OUTBREAKS OF CHOLERA IN NEPAL

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Abstract. This paper presents the study of the etiological agents of diarrhea in children below 14 years of age, this study was conducted from May 1995 to April 1996. One thousand one hundred seven (1,107) children with acute diarrhea receiving Oral Rehydration Therapy(ORT) at National Kanti Children's Hospital were included in this study. Stool samples of these patients were investigated at the Microbiology Laboratory, Department of Microbiology, Institute of Medicine. None of the stool samples showed the growth of *Vibrio cholerae* 0139 synonym Bengal. In Nepal, *V.cholerae* could be isolated from June to November. From December to May, no cases of *V. cholerae* were detected. Therefore, we address to this incidence as outbreaks rather than endemic. Mixed infections along with *V. cholerae* were also seen in 29% of cholera patients. *V.cholerae* 01, Hikojima types were the major isolates in our study followed by Ogawa type. *V. cholerae*, Hikojima and Ogawa serotypes were associated with mixed infection in 16.1% and 12.9% of patients, respectively. These isolates were associated with *Shigella, Salmonella* and pathogenic *E. coli.*

INTRODUCTION

Vibrio cholerae, both biotypes El Tor (biovar eltor) and classical (biovar classical), are capable of widespread disease. Seven cholera pandemics have been described since 1817. True endemic areas of cholera are found in Bangladesh and at present certain parts of Asia, Africa and in some areas of south-eastern USA (Fule *et al*, 1990; Siddique *et al*, 1992; Finelli *et al*, 1992; Isa *et al*, 1992; Dutta, 1993). On October 19, 1992, there was a sudden explosive outbreak of cholera-like diseases due to *V. cholerae* 0139 synonym Bengal in southern and eastern parts of India, and in southern parts of Bangladesh (Albert *et al*, 1993; Ramamurthy *et al*, 1993; WHO, 1993).

In Nepal, gastroenteritis is one of the major killer diseases. Every year 30-40 thousand people die of gastroenteritis. During 1991, *V.cholerae* 01 was isolated from 438 fecal specimens (63% of those examined) and 25 cases of *V. cholerae* 0139 were reported in 1993 and 1994 (Kubo *et al*, 1993; Bista *et al*, 1993; Shrestha, 1995; Amatya, 1995; Dixit, 1995, Ministry of Health, 1996).

MATERIALS AND METHODS

Subjects

A total of 1,107 children below 14 years of age with acute diarrhea were included in this study. They were attending the Oral Rehydration Therapy (ORT) center, at National Kanti Children's Hospital, Kathmandu.

Sample collection

After careful examination of these patients by a pediatrician, the stool samples were collected aseptically into a special stool container (Manufacture from Eiken Chem, Japan) and then brought to the microbiology laboratory immediately.

Culture

These stool samples were processed in order to isolate pathogens, with special interest in *V. cholerae*. The stool samples were therefore inoculated into Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar, Polymixin Mannose Tellurite (PMT) Agar, Eosin Methylene Blue (EMB) Agar, Mac Conkey Agar, *Salmonella-Shigella* Agar, and en-

richment media such as Alkaline Peptone Water pH 8.6, Selenite F, Rapaport broth. Both (direct and after enrichment) specimens were sub-cultured into selective medium. The yellow colonies showing in TCBS media were subcultured into Nutrient Agar, and catalase test, oxidase test and decarboxylase test were performed on overnight culture.

Serotyping and biotyping of *V.cholerae*

Serotyping of *V. cholerae* was done with the use of *V. cholerae* immune sera and monoclonal antibodies (Denka Seiken, Japan). In this test, a slide latex agglutination test was employed; serotyping of *V. cholerae* 01 was based on the following scheme:

Serotype	Antigen Structure	Immune sera			Monoclonal antibody		
		Polyvalent	Anti-Ogawa	Anti-Inaba	A	B	C
Ogawa	AB(C)	+	+	-	+	+	-
Inaba	AC	+	-	+	+	-	+
Hikojima	ABC	+	+	+	+	+	+

For determination of Biotype of *V. cholerae* the following tests were carried out and interpreted as follows:

Biotype	Hemolysis	RBC, agglutination	Polymyxin B (50μ) sensitivity	Colistin (50 μ) sensitivity	VP reaction
Classical type	-	-	+	+	-
El Tor type	+	+	-	-	+

Note: +=Positive; -=Negative; VP=Voges Proskaur

RESULTS

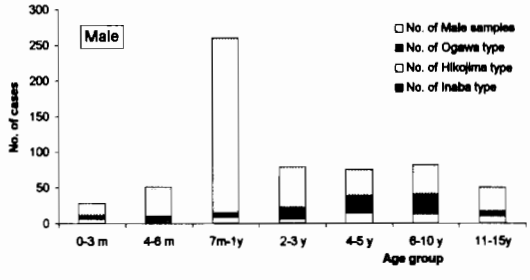
A large number of patients (89.2%) presented with watery diarrhea, followed by abdominal pain, vomiting, dehydration and fever. Mucus in stool and mucus in blood were found in 11.4% and 13.15% respectively (Table1).

