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Consecutive outbreaks of *Vibrio cholerae* O139 and *V. cholerae* O1 cholera in a fishing village near Karachi, Pakistan

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Summary In July 2002 and June 2003, cholera outbreaks were detected by a diarrhoea surveillance system in a village outside Karachi, Pakistan. Specimens were culture confirmed. The first outbreak was caused by *Vibrio cholerae* O139 ($n = 30$) and the second outbreak by *V. cholerae* O1 ($n = 39$). Demographic and clinical features of patients were recorded and case—control studies were conducted following each outbreak. Clinical information was obtained for 29 of the 30 patients in the first outbreak, and 2 of the patients in the second outbreak were either out of the area or lost to follow-up, leaving 29 and 37 cases in the analysis for the first and second outbreak, respectively. Eighteen (49%) of the 37 *V. cholerae* O1 patients were under 2 years of age compared with 6 (21%) of the 29 *V. cholerae* O139 patients ($P = 0.02$). *Vibrio cholerae* O139-infected patients were more likely to be febrile (16/29) than those infected with *V. cholerae* O1 (2/37; $P < 0.001$). A household contact with cholera was a risk factor in both outbreaks; water source was a risk factor in the first outbreak only. Geographically, cases were clustered during the first outbreak but not during the second. Person-to-person contact and water reservoirs appear to be the main transmission routes for cholera in this setting.

1. Introduction

Cholera has been endemic in south Asia throughout recorded history (Sack et al., 2004). During the past 190 years, six cholera pandemics have been caused by *Vibrio cholerae*

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classical biotype. The seventh cholera pandemic was caused by El Tor biotype. In 1992, a newly described non-O1 serogroup of *V. cholerae*, designated O139 Bengal, caused unusual cholera outbreaks in India and Bangladesh (Cholera Working Group, 1993; Ramamurthy et al., 1993). Before the discovery of *V. cholerae* O139, only serogroup O1 was known to cause epidemic cholera, therefore the O139 serotype was essentially a 'new' cause of cholera (Sack et al., 2004). In 1993, isolation of *V. cholerae* serogroup O139 was reported from Karachi, Pakistan (Fisher-Hoch et al., 1993). By 2001, 144 (21%) of 689 *V. cholerae* isolates from a tertiary care hospital in Karachi were serogroup O139 (Jabeen and Hasan, 2003).

The clinical presentation of cholera due to serogroups O1 and O139 is thought to be similar (Bhattacharya et al., 1993; Cholera Working Group, 1993; Dhar et al., 1996). Symptoms are usually abrupt and include watery diarrhoea and vomiting. The most distinctive feature of cholera is the painless purging of voluminous stools resembling rice water (Sack et al., 2004). Without treatment, the case fatality rate (CFR) for severe cholera is approximately 50% (Sack et al., 2004). However, the morbidity and mortality of *V. cholerae* O139 outbreaks cannot be compared with the historical reports on the morbidity and mortality of *V. cholerae* O1 outbreaks because treatment has changed. Oral rehydration therapy (ORT) has had a major impact on mortality. Following the introduction of ORT there were only 6500 deaths among 750 000 cases of cholera in the Americas in 1991—1992 (CFR 0.9%) (Kaper et al., 1995). Little is known about the comparative epidemiology of cholera outbreaks caused by *V. cholerae* serogroups O1 and O139.

In Karachi, cholera outbreaks are known to have occurred as early as the year 1846, but no cholera outbreak has been reported in the scientific literature. Here we compare and contrast two consecutive cholera epidemics caused by *V. cholerae* serogroup O139 and serogroup O1 in a peri-urban village.

2. Methods

2.1. Study site and population

Rehri Goth, a fishing village in the Malir District of Karachi, has a population of 8343 living in 1195 households (7.0 individuals/household). There are 1195 (14%) persons less than 5 years old.

The village is relatively old and has a stable population. The mean duration of households in the village is 51 years. Most of the population speaks Sindhi; 87% are illiterate. The main occupation in the village is fishing, which is seasonal and income is extremely low when weather conditions or law restrictions prevent fishing. In 2001, the mean monthly household income was 4255 rupees (approximately US$85). Parts of Rehri Goth are marshy with brackish water. Seasonal rainfall results in flooding and increases the risk of enteric infections. Tap water is used for drinking and cooking. A reservoir collects water from the adjacent range of hills. The brackish reservoir water serves the neighbouring compounds for washing clothes and cooking utensils. The reservoir is also occasionally used by children for swimming and by herdsman to bath their cattle (Figure 1).

