

## Original Article

# A Cholera Outbreak Associated with Drinking Contaminated Well Water

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## Abstract

**Background:** Cholera has been a significant public health challenge in many communities. An outbreak of acute diarrheal illness occurred among participants in a wedding ceremony in a village in Qazvin, Iran, in 2008. We conducted an epidemiological, environmental and microbiological investigation to determine the causative agent, source and extent of this outbreak.

**Methods:** Clinical and environmental samples were collected and analyzed for the presence of diarrhea-causing bacterial organisms, which included *Vibrio cholerae*. The relationship between the strains was determined using enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR).

**Results:** The attack rate was 21.8%. Clinical and environmental samples were positive for *V. cholerae* serotype Inaba. All tested isolates had a similar ERIC-PCR pattern, which indicated that a single clone of *V. cholerae* was responsible for this outbreak.

**Conclusions:** Our findings demonstrated that well water was the source of this outbreak.

**Keywords:** cholera, outbreak, well water

## Introduction

Gastroenteritis and inflammatory diarrhea, as important public health problems, are common.<sup>1</sup> Cholera is an infectious disease of the gastrointestinal tract caused by certain strains of *Vibrio cholerae*. It is one of the most frequent water-borne diseases in many countries such as Iran.<sup>2-4</sup> It is essentially a disease of poor sanitation and has been linked to consumption of food and water from unsafe sources, such as drinking or bathing in lakes, drinking river water or water from tube-wells, eating at large funeral feasts and the consumption of cold leftover foods.<sup>5</sup>

Many techniques identifying and describing relationships between bacterial isolates are available. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) is a useful technique that employs the enterobacterial repetitive intergenic consensus sequence as the target for PCR. This technique has been widely used for the typing of bacteria in epidemiological and ecological studies.<sup>6</sup>

In October 2008, an acute diarrheal illness was reported among participants in a wedding ceremony in a village in Qazvin, Iran. We conducted an epidemiological, environmental and microbiological investigation to determine the causative agent, source and extent of this outbreak.

## Materials and Methods

A standardized questionnaire was used to interview case patients about age, gender, and type of water and food consumed during the ceremony. The stool samples of all patients were collected and transferred to Iranian Reference Health Laboratories for microbiological analysis.

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logical analysis. Two water samples were also collected and processed on the same day. The samples were analyzed for the presence of diarrhea-causing bacterial organisms that included *Vibrio cholerae*. Enrichment plating methods, using alkaline peptone water (APW, pH 8.6), thiosulfate-citrate-bile-salts-sucrose (TCBS) agar, as well as taurocholate-tellurite-gelatin agar were used for the isolation of *V. cholerae*.<sup>7</sup>

The relationship between strains was determined using ERIC-PCR. Genomic DNA was extracted as described in previous reports.<sup>3,6</sup> The PCR amplifications were conducted using primers, ERIC 1R: 5'-ATG TAA GCT CCT GGG GAT TCA-3' and ERIC 2: 5'-AAG TAA GTG ACT GGG GTG AGC G-3' under the conditions described previously.<sup>6</sup>

## Results

The outbreak occurred between October 19 – 23, 2008. Twelve out of 55 participants experienced cholera disease. The attack rate was estimated at 21.8%. Most case patients reported illness onset on October 19<sup>th</sup>. The age of cases ranged from 3 to 75 years old, however the majority were 20 – 40 years, who accounted for 41% of all cases. Ten of 12 case-patients were female and 3 were hospitalized. Ten out of 12 patients presented with mild disease and the remaining 2 were severe. All severe cases were inpatients, and all but one patient with mild disease were outpatients (Table 1).

Our records showed that all patients consumed well water before the onset of the outbreak. The cultures of stool and well water samples yielded *V. cholerae*. All strains belonged to serotype Inaba. ERIC-PCR produced only a single pattern with 10 DNA band fragments with sizes ranging from 100 to 2000 bp in all human and water samples (Figure 1).

## Discussion

Clonal assay showed that a single clone of *V. cholerae* serotype Inaba was responsible for this outbreak.