Out of 1,107 patients, male patients comprised 56% and female patients 44%. A majority of diarrheal patients belonged to the age group, 7 months to 1 year. A few patients belonged to the age group 0-3 months. *V. cholerae* 01 biotype El Tor serovariety Inaba types were isolated from patients in age groups 4-6 months, 2-3 yeas and 6-10 years whereas Hikojima and Ogawa types were isolated from patients of all age group (Fig 1-1, 1-2). During an outbreak between June to November, a maximum number of cholera cases were seen in the month of August. Inaba type was isolated only in the month of July. Hikojima type was found to be most common in the month of July followed by September, and August, while Ogawa type was isolated from June to September, the greatest number of Ogawa type being seen in July followed by August and June. There were no cases of cholera from December to May. Though cholera cases were detected till November, the detection rate significantly dropped in this month and no cases of cholera could be detected from December to May. From june to November, there were 784 diarrheal patients. Of these 40.1% (314/784) patients showed the growth of *V. cholerae* in their stool samples. The incidence of Ogawa, Hikojima and Inaba types of *V. cholerae* was 25.4% (199/784), 14.2% (111/784), and 0.5% (4/784), respectively. Maximum number of cholerae were seen in the month of August (72%) folowed by July (62%) (Fig 2). Mixed infections were seen in 29% of cholera patients. *V. cholerae* Hikojima and Ogawa serotypes were associated with mixed infection in 16.1% and 12.9% of cases, respectively. These isolates of *V. cholerae* were associated with *Shigella*, *Salmonella* and pathogenic *E.coli*. None of the Inaba types was associated with these pathogenic agents (Table 2). Of the total cholera patients, 11.6% (34) were followed up after therapy.

Table 1

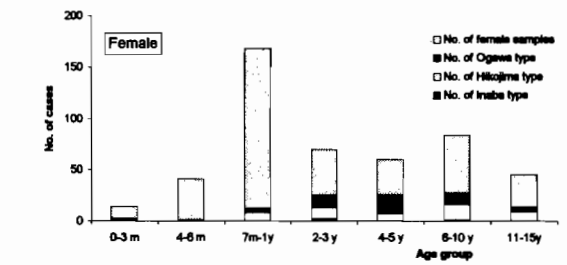
Number of cases by clinical symptom

	N=1,107
Watery diarrhea	987 (89.2%)
Mucous in stool	126 (11.4%)
Mucous and blood	145 (13.1%)
Fever	455 (41.1%)
Abdominal pain	752 (67.9%)
Vomiting	671 (60.6%)
Dehydration	860 (77.7%)



	0-3 m	4-6 m	7m-1y	2-3 y	4-5 y	6-10 y	11-15y
No. of Male	28	51	260	79	75	82	50
Ogawa	5	6	5	16	24	27	6
Hikojima	6	3	9	6	14	13	10
Inaba	0	1	0	0	0	0	0

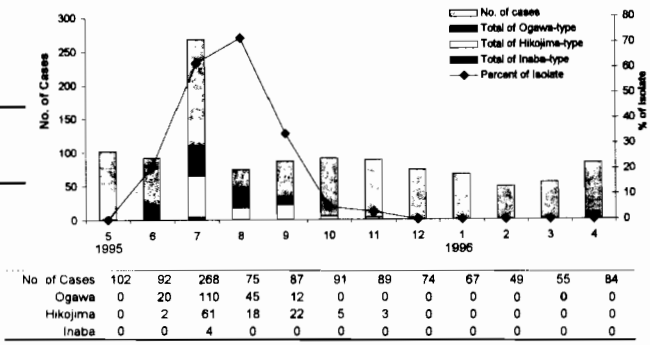
Fig 1 1–Age distribution of patients.



	0-3 m	4-6 m	7m-1y	2-3 y	4-5 y	6-10 y	11-15y
No. of Female	14	41	168	70	60	84	45
Ogawa	2	1	4	25	25	27	5
Hikojima	0	0	8	11	7	15	9
Inaba	0	0	0	2	0	1	0

Fig 1-2 –Age distribution of patients.