In 2001, the healthcare system of Rehri Goth consisted of 2 medical doctors, 11 healthcare providers without medical degrees (quacks) and 3 homeopaths. Drugs are freely available without prescription from two drug vendors. A study treatment centre was established in the village in 1997. During the cholera outbreaks, the centre was open 12 h a day, 7 days a week. Passive surveillance for diarrhoea augmented by weekly household visits was conducted

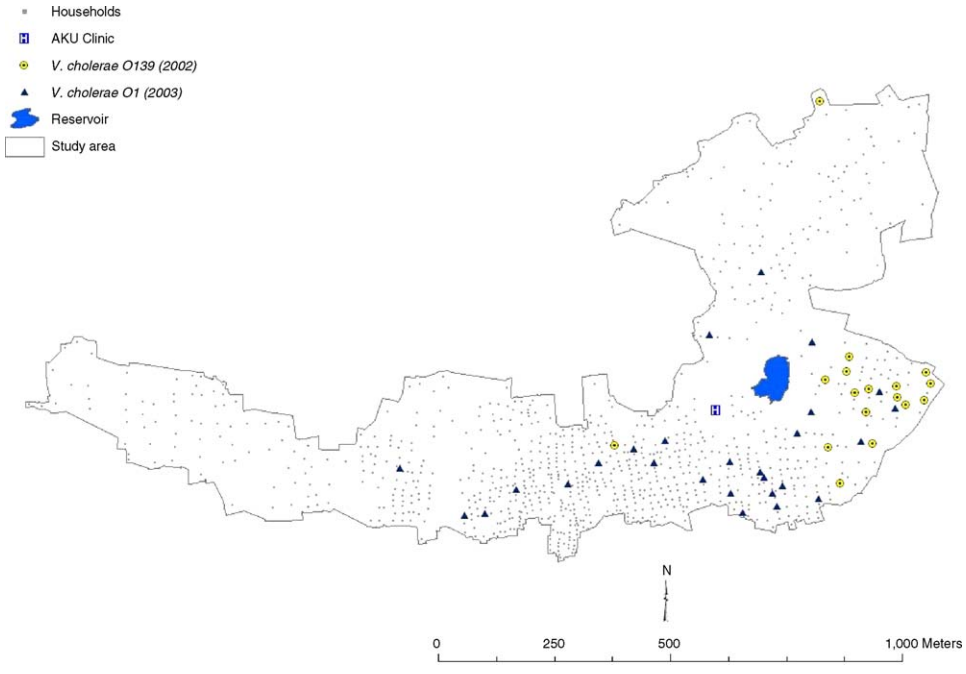

Figure 1 Spatial distribution of cholera cases, 2002—2003, in Rehri Goth, Karachi, Pakistan.

by the study treatment centre during the period of the outbreaks. The same team of healthcare providers assessed diarrhoea patients in the treatment centre in 2002 and 2003, recorded clinical findings on standardised case report forms (CRF) and collected stool specimens or rectal swabs. A weekly household surveillance system was also in place when the outbreaks occurred.

2.2. Definitions

Diarrhoea was defined as three or more loose bowel movements during a 24-h period. Culture-confirmed cholera was defined as a diarrhoeal episode in which *V. cholerae* O1 or O139 was isolated. Fever was defined as an axillary temperature greater than 37.5 °C. A suspected cholera case was defined as a case of watery diarrhoea detected during either outbreak in a resident of the study area. Diarrhoea following 3 days or more free of diarrhoea as defined above was considered a new diarrhoea episode

2.3. Case—control study

A case—control design was used to compare potential risk factors in cases and healthy controls from Rehri Goth. Cases were enrolled at the study treatment centre as follows. After informed consent was obtained, a CRF containing information regarding demographics, medical history and management plan was completed and rectal swabs or a stool specimen were obtained from consenting patients presenting with diarrhoea. Cholera cases were followed up at home by a trained community health worker who completed a questionnaire on potential risk factors, including food intake, water supply and sanitation.

Two age- and sex-matched neighbourhood controls were recruited for each case. In 2002, controls were recruited for all clinically suspected cholera cases before microbiological confirmation was available. In 2003, controls were recruited only for culture-confirmed cholera cases. Controls had to: (1) give consent for study participation (by the parent or guardian for minors); (2) live in Rehri Goth; (3) not have had diarrhoea within 4 weeks of the date of onset of the diarrhoeal illness of his or her matched case; and (4) not have previously served as a control for another case. The search for controls started in the household on the right of the case. If no eligible control resided in the household, the search continued in the next nearest house to the right until two eligible controls were found.

