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Severity of Cholera During Concurrent Infections with Other Enteric Pathogens

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ABSTRACT

In a clinic-based case-control study in Bangladesh we evaluated whether children with diarrhoea due to *V. cholerae* O1 in association with other enteric pathogen(s) are likely to manifest more severe disease as indicated by development of moderate or severe dehydration. Children with moderate or severe dehydration were defined as cases and those with no dehydration were controls; both cases and controls had acute diarrhoea. A systematic sample of 268 dehydrated cases and 699 nondehydrated controls aged 1-35 months with acute watery diarrhoea of 6 days or less was included. In a multivariate analysis it has been shown that infection with *Vibrio cholerae* O1 in association with another diarrhoea pathogen (odds ratio=7.07) was strongly correlated with status of dehydration than those with the *V. cholerae* O1 infection as a single pathogen (odds ratio=3.63). Either group was associated with significant risk of dehydration. The results of the study suggest that more than one enteropathogen may be simultaneously involved in causing severe diarrhoea, and appropriate public health measures to reduce environmental contamination should be beneficial.

Key words: Cholera; *Vibrio cholerae;* Diarrhoea, Infantile; Enteropathogens; Bangladesh; Case-control studies.

INTRODUCTION

An estimated 60 to 70 percent of diarrhoeal death is due to dehydration. Most of them are young children (1). A knowledge of the risk factors that adversely affect the severity of dehydration would be useful in formulating appropriate rehydration therapy for management of diarrhoeal diseases. In aetiological studies on acute diarrhoeal illness, more than one enteric pathogen are identified in 20% patients (2). In these patients it is difficult to ascertain which of the infecting agents contributed significantly to the clinical severity of the disease. It is possible that more than one pathogen concurrently contribute to a diarrhoeal illness.

Furthermore, it is not known whether infection with more than one pathogen is associated with more severe disease than a single enteric pathogen. In a clinic-based case-control study we have evaluated the roles of some enteropathogens in association with *Vibrio cholerae* O1 as risk factors for dehydrating diarrhoea.

MATERIALS AND METHODS

The study was conducted at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) hospital between August 1988 and September 1989. The details of the methods have been reported earlier (3). Briefly, the inclusion criteria were: (a) children aged 1-35 months attending the triage area, and (b) watery diarrhoea for 6 days or less. Children with dysenteric illness (bloody or bloody mucoid diarrhoea) were excluded. Names and identification numbers of all eligible children were recorded round the clock. From among these children a 5% systematic sample was enrolled in both case and control groups. Children with acute watery diarrhoea and moderate or severe dehydration [i.e. with definite decrease in skin elasticity and one or more sign(s) of (a) sunken eyes, (b) failure to urinate for six hours or more, (c) sunken anterior fontanelle in infants and (d) rapid and weak pulse] were enrolled as cases. Children with no detectable signs of dehydration, with or without thirst were considered as controls.

Informed consents were obtained from mothers of all the children before enrollment in the study. After admission a physician evaluated the clinical conditions, including the status of dehydration. Nutritional anthropometric measurements were taken on admission and discharge from the hospital. Demographic, social, economic, dietary and medical histories were obtained from the mothers by trained female interviewers.

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Personnel involved in data collection and patient care activities were kept unaware of the purpose of the study as well as of the classification of the children as cases and control.

Table I. Comparison of acute diarrhoea cases (i.e. with moderate to severe dehydration and acute diarrhoea controls (i.e. with no dehydration) according to enteropathogens detected and confounders

