University of Goteborg, Guldhedsgatan, 10A, S-413 46 Goteborg, Sweden

368. Lee SH. Epidemiology of diarrhoeal disease in Hong Kong. Hong Kong J Paediatr 1995 Sep;6(1 Suppl):37-43. Eng. Department of Community and Family Medicine, The Chinese Unisersity of Hong Kong, Shatin, Hong Kong

369. Li E, Stanley SL. Jr. Parasitic infections of the gastrointestinal tract. Curr Opin Gastroenterol 1996 Jan;12(1):82-7. 47 ref, Eng. Washington University School of Medicine, Campus Box 8051, 660 S. Euclid Avenue, St. Louis, MO 63110, USA

370. Lundkvist K, Ewald U, Lindgren PG. Congenital chloride diarrhoea: a prenatal differential diagnosis of small bowel atresia. Acta Paediatr 1996 Mar;85(3):295-8. 25 ref, Eng. Department of Paediatric Surgery, Uppsala University Hospital, S-751 85 Uppsala, Sweden

371. Mahalanabis D, Faruque ASG, Islam A, Hoque SS. Maternal education and family income as determinants of severe disease following acute diarrhoea in children: a case control study. J Biosoc Sci 1996 Apr;28(2):129-39. 13 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"In a case-control study among the urban poor of Dhaka, Bangladesh, the association of maternal education and family income with severity of disease due to diarrhoea in children was examined. After adjusting for family income, 7 or more years of school education was associated with 54% reduced risk of severe disease as indicated by the presence of dehydration. Income in the uppermost quartile of this population, independently of maternal education, was associated with 41% reduced risk of severe disease compared to the lowest quartile. In the logistic regression model the effect of maternal education remained high after adjustment for several confounders. Based on the concept that socioeconomic variables operate through a set of proximate variables it is contended that maternal education, independently of economic power, through its impact on disease from acute diarrhoea, favourably influences child survival."

372. McKee MI, O'Brien AD. Truncated enterohemorrhagic *Escherichia coli* (EHEC) 0157:H7 intimin (EaeA) fusion proteins promote adherence of EHEC strains to HEp-2 cells. Infect Immun 1996 Jun;64(6):2225-33. 51 ref, Eng. Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20892-4255, USA

373. Makino M, Tanabe Y, Shinozaki K, Matsuno S, Furuya T. Haemorrhagic shock and encephalopathy associated with rotavirus infection (case report). Acta Paediatr 1996 May;85(5):632-4. 16 ref, Eng. Division of Neurology, Chiba Children's Hospital, 579-1 Heta-cho, Midori-ku, Chiba 266, Japan

374. Manjarrez-Hernandez HA, Baldwin TJ, Williams PH, Haigh R, Knutton S, Aitken A. Phosphorylation of myosin light chain at distinct sites and its association with the cytoskeleton during enteropathogenic *Escherichia coli* infection (note). Infect Immun 1996 Jun;64(6):2368-70. 23 ref, Eng. Institute of Infections and Immunity, University Hospital, Nottingham, NG7 2UH, UK

375. Manning PA. The molecular biology of bacterial enteric pathogens. Hong Kong J Paediatr 1995 Sep;6(1 Suppl):17-27. 34 ref, Eng. Microbiology Pathogenesis Unit, Department of Microbiology and Immunology, The University of Adelaide, Adelaide, South Australia 5005

376. McQueen CE, Hicks CB. Treatment of gastrointestinal infections. Curr Opin Gastroenterol 1996 Jan;12(1):54-61. 55 ref, Eng. Department of Gastroenterology, Walter Reed Army Institute of Research, Building 40, Walter Reed Army Medical Center, Washington, DC 20307-5100, USA

377. Mayer CL, Palmer CJ. Evaluation of PCR, nested PCR, and fluorescent antibodies for detection of *Giardia* and *Cryptosporidium* species in wastewater. Appl Environ Microbiol 1996 Jun;62(6):2081-5. 26 ref, Eng. Department of Epidemiology and Public Health (R-669), University of Miami School of Medicine, PO Box 016069, Miami, FL 33101, USA

378. Mazumder RN, Kabir I, Rahman MM, Khatun M, Mahalanabis D. Absorption of macronutrients from a calorie-dense diet in malnourished children during acute shigellosis. J Pediatr Gastroenterol Nutr 1996 Jul;23(1):24-8. 16 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"A metabolic balance study was performed to determine the absorption of macronutrients and energy from different food items in 23 malnourished children aged 12 to 48 months with clinically severe acute dysentery due to shigellosis. In a 72-h balance period, the absorption of carbohydrate, protein, fat, and total energy was determined. All the children received a standard hospital diet; 12 children in the test group were offered an additional calorie-dense milk (5.0 kJ/ml with a protein-energy ratio of 11.0), and 11 children in the control group, on the other hand, received a milk formula with an energy of 2.5 kJ/ml with a protein-energy ratio of 11.0. The intakes (g/kg/day) of protein, fat, carbohydrate, and energy between test and control groups were 4.25 versus 2.32 (p=0.01), 7.63 versus 3.00 (p=0.01), 21.09 versus 11.14 (p=0.01), and 711 kJ/kg/day versus 338 kJ/kg/day (p=0.01), respectively. The coefficients of absorption of protein, fat, carbohydrate, and energy between test and control groups were 61 versus 67% (p=0.45), 69 versus 82% (p=0.11), 77 versus 86% (p=0.13), and 72 versus 82% (p=0.13), respectively. The losses (g/kg/day) of protein, fat, carbohydrate, and energy between the two groups were 1.61 versus 0.76 (p=0.00), 2.44 versus 0.55 (p=0.00), 5.0 versus 1.6 (p=0.00), and 204 kJ/kg/day versus 60 kJ/kg/day, respectively. The results of this study indicate that during the acute stage of shigellosis (with a substantially enhanced total intake of protein, fat, carbohydrate, and energy), by adding calorie-dense meals in malnourished children younger than 5 years, the absorption of macronutrients is not significantly different from that with the usual diet but suboptimal dietary energy intake, as is the case under ordinary treatment conditions."