2.4. Laboratory methods

Two swabs or a stool specimen were obtained from each patient with diarrhoea. One swab was placed in alkaline peptone water (APW) and another was plated immediately onto XLD Agar, Salmonella Shigella (SS) Agar or MacConkey's agar and incubated at 37 °C for 18—24 h before transport. All swabs and stool samples were transported daily to the central laboratory at Aga Khan University, Karachi. The next day, the swabs inoculated in APW were subcultured on tellurite taurocholate gelatine agar. After overnight incubation, suspected colonies on the agar plates were tested biochemically and serologically with polyvalent, Ogawa and Inaba antisera (Difco Laboratories, Detroit, MI, USA). Non-agglutinating strains were tested with antiserum to *V. cholera* O139 (Denka Seiken Ltd, Tokyo, Japan).

2.5. Data management and analysis

2.5.1. Clinical data

The CRFs and risk factor questionnaires were double-entered into custom-made entry programs using FoxPro software (Microsoft, Seattle, WA, USA). The data management programs included typing and range errors as well as internal consistency check programs.

2.5.2. Analysis

All culture-confirmed cases were included in the analysis. During the first outbreak in 2002, 308 controls were approached to match 154 clinically suspected cholera cases (Figure 2). During the 2003 outbreak, 74 controls were approached for the 37 culture-confirmed cholera cases with follow-up data. *Vibrio cholerae* was isolated from 30 clinically suspected cholera cases detected in 2002. The 30 culture-confirmed cholera cases detected in 2002 and the 37 culture-confirmed cholera cases with follow-up data detected in 2003 formed the case series for this study. Clinically suspected cases that could not be confirmed by culture were not included in the analysis because a clinical cholera diagnosis is non-specific. As the controls served as a frequency-matched group to the actual cases, an unmatched analysis was conducted. Because the number of cases was limited, there was a risk of over fitting models if too many variables were included in the logistic regression models (Bagley et al., 2001). Therefore, the models were only adjusted for age, which we considered the most relevant confounder in this study. Environmental and socioeconomic variables were compared between cases and controls. Medical characteristics were compared between cholera cases detected in 2002 and cases detected in 2003. Student's *t*-test was used to compare continuous variables and the χ^2 test for categorical variables. Test statistics were interpreted in a two-tailed fashion. Statistical significance was designated at a *P*-value of <0.05. SPSS (SPSS Inc., Chicago, IL, USA) and Stata/SE 8 (Stata Corp., College Station, TX, USA) software were used for the statistical analyses.

2.5.3. Geographic data

IKONOS satellite images were enhanced using an image processing software package (ERDAS Imagine, Atlanta, GA, USA) to facilitate the digitisation of house parcels in the study area. An extensive ground survey was conducted to assign a household census ID of the house parcels that yielded household geographic information system (GIS) data of the study area (Ali et al., 2004). The household GIS data were accurate enough to pursue any point pattern analysis of the disease data. A GIS software package (ArcGIS; ESRI Inc., Redland, CA, USA) was used to map the spatial distribution of the cholera cases, and cluster analysis was performed using spatial analysis software (ClusterSeer®; TerraSeer Inc., Ann Arbor, MI, USA).

The Cuzick—Edward's k-NN (k-Nearest Neighbors) test was applied to evaluate clustering of cholera cases