Variable	Diarrhoea with moderate to severe dehydration (Cases) n=699 (%)	Diarrhoea with no dehydration (Controls) n=268 (%)	Odds ratio (95 percent C.I.)	p-value
Vibrio cholerae O1 (VC O1) ¶				
With other pathogens	18 (6.7)	8 (1.1)	6.40 (2.93-13.98)	<0.0001 *
As only pathogen	10 (3.7)	8 (1.1)	3.56 (1.34-9.47)	
Not isolated ø	240 (89.6)	683 (97.7)	1.00	
Age of patient (months)†				
1-9	162 (60.4)	325 (46.5)	1.76 (1.32-2.35)	0.0001 *
10-35	106 (39.6)	374 (53.5)		
Weight-for-age (percent)‡				
<60	48 (17.9)	39 (5.6)	5.33 (3.36-8.45)	<0.0001 *
60-74	132 (49.3)	279 (39.9)	2.05 (1.50-2.80)	
>74	88 (32.8)	381 (54.5)	1.00	
Mud floor	199 (74.3)	398 (56.9)	2.18 (1.59-2.98)	<0.0001 *
Cemented floor	69 (25.7)	301 (43.1)		
Withdrawal of breastfeeding during diarrhoea				
Yes	6 (2.2)	3 (0.4	5.31 (1.24-22.79)	0.0165 *
No	262 (97.8)	696 (99.6)		
Duration of diarrhoea prior to admission †				
>36 hours	127 (47.4)	355 (50.8)	.87 (.64-1.18)	0.3443 *
<=36 hours	141 (52.6)	344 (49.2)		

* Chi-square test

† Categorised according to median and less, and more than median

‡ Median is 74 percent

¶ Chi-square test for a trend was also highly significant (X^2=30.2, p=<.0001)

ø Includes those in whom other pathogens were detected

For isolating enteric pathogens, rectal swabs from study children were obtained and plated immediately on bacteriologic culture media. Pathogens were identified using standard laboratory methods (4-13). After completion of the study the data were entered in a microcomputer and analyzed using SPSS software packages (14). Enteropathogens considered in these analyses were *V. cholerae* O1, enterotoxigenic *E. coli* (ETEC), *Campylobacter jejuni*, rotavirus, and other vibrios (mainly *Aeromonas*). To determine the association between *V. cholerae* O1 and other pathogens and presence or absence of moderate or severe dehydration, a univariate statistical analysis was performed. For cholera, patients were divided into three categories: (a) those who had *V. cholerae* O1 in association with one or more of other enteric pathogens, (b) those who had *V. cholerae* O1 only, and (c) those who had no *V. cholerae* O1, in whom either no pathogen was detected, or one or more enteric pathogen(s) other than *V. cholerae* O1 was detected. Patients included in the third group were used as reference category.

Confounding variables (age, quality of house, nutritional status, breastfeeding behaviour during diarrhoea at home, and duration of illness before coming to the hospital) were identified by evaluating the tables of *V. cholerae* O1 and dehydration status stratified by each likely confounder one at a time using Mantel-Haenszel procedure (15). If the stratified odds ratio deviated from the crude odds ratio by 10% or more the variable was considered as confounder and included in the multivariate analysis model (15,16). The basic model (logistic regression) was fitted with dehydration as dependent variable and presence of *V. cholerae* O1 singly or in association with other diarrhoea pathogen(s) as the risk factor of interest. Adjusted odds ratios (OR) and 95% confidence intervals (CIs) for the risk factors and for the confounding variables were estimated with the state of dehydration as the dependent variable. Statistical softwares Epi Info (CDC Atlanta) (17), and EGRET (18) were used.

RESULTS

Of the 967 stool samples, 622 (64%) yielded growth of at least one enteropathogen (Appendix). *V. cholerae* O1 accounted for infection in 2.9% and 4.2% of children as single pathogen or in association with one or more pathogen(s) respectively.

Table II. Adjusted odds ratio and 95 percent confidence interval of factors associated with the presence of dehydration (dependent variable) in 967 children aged 1-35 months with acute diarrhoea in Dhaka, Bangladesh, 1988-1989, a case-control study: multivariate analysis using logistic regression model*