379. Molla AM. Treatment of diarrhoea in children: a review and update. Hong Kong J Paediatr 1995 Sep;6(1 Suppl):126-33. 20 ref, Eng. Faculty of Medicine, Kuwait University, Safat, Kuwait 24923

380. Montilla R, Chowdhury MAR, Huq A, Xu B, Colwell RR. Serogroup conversion of *Vibrio cholerae* non-O1 to *Vibrio cholerae* O1: effect of growth state of cells, temperature, and salinity. Can J Microbiol 1996 Jan;42(1):87-93. 20 ref, Eng. Departmento de Microbiologia, Faculted de Farmacia, Avenida Diagnonal 643, Barcelona 08028, Spain

"Recently, we reported the occurrence of seroconversion from *Vibrio cholerae* non-01 to *V. cholerae* O1, but little is known about the environmental and physiological factors influencing seroconversion. We investigated effects of temperature (4,25, and 35oC) and salinity (<0.5 and 10%), as well as the stage of

growth of cells, on serogroup conversion. Seroconversion of *V. cholerae* occurred under various environmental conditions. However, the rate of seroconversion in natural water (<0.5% salinity) and synthetic seawater microcosms (10% salinity), employing cells harvested from stationary phase culture, was approximately 2 logs higher than cells harvested from cultures in the logarithmic phase (i.e. 105 versus 103 per 1010 cells). Thus, the physiological state of the cells, and to a lesser degree, temperature and salinity, is an important factor in the conversion of *V. cholerae* from non-O1 to O1 serogroup."

381. Mukhopadhyay RM, Chaudhuri SK. Rapid *in vitro* test for determination of anti-amoebic activity. Trans R Soc Trop Med Hyg 1996 Mar-Apr;90(2):189-91. 9 ref, Eng. Organon Research Centre, 7 Wood Street, Calcutta 700016, India

382. Murch SH, Winyard PJD, Koletzko S, Wehner B, Cheema HA, Risdon RA, Phillips AD, Meadows N, Klein NJ, Walker-Smith JA. Congenital enterocyte heparan sulphate deficiency with massive albumin loss, secretory diarrhoea, and malnutrition. Lancet 1996 May 11;347(9011):1299-301. 12 ref, Eng. University Department of Paediatric Gastroenterology, Royal Free Hospital, London NW3 2QG, UK

383. Myaux JA, Unicomb L, Besser RE, Modlin JF, Uzma A, Islam AM, Santosham M. Effect of diarrhea on the humoral response to oral polio vaccination. Pediatr Infect Dis J 1996 Mar;15(3):204-9. 18 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

384. Naber KG. Fleroxacin overview. Chemotherapy 1996;42(1 Suppl):1-9. 27 ref, Eng. Urologic Clinic, Elisabeth Hospital, Straubing University of Munich Medical School, Teaching Hospital, D-94315, Straubing, Germany

385. Nakagawa I, Takahashi I, Kiyono H, McGhee JR, Hamada S. Oral immunization with the B subunit of the heat-labile enterotoxin of *Escherichia coli* induces early Th1 and late Th2 cytokine expression in peyer's patches. J Infect Dis 1996 Jun;173(6):1428-36. 40 ref, Eng. Department of Oral Microbiology, Osaka University, Faculty of Dentistry, 1-8 Yamadaoka, Suita-Osaka, 565, Japan

386. Nath G, Chaudhury A, Shukla BN, Sanyal SC. Intestinal parasites among under five children in a semiurban slum of northern India. Indian J Med Microbiol 1995 Oct;13(4):196-9. 16 ref, Eng. Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

387. Ndip RN, Obi CL, Agbonlahor DE, Igumbor EO, Ayamba LM. Anti-*Plesiomonas shigelloides* agglutinating and complement-fixing antibody titres in normal individuals and diarrhoeal patients in Edo State, Nigeria (short report). J Diarrhoeal Dis Res 1996 Mar;14(1):41-3. 13 ref, Eng. Department of Life Sciences, Faculty of Science, University of Buea, PO Box 63, Buea, Cameroon

"One hundred patients with diarrhoea and 50 asymptomatic individuals attending various hospitals in Edo State, Nigeria, were screened for serum complement-fixing and agglutinating antibodies to *Plesiomonas shigelloides* using the complement-fixation and agglutination tests. Seventy (70%) of the 100 patients and 20 (40%) of the 50 asymptomatic individuals had detectable complement-fixing antibodies at titres ranging from 1:32 to 1:128 and 1:8 to 1:32 respectively. Results suggest that cases of diarrhoea in this environment may be due to *P. shigelloides,* but the demonstration of antibodies in asymptomatic individuals show that they also have serum antibodies against *P. shigelloides*. The exclusive use of antibody responses in the diagnosis of *P. shigelloides* infections should, therefore, be interpreted with caution."

388. Newton T, Murphy MS, Booth IW. Glucose polymer as a cause of protracted diarrhea in infants with unsuspected congenital sucrase-isomaltase deficiency. J Pediatr 1996 Jun;128(6):753-6. 18 ref, Eng. University of Birmingham, Institute of Child Health, Francis Road, Birmingham B16 8ET, UK

389. Nimri LF, Hijazi S. Rotavirus-associated diarrhoea in children in a refugee camp in Jordan. J Diarrhoeal Dis Res 1996 Mar;14(1):1-4. 20 ref, Eng.

"Studies on the rotavirus-associated acute diarrhoeal illness in Jordanian children are non-existent. The present case-control study was conducted to investigate the prevalence of rotavirus diarrhoea among children aged less than 5 years, attending the United Nations Refugee World Aid Clinic in northern Jordan. The potential environmental and behavioural risk factors contributing to the infection were also studied. Using the ELISA technique rotavirus antigens were detected in the stool samples of 35% of the 220 cases of gastroenteritis and in 3% of the control group. The control subjects were matched for age and sex with the cases. The overall prevalence was significantly higher (62%) in children aged less than 24 months [OR=2.4, 95% CI (1.1-5.1)] than those in the older age groups. Severe cases of diarrhoea were rare. Diarrhoea due to rotavirus was more prevalent during the summer months (June-August). Risk factors for acute diarrhoea in these children are related to the infant feeding practices of using unboiled tap water to prepare the formula milk, and the low educational level of the mothers."