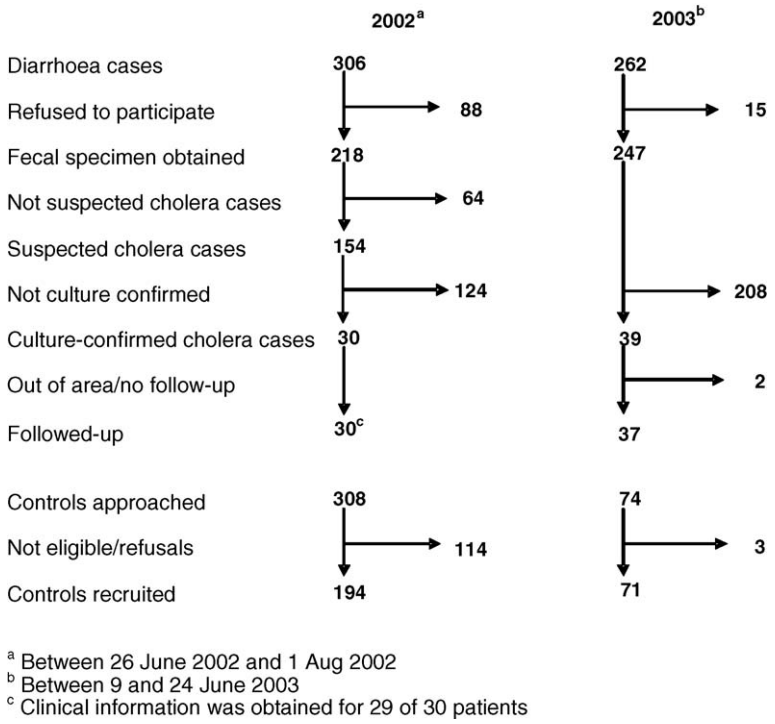

Figure 2 Case and control assembly.

(Cuzick and Edwards, 1990). The method detects global spatial clusters in individual-level case—control data using control locations to reflect the geographic variation in the population density as a whole. Models were developed for 2002 and 2003. All households with at least one cholera case were included in the model as case households and all households without reported cholera cases as control households. In the year 2002 model, 17 case households and 865 control households were analysed. In the year 2003 model, 27 case households and 885 control households were analysed (Simes, 1986).

2.5.4. Ethics

The study was approved by the Ethical Review Committee of Aga Khan University and the Secretariat Committee for Research Involving Human Subjects, WHO, Geneva, Switzerland. Consent was obtained from each participant (or the parent or guardian for children) after the purpose of the study had been explained.

3. Results

Thirty cholera cases were detected between 26 June and 1 August 2002 and all were due to *V. cholerae* O139. The following year, 39 cholera cases were detected between 9 June and 24 June 2003 and all were caused by *V. cholerae* O1 serotype Ogawa. The two outbreaks occurred in almost the same time period of the year and each lasted approximately 2 weeks (Figure 3a and 3b).

Clinical information was obtained for 29 of the 30 patients in the first outbreak, and 2 of the patients in the second outbreak were either out of the area or lost to follow-up, leaving 29 and 37 cases in the analysis for the first and second outbreak, respectively.

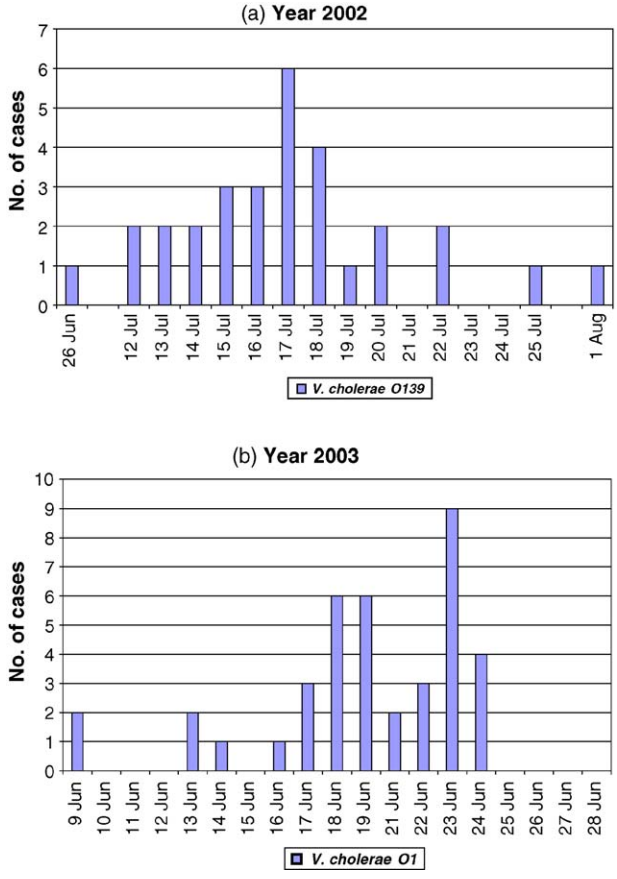

Figure 3 Epidemic curve of (a) *Vibrio cholerae* O139 cases in Rehri Goth in June—August 2002 and (b) *V. cholerae* O1 Ogawa cases in Rehri Goth in June 2003.

