

Epidemic cholera in Guatemala, 1993: transmission of a newly introduced epidemic strain by street vendors*

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D. KOO¹†, A. ARAGON², V. MOSCOSO², M. GUDIEL², L. BIETTI²,
N. CARRILLO², J. CHOJO², B. GORDILLO², F. CANO³, D. N. CAMERON⁴,
J. G. WELLS⁴, N. H. BEAN⁵ AND R. V. TAUXE‡

¹ Preventive Medicine Residency Program, Epidemiology Program Office, Centers for Disease Control and Prevention (CDC), Atlanta, GA

² Ministry of Health, Guatemala

³ Instituto de Nutricion de Centroamerica y Panama, Guatemala City, Guatemala

⁴ Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA

⁵ Biostatistics and Information Management Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA

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SUMMARY

Epidemic cholera reached Guatemala in July 1991. By mid-1993, Guatemala ranked third in the hemisphere in reported cases of cholera. We conducted a case-control study with two age-, sex-, and neighbourhood-matched controls per patient in periurban Guatemala City. Twenty-six patients hospitalized for cholera and 52 controls were enrolled. Seven (47%) of 15 stool cultures obtained after admission yielded toxigenic *Vibrio cholerae* O1. All seven were resistant to furazolidone, sulfisoxazole, and streptomycin, and differed substantially by pulsed-field gel electrophoresis from the Latin American epidemic strain dominant in the hemisphere since 1991. In univariate analysis, illness was associated with consumption of left-over rice (odds ratio [OR] = 7.0, 95% confidence interval [CI] = 1.4–36), flavored ices ('helados') (OR = 3.6, CI = 1.1–12), and street-vended non-carbonated beverages (OR = 3.8, CI = 1.2–12) and food items (OR = 11.0, CI = 2.3–54). Street-vended food items remained significantly associated with illness in multivariate analysis (OR = 6.5, CI = 1.4–31). Illness was not associated with drinking municipal tap water. Maintaining water safety is important, but slowing the epidemic in Guatemala City and elsewhere may also require improvement in street vendor food handling and hygiene.

INTRODUCTION

Epidemic cholera appeared in Peru in January 1991 and spread rapidly throughout South and Central

America [1, 2]. In July 1991, *Vibrio cholerae* O1 was isolated from a man in western Guatemala, its first appearance in that country in over 100 years. Cholera cases were subsequently reported throughout the

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† During the study author was stationed with the Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA. Corresponding author's current affiliation and address: Denise Koo, MD, MPH, Chief, Systems Operations and Information Branch, Division of Surveillance and Epidemiology, Epidemiology Program Office, Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Mailstop C-08, Atlanta, GA 30333.

‡ Requests for reprints: Robert V. Tauxe, MD, MPH, Acting Chief, Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, NE, Mailstop A-38, Atlanta, GA 30333.

country, following the routes of migrating farm-workers. By the end of 1991, all 22 departments of the country had reported a total of 3664 cases of cholera to the Guatemala Ministry of Health.

During 1992, 15861 cases of cholera were reported throughout the country, an annual incidence of 163/100000 population. By 17 July 1993, 12809 cases of cholera had already been reported in Guatemala in 1993. The urban department of Guatemala, which includes Guatemala City, reported nearly 3000 cases, the largest number of any of the departments, and had the sixth highest incidence rate among the 22 departments. We conducted a case-control study to evaluate potential routes of cholera transmission in Guatemala City in order to identify points for intervention.

METHODS

Case-control study

We conducted the case-control study in zones 18 and 6, two periurban zones with similar socioeconomic and physical make-up in Guatemala City. Case-patients were selected from among persons treated at the adult cholera treatment centre (Unidad de Tratamiento de Colera, UTC) of San Juan de Dios General Hospital between 16 July and 29 July, at the height of the epidemic (Fig. 1), who resided in zones 18 or 6. Cases were defined as a diarrhoeal illness with three or more liquid stools within a 24-h period, with duration of no more than 5 days before presentation. Additional criteria included the presence of either (a) dehydration defined by the clinical staff as greater than 5% or (b) a stool culture yielding *V. cholerae* O1. Patients whose stool cultures yielded other pathogenic bacteria were excluded from the study.