390. Nizami SQ, Khan IA, Bhutta ZA. Drug prescribing practices of general practitioners and paediatricians for childhood diarrhoea in Karachi, Pakistan. Soc Sci Med 1996 Apr;42(8):1133-9. 21 ref, Eng. Department of Paediatrics, The Aga Khan University, PO Box no. 3500, Stadium Road, Karachi, Pakistan

"Observations were made of 996 encounters between children with diarrhoea and practitioners (28 paediatricians, 62 general practitioners) in Karachi, Pakistan. Oral rehydration salt (ORS) was prescribed in more than 50% of encounters by 53% of general practitioners (GPs) and 61% of paediatricians. Sixty-six percent of GPs and 50% of paediatricians prescribed antibacterials, 60% of GPs and 28% of paediatricians prescribed antidiarrhoeals and 39% of GPs and 32% of paediatricians prescribed antiamoebics in more than 30% of their encounters. Looking at all encounters, we observed that ORS was prescribed in 52 and 51%, antibacterials in 41 and 36%, antidiarrhoeals in 48 and 29%, and antiamoebics in 26 and 22% of encounters by GPs and paediatricians, respectively. Cotrimoxazole was the most frequently prescribed antibacterial by both types of practitioners. Antidiarrhoeals were prescribed more often by GPs than by paediatricians. In 77% of their encounters. GPs dispensed drug formulations known as `mixtures' made in their own dispensing corners. The mean duration of encounters between patients and GPs was 3 ± 2 minutes and between patients and paediatricians was 9 ± 4 minutes. These results indicate inadequate prescription of ORS and excessive prescription of antibacterials, antidiarrhoeals and antiamoebics. Intervention strategies need to be planned to improve the prescribing practices of both groups."

391. Oberhuber G, Vogelsang H, Stolte M, Muthenthaler S, Kummer AJ, Radaszkiewicz T. Evidence that intestinal intraepithelial lymphocytes are activated cytotoxic T cells in celiac disease but not in giardiasis (short communication). Am J Pathol 1996 May;148(5):1351-7. 31 ref, Eng. Department of Pathology, University of Vienna, AKH-Wein, Medical School Wahringer Gurtel 18-20, A-1090 Vienna, Austria

392. Okafor JI, Okunji PO. Prevalence of *Cryptosporidium* oocysts in faecal samples of some school children in Enugu State, Nigeria. J Commun Dis 1996 Mar;28(1):49-55. 34 ref, Eng. Department of Microbiology, University of Nigeria, Nzukka, Nigeria

393. Okeke TA, Okafor HU, Amah AC, Onwuasigwe CN, Ndu AC. Knowledge, attitude, practice, and prescribing pattern of oral rehydration therapy among private practitioners in Nigeria. J Diarrhoeal Dis Res 1996 Mar;14(1):33-6. 17 ref, Eng. Department of Community Medicine, University of Nigeria Teaching Hospital, PMB 01129, Enugu, Nigeria

"To determine the knowledge, attitude, and practice of oral rehydration therapy (ORT) among private medical practitioners in Enugu, Nigeria, 91 doctors were interviewed using a structured questionnaire. All the doctors had heard of ORT and believed in its efficacy. The commonest source of information on ORT was the medical school (44%). Fifty percent would recommend salt-sugar solution (SSS) rather than oral rehydration solution (ORS). The main reason is its cost-effectiveness and easy availability. Only 55% of the respondents knew how to prepare SSS correctly. The percentage of doctors who prescribe smooth muscle relaxant (spasmolytic use rate) was 41%, and the commonest reason for its use was to reduce bowel movement. The influence of year of medical graduation on spasmolytic use was found to be statistically significant ($p<0.05$). Antibiotics were commonly used, although most (76%) doctors believed that viral infections were a common cause of childhood diarrhoea. All the respondents would recommend continued breastfeeding during diarrhoeal episodes. The study revealed a high rate of inappropriate drug use and a deficiency in the knowledge and practice of ORT."

394. Paredes P, de la Pena M, Flores-Guerra E, Diaz J, Trostle J. Factors influencing physicians' prescribing behaviour in the treatment of childhood diarrhoea: knowledge may not be the clue. Soc Sci Med 1996 Apr;42(8):1141-53. 30 ref, Eng. Instituto de Investigacion Nutricional, PO Box 18-0191, Lima 18, Peru

395. Pedersen C, Danner S, Lazzarin A, Glauser MP, Weber R, Katlama C, Barton SE, Lundgren JD. Epidemiology of cryptosporidiosis among European AIDS patients. Genitourin Med 1996 Apr;72(2):128-31. 23 ref, Eng. The EuroSIDA Coordinating Centre, Department of Infectious Diseases (144), Hvidovre Hospital, 2650 Hvidovre, Denmark

396. Peterson JW, Saini SS, Dickey WD, Klimpel GR, Bomalaski JS, Clark MA, Xu X-J, Chopra AK. Cholera toxin induces synthesis of phospholipase A2-activating protein. Infect Immun 1996 Jun;64(6):2137-43. 38 ref, Eng. Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555-1019, USA

397. Phillips AD. Intractable diarrhoea in developed countries. Hong Kong J Paediatr 1995 Sep;6(1 Suppl):28-36. 46 ref, Eng. Queen Elizabeth Hospital for Children, Hackney Road, London E2 8PS, UK

398. Pickett CL, Pesci EC, Cottle DL, Russell G, Erdem AN, Zeytin H. Prevalence of cytolethal distending toxin production in *Campylobacter jejuni* and relatedness of *Campylobacter* sp. *cdtB* genes. Infect Immun 1996 Jun;64(6):2070-8. 39 ref, Eng. Department of Microbiology and Immunology, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642, USA