Variable of interest	Coefficient	STD error	Adjusted odds ratio (95 percent CI)	p-value
Vibrio cholerae O1				
With other pathogens	1.956	.457	7.07 (2.88-17.34)	<0.001
As single pathogen	1.289	.510	3.63 (1.33-9.86)	0.012
Not isolated			1.00	
Young age (1-9 month=1, 10-35 month=0)	.8343	.161	2.30 (1.68-3.16)	<0.001
Floor (mud=1, cemented=0)	.5659	.170	1.76 (1.26-2.46)	<0.001
Weight-for-age (percent)				
<60	1.569	.261	4.80 (2.88-8.02)	<0.001
60-74	.7116	.169	2.04 (1.46-2.84)	<0.001
>74			1.00	
Withdrawal of breastfeeding during diarrhoea (Yes=1, No=2)	1.334	.748	3.80 (.88-16.45)	0.075
Duration of diarrhoea before hospitalization (>36 hr=1, <=36 hr=0)	-.2691	.156	.76 (.56-1.04)	0.084

* Both cases and controls had acute watery diarrhoea; the former had moderate or severe dehydration, and the latter had none or mild dehydration

C. jejuni was most frequently associated with *V. cholerae* O1 (54%). Enterotoxigenic *E. coli* (ETEC) accounted for infection in 10.5% as a single agent and 13.8% in association with other enteropathogen(s) among children yielding positive isolates. Again, *C. jejuni* was the pathogen most often associated with ETEC (53%). Rotavirus alone was detected in 26.2% and in association with another pathogen in 19.0% of children. *C. jejuni* was found in 20.9% as a single pathogen and in 21.2% as a mixed pathogen among children yielding positive isolates. Among the latter, 38.6% was associated with rotavirus. Other vibrios (mainly *Aeromonas* species) were detected as single pathogen in 4.8% or mixed pathogen in 8.5% children.

A large proportion of children (69%) who were positive for *V. cholerae* O1 was dehydrated. On the other hand, dehydration was observed in 35% for ETEC, 37% for *C. jejuni*, 31% for rotavirus, and 36% for other vibrio infections.

Cases (i.e. with dehydration) and controls (i.e. with no dehydration) were compared according to the characteristics of interest, and results of the univariate analysis are shown in Table I. More cases than controls were positive for *V. cholerae* O1 associated with other pathogens (OR=6.40, 95% CI=2.93-13.98) or as a single pathogen (OR=3.56, CI=1.34-9.47). Chi-square values for trend in those three categories i.e. *V. cholerae* O1 in association with other pathogens, *V. cholerae* O1 as single pathogen, and absence of *V. cholerae* O1 (includes other pathogens) are highly significant (Chi-square=30.2, p=<0.0001).

Cases were younger (OR=1.76, CI=1.32-2.35) and relatively more malnourished (marasmic) than the controls (OR=5.33, CI=3.36-8.45). Poor housing condition (as indicated by mud floor) was more common among cases than the controls (OR=2.18, CI=1.59-2.98). Cessation of breastfeeding during diarrhoea at home was more common among cases than controls. Duration of illness before coming to the hospital was comparable in both the groups.

Table II shows the results of multivariate analysis using logistic regression model. The adjusted odds ratios (OR) and 95% confidence intervals (CIs) for *V. cholerae* O1 as mixed or single isolate were computed where no isolation of *V. cholerae* 01 served as the reference category which includes children with another diarrhoea pathogen or no pathogen. Mixed or single infection with *V. cholerae* O1 was associated with significantly higher risk of developing moderate or severe dehydration (OR=7.07, CI=2.88-17.34, and OR=3.63, CI=1.33-9.86) respectively. A dose-response (i.e. with increasing levels of exposure to the factor, a corresponding increase in disease severity) in risk for dehydration between mixed and single infection was also demonstrated.

DISCUSSION

One special feature of this case-control study design was the choice of the control group i.e. children with diarrhoea but no dehydration were selected as controls instead of non-diarrhoea or healthy children. The study has competed with the ethical issues of clinical studies because both status of dehydration and status of the risk factors of interest were already determined at the time of clinic attendance. This design was partly developed by World Health Organization and has been reported (19).