Two neighbourhood controls matched for sex and age (within 2 years for patients 13–19 years old, within 5 years for patients 20–29 years, and within 10 years for those 30 years or older) were selected for each patient by going door-to-door systematically. Controls were eligible for inclusion if they had not had diarrhoea since the beginning of June. Patients and controls were interviewed about food and beverage exposures and water handling in the home during the 3-day period before the onset of illness in the patient. Persons (patients or controls) who had resided in zone 18 or 6 for less than 1 week before the onset of illness in the patient were excluded from the study.

Univariate matched odds ratios with 95% confidence limits were calculated by the Robins, Green-

land, Breslow method using Epi Info, version 5, software from the Centers for Disease Control and Prevention (CDC) [3]. All *P*-values are 2-tailed. Multivariate logistic regression analysis was performed with risk factors found to be associated with illness in univariate analysis [4].

Cultures

We obtained rectal swabs from eligible patients who were still hospitalized at the time of interview. The swabs were transported in Cary–Blair medium to the Instituto de Nutricion de Centroamerica y Panama (INCAP) for culture using thiosulphate citrate bile salts sucrose (TCBS) agar. At CDC, isolates of *Vibrio cholerae* O1 were serologically confirmed using polyvalent and monovalent O1 antisera and were biochemically identified and biotyped [5]. They were tested by polymerase chain reaction for the gene encoding for the A subunit of cholera toxin [6]. Isolates were tested for susceptibility to ampicillin, chloramphenicol, ciprofloxacin, doxycycline, erythromycin, furazolidone, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole by the disk diffusion technique as described by the National Committee for Clinical Laboratory Standards [7]. Zone size criteria standardized for the *Enterobacteriaceae* were used for the interpretation of antimicrobial susceptibilities since interpretive criteria have not been established for *V. cholerae*.

Pulsed-field gel electrophoresis (PFGE) was performed as described by Cameron and colleagues [8]. Briefly, total genomic DNA was embedded in 1% agarose plugs, lysed by sarkosyl/proteinase K treatment, and digested with restriction endonuclease *Not* I (New England Biolabs, Inc., Beverly, Mass.). The resulting fragments were separated in a 1% fast lane agarose (RMC, Rockland, Maine) gel using a CHEF-DRII system (Bio-Rad, Richmond, Calif.) with a ramp time of 5–50 sec for 20 h at 200 V.

Environmental testing

We visited case and control homes to observe the handling of drinking water in the home and to test chlorine levels in potential drinking water sources. Drinking water samples and samples from municipal water taps were tested for free chlorine using colourimetric OPD chlorine test kits (Hach, Loveland). Levels of free chlorine of ≥ 0.5 mg/l were