399. Pinto RM, Abad FX, Gajardo R, Bosch A. Detection of infectious astroviruses in water (note). Appl Environ Microbiol 1996 May;62(5):1811-3. 16 ref, Eng. Department of Microbiology, University of Barcelona, School of Biology, Av. Diagonal, 645, 08028 Barcelona, Spain

400. Pitkajarvi T, Kujanne E, Sillantaka I, Lumio J. Norfloxacin and *Salmonella* excretion in acute gastroenteritis - a 6-month follow-up study. Scand J Infect Dis 1996;28(2):177-80. 17 ref, Eng. Medical School, University of Tampere, Box 607, FIN-33101 Tampere, Finland

401. Pitman C, Amali R, Kanyerere H, Siyasiya A, Phiri S, Phiri A, Chakanika I, Kampondeni S, Chintolo FE, Kachenje E, Squire SB. Bloody diarrhoea of adults in Malawi: clinical features, infectious agents, and antimicrobial sensitivities. Trans R Soc Trop Med Hyg 1996 May-Jun;90(3):284-7. 18 ref, Eng. Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5 QA, UK

402. Prasad B. Development of oral rehydration therapy. J Nep Med Assoc 1995 Oct-Dec;33(116):407-11. 18 ref, Eng. Child Health Division, Department of Health, Teku, Kathmandu, N**epal**

403. Qadri F, Mohi MG, Azim T, Faruque SM, Kabir AKMI, Albert MJ. Production, characterization and immunodiagnostic application of a monoclonal antibody to Shiga toxin. J Diarrhoeal Dis Res 1996 Jun;14(2):95-100. 29 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"A mouse monoclonal antibody (MAb ICT7) that is specific for Shiga toxin was produced. The MAb neutralises the cytotoxic effects of both purified Shiga toxin and culture extracts of *Shigella dysenteriae* type 1 in HeLa cells. Using MAb ICT7 and polyclonal rabbit antiserum, a sandwich ELISA was developed. This test detects Shiga toxin in both *S. dysenteriae* type 1 bacterial extracts and in stools of patients with *S. dysenteriae* type 1 infection. The ELISA also detects toxin in enterohaemorrhagic *Escherichia coli* (EHEC) strains positive for Shiga like toxin I. The test could detect a minimum of 100 pg of purified Shiga toxin. Furthermore, the ELISA did not detect toxin in non *S. dysenteriae* type 1 *Shigella* species or Shiga like toxin II produced by EHEC strains."

404. Rabalais GP. Recent advances in the prevention and treatment of diarrheal diseases. Curr Opin Infect Dis 1996 Jun;9(3):210-3. 24 ref, Eng. Department of Pediatrics, Kosair Children's Hospital, 200 E. Chestnut Street, Louisville, Kentucky 40292, USA

405. Rabbani GH. Mechanism and treatment of diarrhoea due to *Vibrio cholerae* and *Escherichia coli*: roles of drugs and prostaglandins. Danish Med Bull 1996 Apr;43(2):173-85. 159 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

406. Rahim Z, Aziz KMS. Factors affecting production of haemolysin by strains of *Vibrio fluvialis* (short report). J Diarrhoeal Dis Res 1996 Jun;14(2):113-6. 18 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"The in vitro production of haemolysin by *Vibrio fluvialis* was studied using sheep erythrocyte. The effect of the composition of various media and different concentrations of sodium chloride on the production of haemolysin and heat-stability was investigated. Comparatively higher titre of haemolysin production was noted in brain heart infusion (BHI) broth. Adding 0.5% NaCl to BHI broth reduced the production of haemolysin; adding 5.0% NaCl to the medium totally inhibited it. The highest titre of haemolysin was produced at 30oC and 37oC, whereas no haemolysin was produced at 50oC. Haemolytic activity was totally destroyed when heated at 56oC for 30 minutes. Haemolysin could be assayed easily following this method."

407. Reyes M, Hedlund K-O, Lorenzana I, Ehrnst A. Respiratory infection and iatrogenic diarrhea in Honduras and El Salvador during the 1991-1992 season. Am J Trop Med Hyg 1996 Mar;54(3):260-4. 22 ref, Eng. Division of Clinical Virology, F68, Karolinska Institutet, Huddinge University Hospital, S-141 86 Huddinge, Sweden

408. Ribeiro CD, Thomas MT, Kembrey D, Magee JT, North Z. Resistotyping of campylobacters: fulfilling a need. Epidemiol Infect 1996 Apr;116(2):169-75. 26 ref, Eng. Department of Medical Microbiology and Public Health Laboratory, University Hospital of Wales, Heath Park, Cardiff CF4 4XW, UK

409. Robins-Browne RM. Bacterial infections of the small intestine and colon. Curr Opin Gastroenterol 1996 Jan;12(1):68-75. 55 ref, Eng. Royal Children's Hospital, Department of Microbiology and Infectious Diseases, Flemington Road, Parkville, Victoria 3052, Australia

410. Roy SK, Tomkins AM, Akramuzzaman SM. Current management of persistent diarrhoea and malnutrition in developing countries. Hong Kong J Paediatr 1995 Sep;6(1 Suppl):100-13. 56 ref, Eng. International **Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh**

411. Ruedl C, Rieser C, Kofler N, Wick G, Wolf H. Humoral and cellular immune responses in the murine respiratory tract following oral immunization with cholera toxin or *Escherichia coli* heat-labile enterotoxin. Vaccine 1996 Jun;14(8):792-8. 32 ref, Eng. Institute for General and Experimental Pathology, Medical School, University of Innsbruck, Fritz-Pregl Strabe 3/IV, A-6020 Innsbruck, Austria