Table 1 Comparison of demographic and clinical characteristics of 29 *Vibrio cholerae* O139 patients in 2002[a] and 37 *V. cholerae* O1 patients in 2003 in Rehri Goth, Pakistan

Characteristic	*V. cholerae* O139 [*n* (%)]	*V. cholerae* O1 [*n* (%)]	*P*-value	OR (95% CI)[b]
Under 2 years old	6 (21)	18 (49)	0.02	–
Male	16 (55)	23 (62)	0.16	–
Sick for >2 days prior to presentation	3 (10)	10 (27)	0.09	0.5 (0.1–2.0)
Dysentery	2 (7)	2 (5)	0.80	0.8 (0.1–6.3)
Watery diarrhoea	16 (55)	31 (84)	0.01	0.2 (0.1–0.6)
Increased thirst	26 (90)	21 (57)	0.00	5.9 (1.5–23)
Dehydrated	2 (7)	5 (14)	0.38	0.5 (0.1–2.9)
Abdominal pain	2 (7)	0 (0)	0.11	–
Vomiting	5 (17)	8 (22)	0.66	1.0 (0.3–3.7)
History of fever	16 (55)	2 (5)	0.00	38 (6.9–208)
Fever (>37.5 °C) on presentation	11 (38)	1 (3)	0.00	23 (2.7–195)
Intravenous fluids required	4 (14)	9 (24)	0.28	0.4 (0.1–1.7)

[a] Case report forms were completed for 29 of 30 patients in the 2002 outbreak.
[b] Odds ratio (OR) and 95% CI adjusted for age.

There were important demographic and clinical differences between the 2002 and 2003 cholera outbreaks (Table 1). The cholera patients were between 4 months and 75 years of age. *Vibrio cholerae* O139 patients were older than *V. cholerae* O1 patients (median and interquartile range, 5 years (3–15.5 years) vs. 2 (1–5 years); $P > 0.05$). Eighteen (49%) of the 37 *V. cholerae* O1 patients were under 2 years of age compared with 6 (21%) of the 29 *V. cholerae* O139 patients ($P = 0.02$). Patients with *V. cholerae* O1 had watery diarrhoea more commonly (31/37 (84%) patients) than patients with *V. cholerae* O139 (16/29 (55%); $P = 0.01$). Eleven patients (38%) with *V. cholerae* O139 infection were febrile on presentation compared with only one person (3%) with *V. cholerae* O1 ($P < 0.001$). There was no significant difference in sex, presence of dysentery, abdominal pain or vomiting.

The factors associated with cholera due to *V. cholerae* O139 and *V. cholerae* O1 were compared (Table 2). For both pathogens, a household contact with cholera was significantly associated with disease. Attending a gathering in the week preceding the disease was associated with *V. cholerae* O139 but not with *V. cholerae* O1 illness. Similarly, the use of reservoir water was a risk factor in the infections caused by *V. cholerae* O139 but not for *V. cholerae* O1 infections. Using a safe toilet with a flush system did not confer significant protection against either pathogen.

The geographic distribution of the *V. cholerae* O139 outbreak in 2002 appeared to be contained within several compounds at the eastern end of Rehri Goth. There was a significantly higher probability that the nearest neighbour of a case of *V. cholerae* O139 was a case than the neighbour of a household free of cholera cases (Cuzick–Edward's test, $P < 0.05$). This observation was true for every order of neighbourhood (up to the 10th) tested in the model. The results of the combined tests also show global clustering of cholera cases in 2002 (normal approximation: Bonferroni *P*-value <0.001, Simes *P*-value <0.001; Monte Carlo randomisation (simulations): Bonferroni *P*-value = 0.01, Simes *P*-value = 0.003). In contrast, the *V. cholerae* O1 outbreak in 2003 was more diffuse, spreading from the east to the centre of the village (Figure 1). No statistically significant clustering of cholera cases was observed in 2003. No individuals with cholera episodes in both years were detect. One 45-year-old woman with suspected cholera died in July 2002. No deaths occurred in 2003.

