

## An outbreak of El Tor cholera in Kavre district, Nepal

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### Abstract

Outbreak of Cholera still remains major public health problem in most of the developing countries including Nepal. A prospective study was carried out at Dhulikhel Hospital, Kathmandu University Teaching Hospital, Kavrepalanchok during 1<sup>st</sup> May 2004 to 31<sup>st</sup> October 2004. A total of 148 stool samples from patients with acute diarrhea were collected and further investigated for Cholera. The study was conducted to establish the causes of the outbreak of acute diarrheal disease, antimicrobial profiles of the stool isolates and parasitic co-infection in Cholera cases. The samples were subjected to standard recommended microbial procedures and confirmation of the isolates was done by seroagglutination using *V.cholerae* polyvalent O1 and O139 antisera and monovalent Ogawa and Inaba antisera. Out of the 148 stool samples, 46 cases (31%) were found to be positive for *V.cholerae* serogroup O1, biotype ElTor, serotype Ogawa. Both sexes were equally affected. Young age group of less than 30 years were mostly affected. Brahmin was the most affected ethnic group. The isolates were sensitive to all the antibiotics tested except co-trimoxazole. Among the laboratory confirmed cholera cases 30% exhibited co-infection with other parasites among which *Giardia lamblia* and *Ascaris lumbricoides* were the most common.

**Key words:** Cholera, El Tor, Ogawa, Vibrio, Co-infection, Nepal

**C**holera, an acute intestinal infection caused by the bacterium *Vibrio cholerae* remains a major public health problem in many parts of Africa, Asia and Latin America. In spite of its rarity in developed countries, cholera is still an important infection worldwide.<sup>1</sup>

During the monsoon season outbreaks of cholera are encountered almost every year. El Tor *V. cholerae* has replaced their classic counterpart over the last few decades. Outbreaks due to *V. cholerae* O139 have been reported from various places in India.<sup>2</sup>

*V. cholerae* is a curved Gram-negative bacillus that belongs to the family vibronaceae and shares common characteristics with the family enterobacteriaceae. The species *V. cholerae* includes both pathogenic and nonpathogenic strains, differing in their virulence gene contents and polysaccharide surface antigens. Only *V. cholerae* O1 and O139 are responsible for the disease defined clinically and epidemiologically as cholera. *V.cholerae* O1 is divided into classical and El Tor biotypes, and into 3 sero-subtypes, Ogawa, Inba and Hikojima. *V. cholerae* O139 has characteristics in common with the El Tor biotype, but differs from O1 in its polysaccharide surface antigens.<sup>1</sup>

During summer, it has been noticed that the most common cause of epidemic of diarrhea in Kathmandu Valley is cholera. The organism is transmitted faecorally and food and water pollution are the main

perpetrating factors.<sup>3</sup> Intestinal infection with *V. cholerae* results in the loss of large volume of watery stool leading to severe and rapidly progressing dehydration and shock. Without adequate and appropriate rehydration therapy severe cholera kills about half of affected individuals.<sup>4</sup> In 2004, there was an outbreak of gastroenteritis in Kaverpalanchok district, Nepal during May to October. We undertook this study to investigate the outbreak to identify *V. cholerae* and study their antimicrobial resistance profile.

### Patients and methods

All the diarrhea cases having high frequency and loose stools with or without rice water appearance were studied from 1<sup>st</sup> May to 31<sup>st</sup> October 2004 at Dhulikhel Hospital, Kathmandu University Teaching Hospital (KUTH). The stool specimens were

collected from emergency, medical and pediatric OPD and wards of the hospital.

A total of 148 stool specimens were processed according to the standard procedures in the laboratory during the study period. The findings of the study were tabulated and statistically analyzed.

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### ***Direct examination***

All stool specimens were subjected to direct examination macroscopically and microscopically.

### **Macroscopic examination**

Color, consistency, blood, mucus and parasites were observed and noted.

### **Microscopic examination**

Normal saline and iodine preparation were prepared. Cyst and trophozoites of the protozoa and ova, larva and adult worm of helminths were observed and noted.

### **Hanging drop preparation**

Hanging drop preparation was made from all the specimens to see darting motility of *V. cholerae*.