We compared 268 cases who had acute diarrhoea and moderate or severe dehydration with 699 children presenting with similar diarrhoea but with no or mild dehydration. In univariate analysis, presence of *V. cholerae* O1 both as mixed pathogen and a single pathogen, was associated with higher risk of dehydration. In multivariate analysis, this association remained highly significant, and the magnitude of the excess risk for *V. cholerae* O1 both as a single (OR=3.63, CI=1.33-9.86) or mixed infection (OR=7.07,

CI=2.88-17.34) was large after controlling for the confounding variables. This association was independent of the factors related to host susceptibility (age and nutritional status), environmental factor (poor housing), feeding behaviour at home during diarrhoea, and duration of illness (indicating the stage of illness) before attending the health facility. Evidence from animal experiments suggests that infection in animals simultaneously with two pathogens e.g. *E. coli* and rotavirus produces diarrhoea more consistently than infection by single pathogen (20, 21). A study on weanling diarrhoea in pigs showed that individual infections with either rotavirus or *E. coli* caused mild diarrhoea. However, dual infections with rotavirus and *E. coli* resulted in more fluid loss, and prolonged diarrhoea (20). Enterotoxigenic *E. coli* fed for 24 hours to 26 days old gnotobiotic, colostrum-deprived, or suckling calves did not cause any diarrhoea; in contrast, diarrhoea was consistently induced in 1 to 2-week old calves infected with both enterotoxigenic *E. coli* and rotavirus concurrently (21). Infected animals developed moderate to severe diarrhoea and showed evidence of infection with both microbial agents. A prospective cohort of 153 infants in a poor periurban community in Lima, Peru, showed that diarrhoea was more often associated with multiple pathogens than with a single pathogen (22); a relationship with disease severity was however not evaluated.

The increased risk of dehydration in children having *V. cholerae* O1 associated with other diarrhoea pathogen(s) may be attributed to the effects of more than one microbial agent. Clinical studies have reported that *V. cholerae* O1 alone can cause more severe disease compared to the other diarrhoeagenic pathogens (23). Our findings suggest that such an effect is likely to be more pronounced when *V. cholerae* O1 is associated with other diarrhoea pathogen(s).

Campylobacters were found to be the predominant copathogen associated with *V. cholerae* O1 (Appendix). Detection of mixed infection with diverse enteropathogens indicates that the patient lives in a highly contaminated environment. The enteropathogens are generally transmitted by contaminated food, water, and faeces through family members, objects (feeding bottle), food and water in the house. Moreover, incidence of diarrhoea has been shown to be related to the extent of environmental contamination (24).

The mud floor is an indicator of the socioeconomic condition and perhaps an indicator for the level of environmental contamination the child lives in. This may also indicate why such children should have more than one pathogen from the contaminated environment. The role of mud floor in longer survival, growth, and transmission of enteropathogens has been explained earlier (25).

To explain our findings on mixed infections, we speculate that synergistic effect of one favours simultaneous gut colonization by the other enteropathogen(s) resulting in increased toxin production, mucosal damage, and secretion of water and electrolytes. However, the results of this study should be interpreted with caution for several reasons. The merits of this study are: strength of association (OR=7.07, C.I.=2.88-17.34), response to dose in risk for severity of infection after controlling for confounders, and biological plausibility as supported by results of experiments with animals. The weaknesses are: lack of information on inoculum size, their specific pathogenicity, and serologic response to the individual pathogens (e.g. rising titre). We would like to mention that our study was an epidemiological study using case-control design and as such not the type of study which can address the above mentioned questions. Further studies are suggested for that.