Of these 34 patients, 4, 11, 8, 5, 2 patients reported on day 2,3,4,5,6,7 respectively. The stool samples of the 4 and 5 patients who reported on days 2 and 5 respectively showed all negative for *V. cholerae* whereas patients reporting on days 3,4,6 showed the growth of *V. cholerae* in their stool samples (in about 50% of the total patients). Two patients who



	5	6	7	8	9	10	11	12	1	2	3	4
No. of Cases	102	92	268	75	87	91	89	74	67	49	55	84
Ogawa	0	20	110	45	12	0	0	0	0	0	0	0
Hikojima	0	2	61	18	22	5	3	0	0	0	0	0
Inaba	0	0	4	0	0	0	0	0	0	0	0	0

Fig 2 -Sero-prevalence of *V. cholerae*.

Table 2

Number of cases of mixed infection with *V. cholerae* 01 and other pathogens.

Pathogens	Ogawa-type N-201	Hikojima-type N-93
Shigella		
dysenteriae	2	1
boydii	1	
sonnei	2	1
Salmonella		
typhimurium	2	3
typhi	1	
Pathogenic *E.coli*		
EPEC	3	5
EIEC	2	2
ETEC	4	1
EHEC	9	2
Total cases	26 (26/201-12.9%)	15 (15/93%=16.1%)

EPEC = Enteropathogenic *E.coli*
EIEC = Enteroinvasive *E.coli*
ETEC = Enterotoxigenic *E.coli*
EHEC = Enterohemorrhagic *E.coli*

reported on day 7 showed the growth of *V. cholerae* in their stool samples (Table 3).

DISCUSSION

In Nepal, gastroenteritis is a major killer disease. Every year, 30-40 thousand people die from gastroenteritis (Bista *et al*, 1993). An outbreak of cholera was seen from June to November. In Nepal, this period covers the pre-rainy season, rainy season and post rainy season. We believe this period provided favorable conditions for the growth of *V.*

Table 3

Follow up in patients of *V. cholerae* after treatment.

After date	0	1	2	3	4	5	6	7	Total case
Ogawa type-ve			4	4	3	5	2		18
+ve	34			7	5		2	2	16
	34	0	4	11	8	5	4	2	34

Antimicrobial spectrum of follow up case (positive N=16)

Test discs	Consultation day		Post	
	R*	(%)	R*	(%)
Ampicillin	12	75.0	12	75.0
Cephalexin	3	18.8	3	18.8
Cefuroxime	3	18.8	3	18.8
Ciprofloxacin	1	6.3	1	6.3
Cloxacillin	4	25.0	4	25.0
Chloramphenicol	12	75.0	12	75.0
Co-trimoxazole	16	100.0	16	100.0
Erythromycin	15	93.8	15	93.8
Gentamicin	5	31.3	5	31.3
Lincomycin	13	81.3	13	81.3
Nalidixic acid	11	68.8	10	62.5
Norfloxacin	1	6.3	1	6.3
Ofloxacillin	2	12.5	2	12.5
Penicillin G	16	100.0	16	100.0
Polymyxin B	16	100.0	16	100.0
Streptomycin	4	25.0	4	25.0
Tetracycline	1	6.3	3	18.8

*Resistance

cholerae. In addition to this, the water sanitary system is relatively poor in Nepal which has been a contributory factor to spread of cholera in Kathmandu, the capital of Nepal. We were not able to detect any cholera cases from December to May. Therefore, in Kathmandu Hills (1,000-3,000m) and Mountains (>3,000m) cholera is prevalent but not endemic. This is different from several countries of southern Asia. We do not know what the main reason is for this, but the primary factor may be that

southern Nepal (Plain:<1,000m) belongs to the subtropical zone. It has a cycle of cold season every year, and *V. cholerae* bacilli do not survive for a long period.

A maximum number of cholera patients were seen in the month of July followed by November. This type of pattern was also seen in Solapur and Delhi, India (Fule *et al*, 1990; Sachedeva *et al*, 1995). After the outbreaks of cholera in the month of July 1994 (Isa *et al*, 1994), there has been a second outbreak of cholera in the Kathmandu valley in the same period 1 year later. The isolates belonged to *V. cholerae* 01, biotype El Tor. We could not detect any 01 *V. cholerae* despite being greatly interested in this organism since 1994 in Nepal. In the year 1994, all of the isolates of *V. cholerae* 01 belonged to biotype El Tor and serotype Ogawa. In this present study, Hikojima types of *V. cholerae* were seen in the months from July to November 1995. However, a maximum number of Hikojima serotype was seen in the month of July. Inaba serogroup of *V. cholerae* was seen only in the month of July. Of the total Ogawa isolates, a maximum number of Ogawa was seen in July followed by August (Fig 2). Considering the antigenic characterization of Ogawa and Inaba, we believe Inaba and Ogawa types have similar antigenic structure which might have contributed for such a variation. Whereas Hikojima is a medium type, which is unlikely to be shifted to another type. Hikojima type of *V. cholerae* has been the first to be isolated in such a large number from Nepal. About 29% isolates of *V. cholerae* (including both Ogawa and Hikojima type) were associated with other bacterial agents such as *Shiglla, Salmonella* and pathogenic *E. coli* (Table 2).