412. Saini S, Arora DR, Sikka R, Kundra N. Bacteriological study of cholera in Rohtak for five years. Indian J Med Microbiol 1995 Oct;13(4):187-8. 10 ref, Eng. Department of Microbiology, Pt. B.D. Sharma Post-graduate Institute of Medical Sciences, Rohtak 124001, India

413. Sanderson K, Ghazali FM, Kirov SM. Colonization of streptomycin-treated mice by *Aeromonas* species. J Diarrhoeal Dis Res 1996 Mar;14(1):27-32. 24 ref, Eng. Department of Pathology, University of Tasmania, GPO Box 252C, Hobart, Tasmania, Australia 7001

"Streptomycin-treated adult mice were investigated as a possible model for studying the enteropathogenicity of *Aeromonas* species. C57BL mice pre-treated with streptomycin (5.0 g/L drinking water, 48 hours) received a single intragastric dose (1010 bacteria /10.5 mL) of one of six well-characterized, toxin-producing, human diarrhoeal isolates of *A. veronii* biovar sobria (n=3) or *A. hydrophila* (n=3). Their faeces were examined for *Aeromonas* for 10 days post-challenge. All strains colonized the antibiotic-treated mice. Colonization did not occur in mice which did not receive streptomycin. Strains of *A. hydrophila* were recovered in greater numbers than strains of *A. veronii* biovar sobria, and colonized (103 cfu/g of faeces) a greater proportion of mice at day 10. Strains of the latter species, however, were more adherent in cell line assays used as models of intestinal adhesion. *A. hydrophila* strains localized in the large intestine and appeared not to be cell associated. This study, therefore, points to species-related differences in intestinal colonization mechanisms. The streptomycin-treated adult mouse model may prove useful for further investigation of some of these mechanisms. Diarrhoeal symptoms were, however, not produced in this model.

414. Sarker SA, Rahman MM, Mahalanabis D, Bardhan PK, Hildebrand P, Beglinger C, Gyr K. Prevalence of *Helicobacter pylori* infection in infants and family contacts in a poor Bangladesh community. Dig Dis Sci 1995 Dec;40(12):2669-72. 25 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"Although *H. pylori* is well established as an etiological agent of type B gastritis and a predisposing factor for peptic ulcer, knowledge about its transmission is unclear. In this study we examined the prevalence of *H. pylori* infection in the family members of index infants infected with this organism as indicated by positive [13C]-urea breath test (UBT). We performed UBT among family members of 15 predominantly breastfed infants, eight with and seven without *H. pylori* infection. Infection rates were 82% and 91% in family contacts of the infected and noninfected infants respectively, the average infection rate being 85%, which is rated to be high. There was no difference in infection rates among the parents of the infected and noninfected infants. Fifty percent and 70% families belonging to infected and noninfected infants, respectively, were found to have all members infected with *H. pylori*. No evidence of sex predilection of infection was found. We conclude that in communities with high prevalence of *H. pylori* infection, there is almost an equal infection rate among the family contacts of infected and noninfected infants, suggesting that environmental factors may be more important than intrafamilial transmission."

415. Schmidt T, Hackelsberger N, Widmer R, Meisel C, Pfeiffer A, Kaess H. Ambulatory 24-hour jejunal motility in diarrhea-predominant irritable bowel syndrome. Scand J Gastroenterol 1996 Jun;31(6):581-9. 44 ref, Eng. Department of Gastroenterology, Hospital Bogenhausen, Englschalkingerstrasse 77, D-81925 Munich, Germany

416. Sciortino CV. Protection against mortality due to *Vibrio cholerae* infection in infant rabbits caused by immunization of mothers with cholera protective antigen. J Diarrhoeal Dis Res 1996 Mar;14(1):16-26. 37 ref, Eng. Department of Pathology and Laboratory Medicine, Department of Veterans Affairs Medical Center, Louisville, KY 40206, USA

"Vaccination of female rabbits with cholera protective antigen (CPA) protected their F1 progeny from lethal challenge with *Vibrio cholerae*. Protection was determined by the choleragenic score and survival rates. Serum and milk IgG, IgM, IgA titres to CPA, cholera toxin, and LPS were determined. At 8 and 20 weeks post-immunization, mothers' milk, sera, and infants' sera showed elevated CPA-specific IgG and IgA, and infants were protected. Mothers' serum and milk antibody remained elevated for 36 weeks. At 26 weeks, mothers were re-bred, but their progeny were swapped and cross-fed. Infants born to the placebo-vaccinated mothers and nursed by CPA-immune nannies were partially protected from challenge. Infants born to CPA-immune mothers and cross-fed by the placebo-vaccinated nannies were less protected. CPA stimulated both transplacental and milk antibody, but passive immunity was primarily milk-derived. A 36-week booster vaccine stimulated an anamnestic serological response that did not provide protection equivalent to the original vaccine. CPA provided partial protective immunity to the milk-fed infant rabbits that suggests that CPA may be important in the development of a cholera vaccine."

417. Scotland SM, Smith HR, Cheasty T, Said B, Willshaw GA, Stokes N, Rowe B. Use of gene probes and adhesion tests to characterise *Escherichia coli* belonging to enteropathogenic serogroups isolated in the United Kingdom. J Med Microbiol 1996 Jun;44(6):438-43. 27 ref, Eng. Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK

418. Shahid NS, Greenough WB, III, Samadi AR, Huq MI, Rahman N. Hand washing with soap reduces diarrhoea and spread of bacterial pathogens in a Bangladesh village. J Diarrhoeal Dis Res 1996 Jun;14(2):85-9. 27 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"Hand washing with soap and water can prevent the spread of diarrhoeal diseases in areas where comparatively costly interventions, such as supply of safe water and improved sanitation, are not possible. In this study, the practice of hand washing with soap and water was instituted in a periurban slum of Dhaka city, and the surveillance for diarrhoea sustained for a one-year period. Rates of primary and secondary attacks were compared to those of a non-intervention area similar in age structure, economic status, education, and other relevant variables. Rectal swabs of cases and contacts established aetiologies. There was a large (2.6 fold) reduction in diarrhoeal episodes in the intervention area during the observation period. Rates of bacterial pathogens were also lower in the intervention area. Significant reduction in diarrhoeal incidences was observed in all age groups for all pathogens except for rotavirus. These observations if implemented as health policy could reduce the spread of diarrhoeal diseases at low cost in high risk areas."