### ***Culture***

Stool specimens were plated on MacConkey Agar (MA), Deoxycholate citrate ager (DCA) and thiosulfate citrate bile salt sucrose (TCBS) agar plates (Hi-Media, Mumbai, India) and incubated overnight at 37<sup>0</sup> C. The samples were also inoculated in alkaline peptone water (APW) and selenite F (SF) broth and incubated overnight at 37<sup>0</sup> C. Sub-cultures were made from APW on TCBS agar and from SF broth on MA and DCA.

### ***Biochemical test***

Suspected non lactose fermenting colonies which were oxidase positive and morphologically resembling *V. cholerae* were identified by a battery of biochemical tests using standard recommended procedure including catalase test, indole test, Voges proskauer (VP) test, nitrate reduction test, cholera red reaction, motility test, triple sugar iron agar (TSI) test, citrate test and methyl red (MR) test.

### ***Biotyping***

Biotyping of the *V. cholerae* isolates was done by haemolysis test on Blood agar (BA), agglutination with chicken RBC and Polymixin B sensitivity test (50unit) following standard procedure.

### ***Serological test***

Confirmation of the *V. cholerae* isolates was done by serotyping of the *isolates* by slide agglutination test using polyvalent *V. cholerae* O1 and *V. cholerae* O139 antisera and monovalent Ogawa and Inaba antisera (Denka Seiken co-Ltd, Japan).

### ***Antibiotic sensitivity test***

Antibiotic sensitivity tests of *V. cholerae* isolates were done following standard procedure using Muller Hinton agar (Hi-Media, India) by disc diffusion

method of Kirby and Bauer. The zone of inhibition was measured in millimeter and interpreted as whether the antibiotic was sensitive, partially sensitive or resistant. The antibiotics tested were Tetracycline (30mcg), Ampicillin (10mcg), Co-trimoxazole (25mcg), Ciprofloxacin (5mcg) and Doxycycline (5mcg). The discs were obtained from Hi-Media, Mumbai, India. The *E. coli* ATCC 25922 was used as the quality control strain.

### ***Results***

Out of the 148 samples, *Vibrio* was isolated from 46 samples. Of the 46 *Vibrio* isolates all of them were *V. cholerae* O1, none of the isolates were identified as *V. cholerae* O139. All of the *V. cholerae* isolates were identified as El Tor biotype, serotype O1, sub-serotype Ogawa strain. Inaba and Hikojima sero subtypes were not found in this study. Nonagglutinable (NAG) *Vibrios* were totally absent. Thus *V. cholerae* O1 El Tor Ogawa was the only isolate during this outbreak. Since there was not a single isolate of *V. cholerae* O139, this strain seems to have been completely replaced by *V. cholerae* O1 El Tor Ogawa.

Of the 46 isolates, almost all (98%) of the patients were from the villages of Kavrepalanchok district. Males and females were equally affected. The cases of cholera were most common in the age group of 11-20 years, followed by 21-30 years of age group. But no age was exempt, even 75 years old man and woman were affected.

Among the laboratory confirmed 46 *V. cholerae* cases Brahmin accounted for the highest number of cases followed by Chhetri and Tamang. The number of cases started from May 2004 and peaked in June. There was a second peak in September 2004 after declining in August.

The antibiotic sensitivity profile showed that all of the isolates were sensitive to all of the drugs tested except Co-trimoxazole, which was resistant to all of the isolates.

Out of 46 laboratory confirmed cases of cholera darting motility characteristics of *V cholerae* was positive in 45 of the cases.

Out of 148 total stool specimens processed, parasitic infection was found in 37 of them. In 3 cases multiple parasitic infestation was found. Among the protozoa *E histolytica* was the commonest and among helminths *A lumbricoides* was the commonest.

Among the laboratory confirmed cholerae cases 14 of them were also co-infected with the parasites. Among the parasites, *Giardia lamblia* was the commonest.

**Table 1** Profile of pathogens isolated from stool culture

S.No.	Pathogens	No. of isolates	Percentage	Remarks
1	<i>Vibrio cholerae</i>	46	85.1	
2	<i>Escherichia coli</i>	6	11.1	♣♣
3	<i>Salmonella</i>	1	1.9	
4	<i>Shigella</i>	1	1.9	
	Total	54	100	

♣♣: *E coli* was reported only in infants and children below 2 years.