Sensitivity tests for *V. cholerae* 01 was done using various antibiotics such as cephalexin, cefuroxime, ciprofloxacin, cloxacillin, chloramphenicol, co-trimoxazole, erythromycin, gentamicin, lincomycin, nalidixic acid, norfloxacin, ofloxacin, penicillin G, polymyxin B, streptomycin, and tetracycline by Kirby Baur disc diffusion technique (Bauer *et al*, 1996) Tetracycline, norfloxacin and ciprofloxacin were the most effective antibiotics to treat these *V. cholerae*. Only 6.3% were found to be resistant to tetracycline whereas isolates from post treatment showed greater degree of resistance (18.8%) to this antibiotic. Ciprofloxacin, ofloxacin were found to be the next most effective antibiotics after tetracycline. Cotrimoxazole, erythromycin, polymixin B, Penicillin G did not show any inhibitory effect to *V. cholerae*, isolated from patients after pre and post treatment. Chloramphenicol, lincomycin were found to be effective in about 25% cases of *V. cholerae* and cloxacillin, and streptomycin in 75% of *V. cholerae*. More than 60% *V. cholerae* were resistant to nalidixic acid (Table 3).

REFERENCES

Albert MJ, *et al.* Large outbreak of clinical cholera due to *Vibrio cholerae* non-01 in Bangladesh. *Lancet* 1993;341:704.

Amatya S. *Vibrio cholerae* 0139 isolated in 1993 and 1994. 1st Congress of Association of Clinical Pathologists of Nepal, 1995.

Bauer AW, *et al.* Antibiotic susceptibility testing by standardized single disc method. *Am J Clin Pathol* 1966;45:493.

Bista MB, *et al.* Diarrheal diseases. An Epidemiological Review-Epidemiology Division Ministry of Health, Nepal, 1993.

Dixit H. Cholera. The Quest for Health, Nepal: Enterprise (P) Ltd, 1995;105.

Dutta K. El Tor cholera in India. *J Assoc Physicians India*, 1993; 4105;315.

Finelli L, *et al.* Outbreak of cholera associated with crab brought from an area with epidemic disease. *J Infect Dis* 1992;166:1433.

Fule RP, *et al.* Cholera epidemic in Solapur during July-August, 1998. *Indian J Med Res* 1990;90;24.

Isa AR, *et al.* Cholera outbreak in Tumpak, Kelantan-1990. *Med J Malaysia*, 1990:45:187.

Janda JM, *et al.* Current perspectives on epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clin Microbiol Rev* 1988;1:245.

Kubo T, *et al.* The isolation and identification of *Vibrio cholerae* at TUTH. *J Inst Med* 1993;15:373-9.

Ministry of Health, Department of Health Services, Nepal. Annual Report, 1996.

Ramamurthy T, *et al.* Emergence of novel strain of *Vibrio cholerae* with epidemic potential in southern and eastern India. *Lancet*, 1993;341:703

Sachdeva V, *et al.* Widespread emergence of *Vibrio cholera* 0139 in India. *Southeast Asian J Trop Med Public Health*, 1995;26:342.

Sakazaki R, *et al.* Serological studies on cholera group of

Vibrios. *Jpn J Med Sci Biol* 1970;23:13.

Shimada T, *et al.* Extended serotyping scheme for *Vibrio cholera*. *Curr Microbiol* 1994;28:175.

Shimada T, *et al.* Outbreak of *Vibrio cholerae* non 01 in India and Bangladesh. *Lancet* 1993;341:1347.

Shrestha J. Prevalence of the new epidemic strain of *Vibrio cholerae* 0139 in Nepal. 1st Congress of Association of Clinical Pathologists of Nepal, 1995.

Siddique AK, *et al.* Cholera epidemics in Bangladesh. *J Diarrhoeal Dis Res* 1992;10:79.

WHO, Epidemic diarrhoea due to *Vibrio cholerae* non-01. *WHO Weekly Epidemiol Rec*, 1993;68:141.