419. Shepherd KM, Wyn-Jones AP. An evaluation of methods for the simultaneous detection of *Cryptosporidium* oocysts and *Giardia* cysts from water. Appl Environ Microbiol 1996 Apr;62(4):1317-22. 11 ref, Eng. School of Health Sciences, The University, Sunderland SR1 3SD, UK

420. Sherchand JB, Shrestha MP. Prevalence of *Cryptosporidium* infection and diarrhoea in Nepal. J Diarrhoeal Dis Res 1996 Jun;14(2):81-4. 24 ref, Eng. Department of Community Medicine and Community Health Research Laboratory, Tribhuvan University Institute of Medicine, Kathmandu, Nepal

"Three hundred and fifty-four soft, loose or watery stool specimens from patients with acute diarrhoea were screened for the presence of *Cryptosporidium* oocysts. A modified Ziehl Neelsen with DMSO staining method was used for detecting *Cryptosporidium* oocysts in the stool samples. The oocysts were identified in 24 (6.8%) of the samples, while 46 samples (13%) showed mixed infections. Children aged between 2 and 10 years were mostly infected by this parasite, while infection was more prevalent in females than in males for all the age groups. These findings suggest that *Cryptosporidium* is one of the important aetiologic agents of diarrhoea in this population and should be looked for during laboratory investigation of diarrhoeal stool samples."

421. Shier RP, Dollimore N, Ross DA, Binka FN, Quigley M, Smith PG. Drinking water sources, mortality and diarrhoea morbidity among young children in Northern Ghana. Trop Med Int Health 1996 Jun;1(3):334-41. 14 ref, Eng. London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

422. Sibal A, Patwari AK, Anand VK, Chhabra AK, Chandra D. Associated infections in persistent diarrhoea - another perspective. J Trop Pediatr 1996 Apr;42(2):64-7. 20 ref, Eng. Diarrhoea Training and Treatment Unit, Kalawati Saran Children's Hospital, Lady Hardinge Medical College, New Delhi 110001, India

"Seventy-eight children diagnosed as cases of persistent diarrhoea (PD) from 1 month to 5 years of age (mean age 8.92 months) hospitalized during a 2-year study period were screened for the presence of non-gastrointestinal infections. Clinical screening suggested acute respiratory infection (ARI) in 30 per cent cases, urinary tract infection (UTI) in 19 per cent and acute suppurative otitis media (ASOM) in 10 per cent of cases. Investigations revealed pneumonia on chest X-ray (39 per cent), positive urine culture (32 per cent), leucocytosis (31 per cent) and positive blood culture (22 per cent). Seven cases (9 per cent) of pneumonia and 10 cases (13 per cent) diagnosed to have UTI were not identified on clinical screening and could be detected only after investigations. *E. coli* was the commonest organism isolated from urine culture (23 per cent) and blood culture (14 per cent); 54 per cent of cases had one or the other associated infection and 28 per cent were suffering from more than one infection. Bacterial pathogens were more frequently isolated from blood in children <6 months ($P<0.01$), with vomiting ($P<0.001$), and severe malnutrition ($P<0.05$); from urine in association with fever ($P<0.001$), duration of diarrhoea >4 weeks ($P<0.05$), and vomiting ($P<0.001$). Pneumonia was detected on chest radiograph more frequently in children with severe malnutrition ($P<0.001$). Sixty eight per cent of cases were successfully treated with dietary management and appropriate treatment of associated infections and 18 per cent of cases died. Mortality was highest in association with severe oral thrush, severe malnutrition, septicaemia, and ARI. Our results suggest that majority of cases of PD are associated with one or the other non-gastrointestinal infections particularly UTI and ARI which may be missed on clinical examination unless efforts are made to investigate these children. Early detection and appropriate management of these infections can considerably modify hospital course and outcome."

423. Siddique AK, Akram K, Zaman K, Mutsuddy P, Eusof A, Sack RB. *Vibrio cholerae* O139: how great is the threat of a pandemic? Trop Med Int Health 1996 Jun;1(3):393-8. 22 ref, Eng. International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"The emergence of the new strain *Vibrio cholerae* O139 and its rapid spread in Bangladesh and India together with its detection in several other countries, have raised the question whether this constitutes the beginning of the eighth pandemic of cholera, and if so, how large a threat it poses. In an attempt to answer this question, epidemic spread patterns of *Vibrio cholerae* O139 strain in Bangladesh were studied. Initially the epidemic moved quickly and affected the entire coastal and estuarine tidal plains of southern Bangladesh. In the flood plains of the northern regions it affected mostly the north-eastern and

north-central areas, at a slower pace than in the southern areas. In the beginning the new strain totally displaced both biotypes (classic and *El Tor*) of *Vibrio cholerae* O1. Nearly 2 years after its initial detection, striking differences in the distribution of *V. cholerae* O139 and O1 were observed. In most northern areas, the new strain was replaced by *V. cholerae* O1, whereas in the southern areas, the new strain was replaced by *V. cholerae* O1, whereas in the southern coastal regions, the O139 strain continues to dominate epidemics. The study suggests that the O139 strain may become endemic in the coastal ecosystem. The threat of a pandemic, therefore, may not be as large as it first seemed."