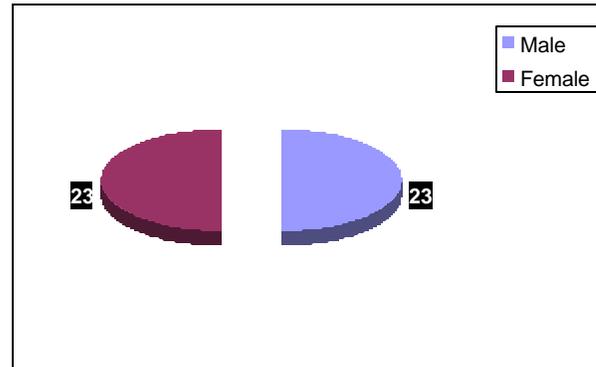
**Table 2** Sensitivity pattern of *V. cholerae* isolates (n=46)

S.no	Antibiotics	Sensitive (%)	Resistant (%)
1	Ampicillin (10mcg)	46 (100)	0 (0)
2	Chloramphenicol (30mcg)	46 (100)	0 (0)
3	Co-trimoxazole (25mcg)	0 (0)	46 (100)
4	Tetracycline (30mcg)	46 (100)	0 (0)
5	Doxycycline (30)	46 (100)	0 (0)
6	Ciprofloxacin (5mcg)	46 (100)	0 (0)

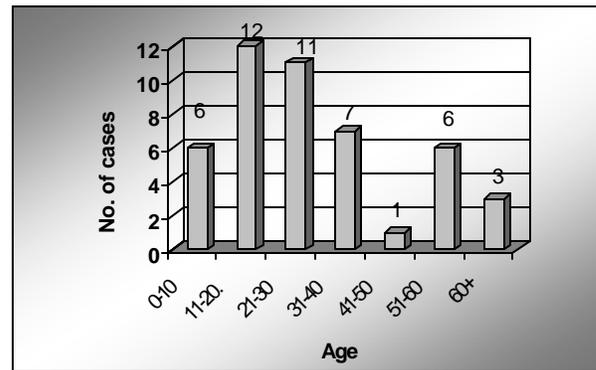
**Table 3** Parasitic infestation found in association with diarrhoea (n=37)

SN	Parasites	Cases
1	<i>E histolytica</i>	20
2	<i>G lamblia</i>	6
3	<i>A lumbricoides</i>	3
4	<i>A duodenale</i>	2
5	<i>T trichuria</i>	2
6	<i>S stercoralis</i>	1
7	Multiple parasites	3

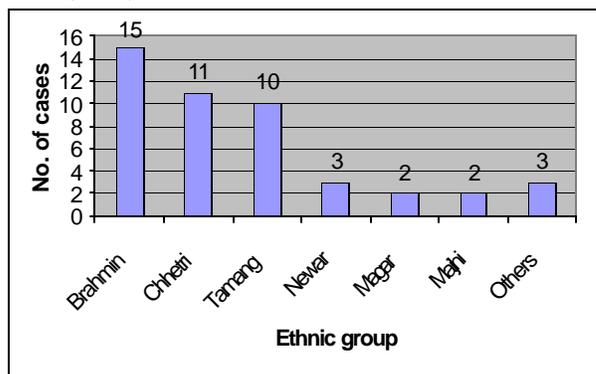
**Fig 1:** Sex wise distribution laboratory confirmed cholera cases (n=46)