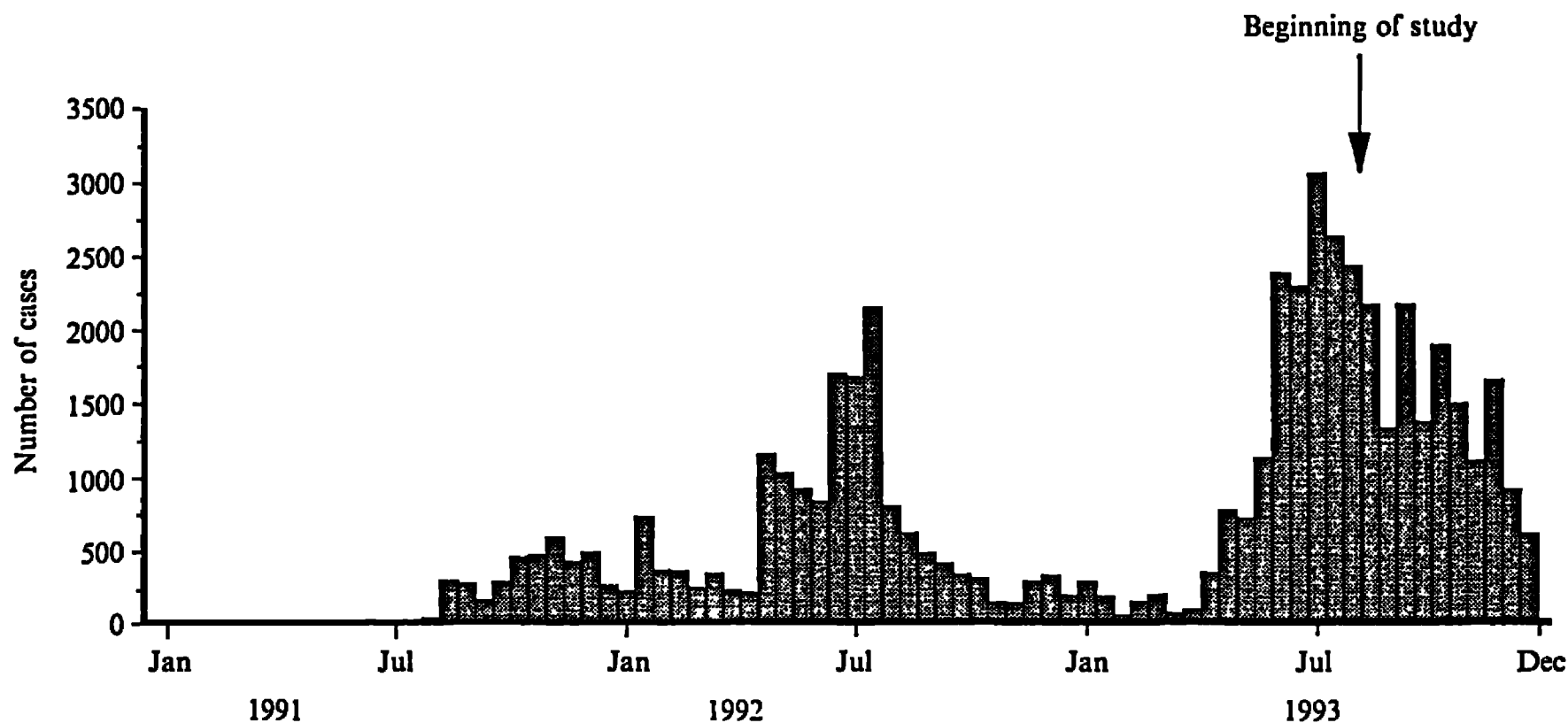


Fig. 1. Biweekly reported cases of cholera - Guatemala, 1991-3.

considered adequate for microbial decontamination. We considered drinking water to be safe if it was either boiled, adequately chlorinated, or commercially bottled.

We purchased a convenience sample of helados sold in zones 6 and 18, and in downtown Guatemala City on 2 August. These homemade frozen popsicles (flavoured ices, or ice lollies) were transported frozen to INCAP and tested for pH, faecal coliforms, and *Escherichia coli* by the Most Probable Number (MPN) technique, three tube method [9].

RESULTS

Case-control study

During the study period, 279 persons entered the UTC for treatment of suspected cholera. Their ages ranged from 13-85 years (mean 41.0); 129 (46%) were female. Of 59 persons seen at the UTC who lived in the study areas of the city, 26 were enrolled in the study. Of the remaining 33, 25 were treated and discharged before we could interview them. Six of the remaining 8 patients were interviewed at the hospital, but 2 had only 5% dehydration and were culture-negative, 1 had a culture that grew *V. cholerae* non-O1, and we could not locate the homes of the other 3. Two patients with co-existing medical illness had been referred to other wards for treatment. No eligible patients or controls refused to participate.

Twenty-six patients and 52 controls were interviewed. Patients ranged in age from 15-84 years (mean 45.2, median 43); the age of controls ranged from 13-79 years (mean 43.2 and median 43.5).

Thirteen patients (50%) were female. Patients were interviewed an average of 2.0 days (range 0-10) after the onset of their illness. Controls were interviewed an average of 2.3 days (range 0-4) after the onset of the patient's illness.

By definition, all 26 patients had diarrhoea. Of these, 25 (96%) reported vomiting, 23 (89%) cramps, 16 (62%) stomach pain, and 4 (15%) a sensation of fever. Of 25 patients who could recall, 10 (40%) were treated with an oral rehydration solution before arrival at the UTC, and 1 (4%) had received an antibiotic before arrival. Nine patients (35%) were seen and treated at the UTC on the same day their illness began, while 11 (42%) presented the following day, 3 (12%) on the second day after onset, and the remaining 3 (12%) were seen 3 days after illness onset. Seventeen (65%) patients had severe dehydration.

Patients were significantly more likely than controls to have consumed left-over rice, non-factory-produced popsicle-like flavoured ices ('helados'), non-carbonated beverages purchased on the streets, and foods bought from street vendors (Table 1). Seventy-seven percent of patients and 44% of controls consumed at least one street-vended food or beverage item. All but one person who recalled eating helados recalled purchasing them in the streets. Street-vended food items were the only risk factor that remained significantly associated with illness in a multivariate analysis with rice and helados (OR 6.5, CI 1.4-31, $P = 0.02$).