424. Silva TMJ, Schleupner MA, Tacket CO, Steiner TS, Kaper JB, Edelman R, Guerrant RL. New evidence for an inflammatory component in diarrhea caused by selected new, live attenuated cholera vaccines and by El Tor and 0139 *Vibrio cholerae* (note). Infect Immun 1996 Jun;64(6):2362-4. 14 ref, Eng. Division of Geographic and International Medicine, University of Virginia School of Medicine, Box 485 HSC, Charlottesville, VA 22908, USA

425. Singh J, Khanna KK, Dhariwal AC, Bhattacharjee J, Singh M, Jain DC, Datta KK. Unusual occurrence of cholera in Delhi during January 1994: epidemiological investigations (short report). J Diarrhoeal Dis Res 1996 Jun;14(2):107-9. 8 ref, Eng. National Institute of Communicable Diseases, 22 Shamnath Marg, Delhi 110054, India

"Hundreds of laboratory confirmed cholera cases occur every year in Delhi. However from 1965 through 1993, no cases of cholera nor carriers of *Vibrio cholerae* have been detected in the months January and February of all these years. Nevertheless, two cases occurred in January 1994. Both were children who acquired their infection locally. Six hundred fifty-eight rectal swabs collected from possible contacts were negative for *V. cholerae*. The next isolations could be made only in April, which is the usual beginning of the cholera season. The study suggests that cholera transmission can occur during the winter months in Delhi, but that it is not sustained."

426. Sohel I, Puente JL, Ramer SW, Bieber D, Wu C-Y, Schoolnik GK. Enteropathogenic *Escherichia coli*: identification of a gene cluster coding for bundle-forming pilus morphogenesis. J Bacteriol 1996 May;178(9):2613-28. 110 ref, Eng. Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California 94305, USA

427. Son R, Ansary A, Rusul G, Karim MIA. Isolation of verotoxin-producing *Escherichia coli* associated with diarrhoea in Malaysia containing plasmids showing homology with biotinylated Shiga-like toxin DNA gene probes. World J Microbiol Biotechnol 1996 May;12(3):243-6. 19 ref, Eng. Department of Genetics and Cellular Biology, Faculty of Science, University of Malaya, 59100 Kuala Lumpur, Malaysia

428. Taneja DK, Lal P, Aggarwal CS, Bansal A, Gogia V. Diarrhea management in some Jhuggi clusters of Delhi (brief report). Indian Pediatr 1996 Feb;33(2):117-9. 6 ref, Eng. Department of Preventive and Social Medicine, Maulana Azad Medical College, New Delhi 110002, India

429. Tao H. Human group B rotavirus: adult diarrhea rotavirus. Chin Med J 1996 Jan;109(1):11-2. Institute of Virology, Chinese Academy of Preventive Medicine, Beijing 100052, People's Republic of China

430. Tarr PI, Christie DL. Gastrointestinal infections in childhood. Curr Opin Gastroenterol 1996 Jan;12(1):88-94. 75 ref, Eng. Children's Hospital and Medical Center, 4800 Sand Point Way NE, Seattle, WA 98105-0371, USA

431. Taylor MB, Parker S, Grabow WOK, Cubitt WD. An epidemiological investigation of norwalk virus infection in South Africa. Epidemiol Infect 1996 Apr;116(2):203-6. 27 ref, Eng. Department of Medical Virology, University of Pretoria, PO Box 2034, Pretoria, South Africa 0001

432. Timenetsky MDCST, Gouvea V, Santos N, Alge ME, Kisiellius JJ, Carmona RCC. Outbreak of severe gastroenteritis in adults and children associated with type G2 rotavirus. J Diarrhoeal Dis Res 1996 Jun;14(2):71-4. 28 ref, Eng. Virology Laboratory, Instituto Adolfo Lutz, Sao Paulo, SP 01246-902, Brazil

"An outbreak of severe gastroenteritis affecting 132 adults and children occurred in the small city of Mirassol, Sao Paulo, Brazil, in 1992. The outbreak of diarrhoeal disease had an abrupt onset and afflicted all age segments of the population. Group A rotavirus was the only pathogen associated with the epidemic. It was detected in 12 of the 27 (44%) stool specimens analyzed and was identified as serotype G2 rotavirus. Severe dehydration was common among adults and older children, and 35% of all the notified cases were hospitalized for parenteral rehydration. Contamination of the main water supply was the most likely source."

433. Tomkins A, Benrens R, Roy SK. Micronutrient supplements for diarrhoeal disease. Hong Kong J Paediatr 1995 Sep;6(1 Suppl):95-9. 18 ref, Eng. Centre for International Child Health, Institute of Child Health, University of London 30 Guilford Street, London WC1N 1EH, UK

434. Torres JM, Alonso C, Ortega A, Mittal S, Graham F, Enjuanes L. Tropism of human adenovirus type 5-based vectors in swine and their ability to protect against transmissible gastroenteritis coronavirus. J Virol 1996 Jun;70(6):3770-80. 59 ref, Eng. Department of Molecular and Cell Biology, Centre Nacional de Biotecnologia, Consejo Superior de Investigaciones Cientificas, Campus Universidad Autonoma, Cantoblanco, 28049 Madrid, Spain

435. Turvill JL, Farthing MJG. Water and electrolyte absorption and secretion. Curr Opin Gastroenterol 1996 Mar;12(2):129-33. 32 ref, Eng. Digestive Diseases Research Centre, St. Bartholomew's and The Royal London Hospital School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, UK

436. Van Bergen JEAM. Epidemiology and health policy - a world of difference?: a case-study of a cholera outbreak in Kaputa district, Zambia. Soc Sci Med 1996 Jul;43(1):93-9. 33 ref, Eng. SAD-Schorer Stichting, PC. Hooftstraat 5, 1071BL, Amsterdam, The Netherlands

"The relationship between epidemiology and health policy is an area of considerable debate. This article demonstrates the use of epidemiological methods to make health policy formulation more `policy significant' and `down to earth'. A case-study of a cholera outbreak in Kaputa district, Zambia is used as an illustration. A closer liaison between epidemiology and social sciences is advocated. Epidemiological data should be supplied as feed back to the study population to facilitate community-based action for health."