Recently, some cholera outbreaks caused by serotype Inaba have been reported in many parts of Iran.<sup>2,3</sup> However more recent cholera outbreaks in our country have been due to dissemination of

**Table 1.** Clinical features and dates of onset of the cholera outbreak, Qazvin, Iran, October 2008.

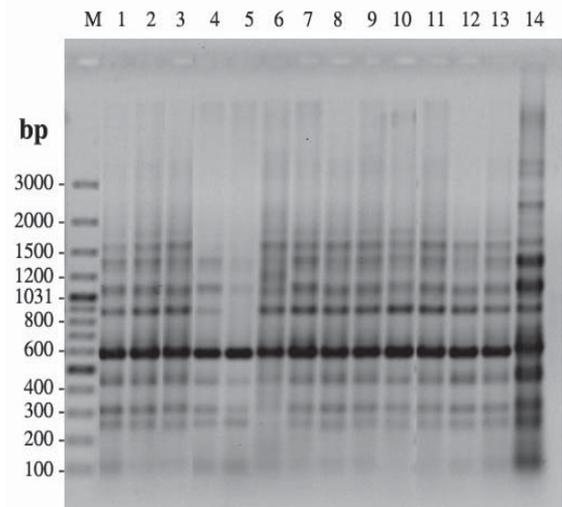
Case No.	Age (years)	Sex	Onset date (day)	Clinical condition	Treatment
1	3	Female	22	Mild	Outpatient
2	6	Male	21	Mild	Inpatient
3	13	Female	19	Mild	Outpatient
4	20	Female	19	Mild	Outpatient
5	22	Female	22	Mild	Outpatient
6	25	Male	23	Mild	Outpatient
7	26	Female	22	Mild	Outpatient
8	39	Female	19	Mild	Outpatient
9	44	Female	21	Mild	Outpatient
10	46	Female	20	Severe	Inpatient
11	75	Female	20	Mild	Outpatient
12	75	Female	19	Severe	Inpatient

other *V. cholerae* serotypes. For example, the cholera outbreak that occurred in different districts of Tehran, Iran in the summer of 1998 was attributed to *V. cholera* O1 biotype El Tor serotype Ogawa.<sup>4</sup> Pourshafie and his colleagues have recently reported that cholera outbreaks which occurred in several provinces of Iran, including Golestan, Ghom, Tehran and Zahedan in the year 2005 were caused by *V. cholerae* serotype Inaba.<sup>2</sup> In a previous investigation, we also found that most of the epidemic *V. cholerae* strains isolated in some provinces of Iran in 2005 were attributed to two close clusters of *V. cholerae* serotype Inaba.<sup>3</sup>

Cholera is known as a warm-climate disease, however our report is interesting while the outbreak described here occurred in October, in an area with cold weather. Here we found that the source of the infection was well water. Groundwater sources such as wells and springs are often believed to be of good quality with regard to bacterial pathogens transmitted by the fecal-oral route. However, such sources are readily contaminated by fecal material, especially where there are potential sources of contamination nearby or where contaminants may be carried by surface waters.

Several community-wide outbreaks resulting from contamination of public water systems with *V. cholera* have been reported in other countries. In most of these outbreaks, an epidemiological link to a contaminated community water supply has been established.<sup>8-10</sup> An outbreak of *V. cholerae* reported from Goma, Zaire was linked to poor water supply. Of approximately 500,000 – 800,000 refugees in Goma, Zaire in 1994, an estimated 50 000 died within a 21-day period due to explosive epidemics of cholera and shigellosis.<sup>8</sup> This is in accordance with Bhunia et al. (2009) who found that the cholera outbreak in West Bengal was due to contamination of a municipal piped water supply by *V. cholera* O1 Inaba.<sup>9</sup> Das et al. (2009) studied an outbreak of cholera that struck the village of Parbatia in Orissa, Eastern India in November 2003, and found that the outbreak was caused by well water that had been contaminated by an initial case-patient.<sup>10</sup>

The establishment of physical barriers to prevent contamination of the water used for recreation with drinking water sources, providing potable water and ensuring proper management of excreta to avoid contamination of other water sources are important measures to reduce cholera transmission. It is strongly recommended

**Figure 1.** Genomic typing of *V. cholerae* by ERIC-PCR fingerprinting. Lanes 1 – 12 and 13 – 14 are identical patterns of the strains isolated from clinical and well water samples. Lane M is a molecular size marker (100 bp).

to provide education for the population at risk to improve appropriate hygienic practices. Identification of local customs that place people at risk is also important in order to eliminate such practices. It is necessary to study and understand *V. cholera* ecologically and epidemiologically in order to develop a control mechanism. Water sources and sanitation need to be improved, surveillance and health care facilities have to be expanded and appropriate vaccines are necessary in order to solve the cholera problem as a major concern.

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