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REFERENCES

1. The management of diarrhoea and use of oral rehydration therapy. A Joint WHO/UNICEF Statement. Second edition. Geneva: World Health Organization, 1985.
2. Black RE, Merson MH, Rahman ASMM, Yunus M, Alim ARMA, Huq MI, *et al.* A two-year study of bacterial, viral, and parasitic agents associated with diarrhoea in rural Bangladesh. *J Infect Dis* 1980;142: 660-4.
3. Faruque ASG, Mahalanabis D, Islam A, Hoque SS, Hasnat A. Breastfeeding and oral rehydration at home during diarrhoea to prevent dehydration. *Arch Dis Child* 1992;67:1027-9.
4. Ewing WH. Edwards and Ewing's identification of *Enterobacteriaceae*. 4th ed. New York: Elsevier, 1986.
5. Monsur KA. Bacteriological diagnosis of cholera under field conditions. *Bull WHO* 1963;28:387-9.
6. Colwell RR. Polyphasic taxonomy of the genus *Vibrio*: numerical taxonomy of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and related *Vibrio* species. *J Bacteriol* 1970;104:410-33.
7. Lennette EH, Ballows A, Hausler WJ, Shadomy HJ, editors. Manual of clinical microbiology. 4th ed. Washington, D.C.: American Society for Microbiology, 1985.
8. Blaser MJ, Glass RI, Huq MI, Stoll B, Kibriya GM, Alim ARMA. Isolation of *Campylobacter fetus* subsp *jejuni* from Bangladeshi children. *J Clin Microbiol* 1980;12:744-7.

APPENDIX

Diarrhoea pathogens in 622 children positive for one or more pathogen(s); case-control study on 1-35 months old children with acute diarrhoea in Dhaka, Bangladesh, 1988-1989

Pathogen	Total number	Percent of those positive for pathogens
As a single pathogen		
Vibrio cholerae O1	18	2.9
Enterotoxigenic *E. coli*	65	10.5
Campylobacter jejuni	130	20.9
Rotavirus	163	26.2
Other vibrios	30	4.8
(*Aeromonas hydrophila*=16, *Aeromonas sobria*=10, *Plesiomonas shigelloides*=2, other=2)		
Shigella	16	2.6
Salmonella	0	0
More than one pathogen		
Vibrio cholerae O1 (VC) and others (VC+Campy=12, VC+ETEC=3, VC+Rota=3, VC+Oth vib=2, one each for: VC+Shig, VC+ETEC+Rota, VC+ETEC+Campy, VC+ETEC+Shig, VC+Rota+Oth vib, VC+Rota+Campy)	26	4.2
Enterotoxigenic *E. coli* (ETEC) and others (ETEC+Campy=29, ETEC+Rota=20, ETEC+Rota+Campy=11, ETEC+Oth vib=9, ETEC+Rota+Oth vib=4, ETEC+VC=3, ETEC+Campy+Oth vib=3, ETEC+Shig=2, One each for: ETEC+VC+Shig, ETEC+VC+Campy, ETEC+VC+Rota, ETEC+Campy+Oth vib+Rota, ETEC+Campy+Shig+Rota)	86	13.8
Rotavirus (Rota) and others (Rota+Campy=51, Rota+ETEC=20, Rota+Oth vib=14, Rota+Campy+ETEC=11, Rota+Shig=4, Rota+Oth vib+ETEC=4, Rota+Campy+Oth vib=4, Rota+VC=3, one each for: Rota+Salm, Rota+ETEC+VC, Rota+VC+Oth vib, Rota+VC+Campy, Rota+Oth vib+Shig, Rota+Campy+Oth vib+ETEC, Rota+Campy+Shig+ETEC)	118	19.0
Other vibrios (Oth vib) and others (Oth vib+Rota=14, Oth vib+Campy=12, Oth vib+ETEC=9, Oth vib+ETEC+Rota=4, Oth vib+Rota+Campy=4, Oth vib+ETEC+Campy=3, Oth vib+VC=2, one each for: Oth vib+Shig, Oth vib+VC+Rota, Oth vib+Rota+Shig, Oth vib+Shig+Campy, Oth vib+ETEC+Rota+Campy)	53	8.5
Campylobacter jejuni (Campy) and others (Campy+Rota=51, Campy+ETEC=29, Campy+VC=12, Campy+Oth vib=12, Campy+ETEC+Rota=11, Campy+Shig=5, Campy+Rota+Oth vib=4, Campy+ETEC+Oth vib=3, one each for: Campy+VC+ETEC, Campy+VC+Rota, Campy+Shig+Oth vib, Campy+ETEC+Oth vib+Rota, Campy+ETEC+Shig+Rota)	132	21.2
Shigella (Shig) and others (Shig+Campy=5, Shig+Rota=4, Shig+ETEC=2, one each for: Shig+VC, Shig+Oth vib, Shig+VC+ETEC, Shig+Rota+Oth vib, Shig+Campy+Oth vib, Shig+Rota+ETEC+Campy)	17	2.7
Salmonella (Salm) and others (Rota+Salm=1)	1	<1.0