**Fig 2:** Age wise distribution of laboratory confirmed cholera cases (n=37)

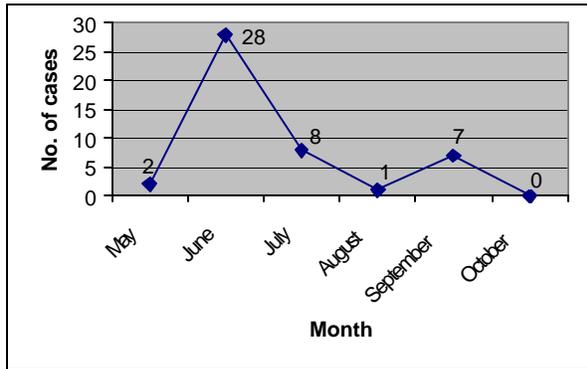


**Fig 3:** Ethnicity of laboratory confirmed cholera cases (n=46)

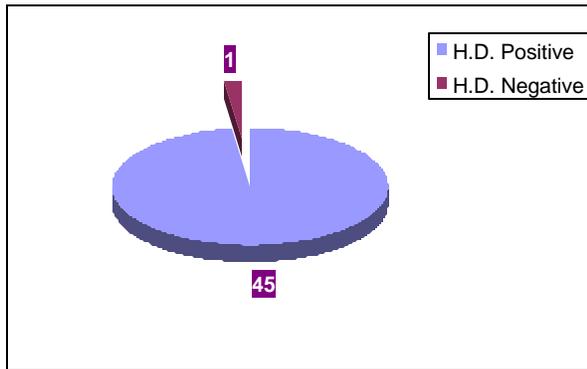


Others include Mahurati, Danuwar, and Pariyar

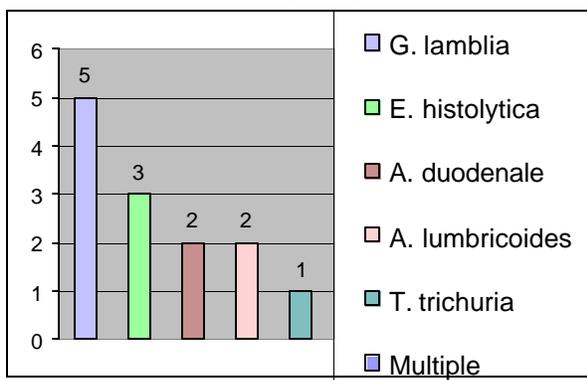
**Fig 4:** Month wise distribution of laboratory confirmed cholera cases (n=46)



**Fig 5:** Correlation of the hanging drop preparation among culture positive cases (n=46)



**Fig 6:** Parasitic infestation among laboratory confirmed cholera cases (n=14)



**Discussion**

Cholera continues to be major public health problem among many poorer and vulnerable communities, despite the fact that the bacteriology, epidemiology and public health aspects of the disease were

described in detail over a century ago.<sup>5</sup> We investigated 46 laboratory confirmed *V. cholerae* cases, which constituted 31% of 148 cases of watery diarrhoea. This is the first report on the isolation of cholera from this area.

Of the 46 *Vibrio* isolates all of them were *V. cholerae* Serogroup 01, El Tor biotype, serotype Ogawa. Serotypes Inaba and Hikojima and *V. cholerae* 0139 were not found in this study. Nonagglutinable (NAG) *Vibrios* were also absent. Males and females were equally affected. The cases of cholera were most common in the age group of 11-20 years. Among the laboratory confirmed 46 *V. cholerae* cases Brahmins accounted for the highest number of cases. The epidemic peaked in June. The antibiotic sensitivity profile showed that all of the isolates were sensitive to all of the drugs tested except Co-trimoxazole.

In Kathmandu valley the same season of the year had been affected by the cholera outbreaks. In 1991 Shrestha A.D. made similar observations in an epidemic of cholera in Kathmandu. In contrast to our finding the isolation rate of *V. cholerae* was much higher.<sup>6</sup> Where as Shrestha C et al in 1996 reported similar findings.<sup>3</sup>

In one of the recent studies in 2004 Kansakar P et al reported *V. cholerae* 01 Eltor Ogawa was responsible for cholera outbreak in Kathmandu Valley.<sup>9</sup> The findings of these studies can be implied that the same strain of *V. cholerae* has been propagated in and across the Kathmandu valley and neighbouring districts causing most of the cholera outbreaks in summer-monsoon season.

In contrast to our findings, in one of the outbreaks of cholera in and around Nagpur, India during June to October 2003 the isolates were multidrug resistant.<sup>2</sup> Similarly, Urassa et al in 2000 reported significant increase in the proportion of *V. cholerae* 01 isolates resistant to Tetracycline, Ampicillin, Nalidixic acid and to erythromycin during two cholera outbreaks in Dar es Salam, Tanzania between 1997 and 1999.<sup>10</sup> Though *V. cholerae* non 01 (i.e.0139) and NAG vibrios have been implicated in cholera outbreaks in India, Bangladesh and Iran recently.<sup>7, 8, 4, 1</sup> they have not been isolated in this outbreak.

**Conclusion**

Cholera remains an epidemic or endemic disease in much of the world including Nepal. The present outbreak of cholera in Kavre district was due to *V. cholerae* 01 El Tor Ogawa strain. Ciprofloxacin and Tetracycline should be used as the drug of choice for the treatment of cholera cases. Adequate measures to improve hygiene and sanitation and supply of safe water are needed to prevent any future epidemic of

cholera. This study reflects the importance of continued monitoring and surveillance of all cholera outbreaks.

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