4. Discussion

The unique occurrence of two cholera outbreaks in the same site provided insight into the epidemiology and clinical features of two aetiological agents, *V. cholerae* O1 and O139. Although it is tempting to generalise from a single outbreak and to extrapolate to other outbreaks caused by these organisms, this may not be legitimate. Either outbreak described here could have been an exception. Keeping this limitation in mind, it is of interest that both the clinical features and the epidemiology of the *V. cholerae* O139 outbreak differed from the *V. cholerae* O1 outbreak in several ways. *Vibrio cholerae* O139 patients were more likely to have fever, were significantly older and were less likely to have watery diarrhoea than the *V. cholerae* O1 patients. The observation that *V. cholerae* O139 disease was more frequently associated with fever on presentation was unexpected. We found just one report in the literature in which patients with *V. cholerae* O139 were more likely to have a temperature >36 °C than patients with *V. cholerae* O1 infection (Dhar et al., 1996). A possible explanation for this finding could be the invasive potential of *V. cholerae* O139. Septicaemia due to *V. cholerae* O139 has been reported and may be associated with the polysaccharide capsule of this organism (Jesudason et al., 1993; Kaper et al., 1995). The cell-mediated immune response provoked by both organisms is similar (Qadri et al., 1997). In contrast to cholera caused by *V. cholerae* O1, there may have been little or no exposure to *V. cholerae* O139 before this outbreak. This hypothesis is supported by the observation that a visitor from Bangladesh was diagnosed with cholera in Rehri Goth in June 2002 and may well have been the index case. An alternative explanation for the observed clinical differences could have been co-infection of the patients by enteric organisms that our

Table 2 Age-adjusted odds ratios (OR) with 95% CI of risk factors for *Vibrio cholerae* O139 (30 cases and 194 controls) and *V. cholerae* O1 Ogawa infections (37 cases and 71 controls) in Rehri Goth, Karachi, Pakistan, in 2002 and 2003

Characteristic	Categories	*V. cholerae* O139				*V. cholerae* O1			
		Cases (%)	Controls (%)	*P*-value	OR (95% CI)	Cases (%)	Controls (%)	*P*-value	OR (95% CI)
Household member with cholera	Yes	18 (60)	21 (11)	<0.001	1.3 (5.2–29.2)	15 (41)	11 (15)	0.005	3.8 (1.5–9.6)
	No/don't know	12 (40)	173 (89)			22 (59)	60 (85)		
Attended any gathering	Yes	8 (27)	11 (6)	<0.001	6.7 (2.4–18.9)	7 (19)	12 (17)	0.8	1.1 (0.4–3.2)
	No/don't know	22 (73)	183 (94)			30 (81)	59 (83)		
Source of water	Reservoir water	22 (73)	54 (28)	<0.001	7.1 (3.0–16.9)	2 (5)	7 (10)	0.1	0.3 (0.1–1.5)
	Any other source	8 (27)	140 (72)			35 (95)	64 (90)		
Type of toilet used	Toilet other than flush system	6 (20)	22 (11)	0.27	1.9 (0.7–5.2)	17 (46)	31 (44)	0.1	2.5 (0.8–8.4)
	Flush system	24 (80)	172 (89)			20 (54)	40 (56)		

microbiological methods would not detect, for example enteric viruses.

Not only did the clinical features of the two serogroups differ, but the risk factors associated with the organisms were also significantly different. For example, the use of reservoir water was only associated with *V. cholerae* O139. However, this finding could be due to a reduction in the use of reservoir water between August 2002 and June 2003. Reservoir water is salty and only used for washing utensils and clothes and for bathing. Washing soiled clothes of infected persons may have contributed to the contamination of the reservoir with the pathogen.

A comparison of the geographic distribution of the *V. cholerae* O139 and *V. cholerae* O1 showed clustering of cases infected by the first but not the second organism — perhaps pointing towards a single source for the first outbreak. Combined with the association of a water source as a risk factor during the first but not during the second outbreak, it seems possible that the reservoir water was the source for the first outbreak.

Our study had several limitations. First, it is possible that some cases may not have presented to the study treatment centre and may have therefore been missed by the surveillance. Thus, the cases described may only be a selection, perhaps of the more severe cases. Nevertheless, concomitant house-to-house surveillance in the whole area was also in place so we think it unlikely that a significant number of cases were missed. Second, some interviewers who completed questionnaires pertaining to risk factors for cholera were aware of the case or control status of the person being interviewed, which could have introduced bias in the assessment of risk factors. In contrast, the clinical features during both outbreaks were recorded by the same team prior to the laboratory diagnosis. It is therefore unlikely that the clinical observations could have been biased.

In Rehri Goth, cholera is transmitted within the household as evidenced by an increased risk of cholera for members of affected households. It seems likely that food contaminated within a household and use of the water reservoir are the most likely transmission routes for cholera. Public health measures, including improvement of the water supply and personal hygiene, specifically hand washing, should help to prevent future cholera outbreaks. In the absence of a safe water supply and adequate sanitation in the short term, mass vaccination should be considered for such exceptionally high-risk communities. Because infection with serogroup O139 does not produce immunity against serogroup O1, a bivalent vaccine would be required to prevent further outbreaks in this setting (Trach et al., 2002).

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

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