9. Blaser MJ, Berkowitz ID, LaForce FM, Cravens J, Reller LB, Wang W-L. *Campylobacter* enteritis: clinical and epidemiologic features. *Ann Intern Med* 1979; 91:179-85.
10. Merson MH, Sack RB, Kibriya AKMG, Al-Mahmood A, Ahmad QS, Huq I. Use of colony pools for diagnosis of enterotoxigenic *Escherichia coli* diarrhea. *J Clin Microbiol* 1979;9:493-7.
11. Guerrant RL, Brunton LL, Schnaitman TC, Rebhun LI, Gilman AG. Cyclic adenosine monophosphate and alteration of Chinese hamster ovary cell morphology: a rapid, sensitive *in vitro* assay for the enterotoxin of *Vibrio cholerae* and *Escherichia coli*. *Infect Immun* 1974;10:320-7.
12. Dean AG, Ching Y-C, Williams RG, Harden LB. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. *J Infect Dis* 1972;125:407-11.
13. Yolken RH, Kim HW, Clem T, Wyatt RG, Kalica AR, Chanock RM, *et al.* Enzyme-linked immunosorbent assay (ELISA) for detection of human reovirus-like agent of infantile gastroenteritis. *Lancet* 1977;2:263-6.
14. SPSS Inc. SPSS/PC+ 4.0 Base Manual for the IBM PC/XT/AT and PS/2. Chicago, IL: SPSS Inc, 1990.
15. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959;22:719-48.
16. Breslow NE, Day NE. Statistical methods in cancer research. vol. 1. The analysis of case-control studies. Lyon: International Agency for Research on Cancer, 1980. (IARC scientific publication no. 32).
17. Epi Info Version 5.01a. Public Domain Software for Epidemiology and Disease Surveillance. Atlanta, Georgia: Centers for Disease Control, 1991.
18. Mauritsen R. EGRET software program. Seattle, WA: Statistics and Epidemiology Research Corporation, 1986.
19. Rodrigues L, Kirkwood B. Case-control designs in the study of common diseases: updates on the demise of the rare disease assumption and the choice of sampling scheme for controls. *Int J Epidemiol* 1990;19:205-13.
20. Lecce JG, Balsbaugh RK, Clare DA, King MW. Rotavirus and haemolytic enteropathogenic *Escherichia coli* in weanling diarrhea of pigs. *J Clin Microbiol* 1982;16:715-23.
21. Tzipori SR, Makin TJ, Smith ML, Krautil FL. Clinical manifestations of diarrhea in calves infected with rotavirus and enterotoxigenic *Escherichia coli*. *J Clin Microbiol* 1981;13:1011-6.
22. Black RE, deRomana GL, Brown KH, Bravo N, Bazalar OG, Kanashiro HC. Incidence and etiology of infantile diarrhea and major routes of transmission in Huascar, Peru. *Am J Epidemiol* 1989;129:785-99.
23. Stoll BJ, Glass RI, Huq MI, Khan MU, Holt JE, Banu H. Surveillance of patients attending a diarrhoeal disease hospital in Bangladesh. *Br Med J* 1982;285:1185-8.
24. Black RE, Brown KH, Becker S, Alim ARMA, Merson MH. Contamination of weaning foods and transmission of enterotoxigenic *Escherichia coli* diarrhoea in children in rural Bangladesh. *Trans R Soc Trop Med Hyg* 1982;76:259-64.
25. Feachem RG, Bradley DJ, Garelick H, Mara DD. Sanitation and disease: health aspects of excreta and wastewater management. Chischester: John Wiley, 1983.