437. van Loon FPL, Banik AK, Nath SK, Patra FC, Wahed MA, Darmaun D, Desjeux J-F, Mahalanabis D. The effect of L-glutamine on salt and water absorption: a jejunal perfusion study in cholera in humans. Eur J Gastroenterol Hepatol 1996 May;8(5):443-8. 41 ref, Eng. Centers for Disease Control and Prevention Mail Stop E62, 1600 Clifton Road, Atlanta, Georgia 30333, USA

"Objectives: To assess the efficacy of an t-glutamine solution on jejunal salt and water absorption in cholera patients. Design: A randomized double-blind jejunal perfusion study. Setting: International Centre for Diarrhoeal Disease Research, Bangladesh. Patients: Nineteen adults with acute cholera. Interventions: Perfusion of balanced salt solutions alternated with defined glucose salt solution and glutamine glucose salt or alanine glucose salt solutions. Main outcome measures: Net jejunal water and sodium secretion. Results: Perfusion of glutamine in the presence of glucose significantly reduced net

water secretion (JnetH2O=-2.6±1.3 ml/h/cm) and also reduced net sodium secretion (JnetNa=213±153 µmol/h/cm). Similar results were observed during the perfusion of solutions that contained alanine in addition to glucose (JnetH2O=-4.2±1.1 ml/h/cm and (JnetNa=-444U±142 µmol/h/cm, respectively) or glucose alone ((JnetH2O=-4.3±1.7 ml/h/cm and (JnetNa=-452±212 µmol/h/cm, respectively). In addition, a higher basal secretion was associated with a greater stimulation of water absorption (F=17, P<0.001). Conclusion: Glutamine in the presence of glucose significantly reduces net water secretion and also reduces sodium secretion; higher basal secretion is associated with greater water absorption. As glutamine is able to stimulate water absorption to the same degree as glucose and alanine, and because it has the theoretical advantage of providing fuel for the mucosa, the inclusion of glutamine as the sole substrate in oral rehydration solution warrants further study."

438. Vijayalakshmi N, Ananthakrishnan S. Intestinal parasitic infestations: consequences and control options. Indian J Med Microbiol 1995 Oct;13(4):163-7. 38 ref, Eng. Department of Microbiology, Jawaharlal Institute of Post-graduate Medical Education and Research, Pondicherry 605006, India

439. Voss E, Manning PA, Attridge SR. The toxin-coregulated pilus is a colonization factor and protective antigen of *Vibrio cholerae* El Tor. Microbial Pathogen 1996 Mar;20(3):141-53. 25 ref, Eng. Microbial Pathogenesis Unit, Department of Microbiology and Immunology, The University of Adelaide, Adelaide, South Australia 5005, Australia

440. Weili Y. Diarrhoea in minorities in China. Hong Kong J Paediatr 1995 Sep;6(1 Suppl):14-6. 5 ref, Eng. Ministry of Public Health, 44 Houbari Beiyan, Beijing, People's Republic of China

441. Wenisch C, Parschalk B, Hasenhundl M, Hirschl AM, Graninger W. Comparison of vancomycin, teicoplanin, metronidazole, and fusidic acid for the treatment of *Clostridium difficile*-associated diarrhea. Clin Infect Dis 1996 May;22(5):813-8. 25 ref, Eng. Department of Infectious Diseases, Internal Medicine 1, University Hospital of Vienna, Wahringer Gurtel 18-20, A-1090 Vienna, Austria

442. Wilson IG, Moore JE. Presence of *Salmonella* spp. and *Campylobacter* spp. in shellfish. Epidemiol Infect 1996 Apr;116(2):147-53. 41 ref, Eng. Northern Ireland Public Health Laboratory, Bacteriology Department, Belfast City Hospital, Lisburn Road, Belfast BT9 7AD, UK

443. Witham PK, Yamashiro CT, Livak KJ, Batt CA. A PCR-based assay for the detection of *Escherichia coli* Shiga-like toxin genes in ground beef. Appl Environ Microbiol 1996 Apr;62(4):1347-53. 43 ref, Eng. Stocking Hall, Cornell University, Ithaca, NY 14853, USA

444. Wong H-C, Lu K-T, Pan T-M, Lee C-L, Shih DY-C. Subspecies typing of *Vibrio parahaemolyticus* by pulsed-field gel electrophoresis. J Clin Microbiol 1996 Jun;34(6):1535-9. 8 ref, Eng. Department of Microbiology, Soochow University, Taipei, Taiwan 11102, Republic of China

445. Yao R, Guerry P. Molecular cloning and site-specific mutagenesis of a gene involved in arylsulfatase production in *Campylobacter jejuni* (note). J Bacteriol 1996 Jun;178(11):3335-8. 25 ref, Eng. Naval Medical Research Institute Annex, 12300 Washington Ave., Rockville, MD 20852, USA

446. Yoon PW, Black RE, Moulton LH, Becker S. Effect of not breastfeeding on the risk of diarrheal and respiratory mortality in children under 2 years of age in Metro Cebu, The Philippines. Am J Epidemiol 1996 Jun 1;143(11):1142-8. 14 ref, Eng. Department of International Health, The Johns Hopkins School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA

The effects of not breastfeeding on mortality due to diarrhea and acute lower respiratory infection (ALRI) in children under 2 years of age were examined using data from a 1988-1991 longitudinal study of 9,942 children in Metro Cebu, The Philippines. Cox regression methods were used to study the magnitude of

the risks, possible interactions with birth weight and nutritional status, and the effect of additional confounding factors. Not breastfeeding had a greater effect on diarrheal mortality than on ALRI mortality. In the first 6 months of life, failing to initiate breastfeeding or ceasing to breastfeed resulted in an 8- to 10-fold increase in the rate of diarrheal mortality. The rate of mortality associated with both ALRI and diarrhea was increased nearly six times by not breastfeeding, but the rate of ALRI mortality alone was not increased. The data also suggested that the risk of mortality associated with not breastfeeding was greater for low birth weight infants and infants whose mothers had little formal education. After age 6 months, the protective effects of breastfeeding dropped dramatically. These findings underscore the importance of promoting breastfeeding, especially during the first 6 months of life, and of targeting high risk groups such as low birth weight babies and those of low socioeconomic status.

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