Always boiling drinking water or using only commercial, non-carbonated bottled water for drinking was slightly protective against illness (Table 1),

Table 1. *Univariate analysis of risk factors for cholera, case-control study, Guatemala City, 16–29 July, 1993*

Risk factor	Proportion exposed				Matched OR	95% CI	P-value
	Patients	Percent	Controls	Percent			
Food/drink items							
Untreated water	13/25	52	16/52	31	2.7	0.9–8.2	0.13
Left-over rice	10/26	38	8/52	15	7.0	1.4–36	0.025
Helados	11/26	42	9/51	18	3.6	1.1–12	0.036
Drinks purchased on streets	15/26	58	16/52	31	3.8	1.2–12	0.032
Foods purchased on streets	15/26	58	10/52	19	11.0	2.3–54	0.001
Any item from streets	20/26	77	23/52	44	6.3	1.4–29	0.013
Water storage/manipulation							
Bottled water	5/26	19	18/52	35	0.5	0.2–1.4	0.25
Always boil	9/26	35	22/52	42	0.8	0.3–1.9	0.69

although not significantly so. Always chlorinating or always either boiling or chlorinating drinking water was not statistically associated with protection from illness.

Culture results

V. cholerae O1 was recovered from 7 (47%) of the 15 patients who had stools cultured. All stool cultures were obtained after onset of antibiotic treatment. All 7 isolates were toxigenic, biotype El Tor, serotype Ogawa, and resistant to furazolidone, sulfisoxazole, and streptomycin. These isolates were different from the Latin American epidemic strain, which is generally susceptible to antibiotics. These isolates also had a distinct and uniform PFGE pattern, indistinguishable from that of isolates from Mexico in 1993. A similar pattern differing by only two bands was seen in isolates from Mexico in 1991; they are considered to be the same strain [10]. All of these 1993 isolates differed by at least six bands from the Latin American epidemic strain [10]. Toxigenic *V. cholerae* O1 strains isolated in Guatemala in 1991 and studied at CDC were susceptible to all antimicrobial agents tested and had PFGE, patterns indistinguishable from the Latin American epidemic strain [8].

Environmental test results

At 44 case and control homes, investigators obtained a spot sample of water directly from a municipal water supply, whether from a private or public faucet. Twenty-seven (61%) samples contained adequate levels of free chlorine, 8 (18%) had detectable chlorine below 0.5 p.p.m., and 9 (20%) contained no detectable chlorine. Free chlorine levels

in all samples ranged from 0.0–2.4 p.p.m. (median 0.6). Case and control homes did not differ significantly with regard to the presence of adequately chlorinated municipal water or the level of chlorination (medians 0.6 for both groups). There was no difference in the proportion of case and control homes with safe water (reported to be boiled, demonstrated to be adequately chlorinated, or commercially bottled). Safe drinking water was present in 24 (92%) of 26 case homes.

None of 40 'helados' sold in downtown Guatemala City and in zones 6 and 18 grew *V. cholerae*. However, 16 (40%) contained detectable faecal coliform bacteria, and 7 had detectable *Escherichia coli*. Faecal coliform counts ranged from 4/g to > 1100 MPN/g. *E. coli* counts ranged from 4 to > 1100 MPN/g. pH ranged from 2.9 to 6.9 (median 4.0); 16 (40%) of the helados samples had a pH measuring > 4.5. Twenty-four (60%) samples either contained faecal coliforms or had a pH > 4.5.

DISCUSSION

Waterborne transmission of *V. cholerae* O1 has been documented in each previous case-control study performed in Latin America during the epidemic, sometimes in combination with foodborne transmission [11–14]. Aware of the risk of drinking unchlorinated municipal water, the Guatemalan Ministry of Health made substantial efforts early in the cholera epidemic to chlorinate the municipal water supply and to educate the public regarding the importance of boiling or chlorinating their drinking water. In this study in Guatemala City, in the third year of the epidemic, illness was not associated with drinking municipal tap water, with or without

additional water treatment in the home. These results, along with the largely adequate chlorine levels measured in municipal water, indicate that efforts to improve the safety of drinking water in the city have been successful in preventing waterborne transmission of *V. cholerae* O1.

Transmission in this study occurred through food, including left-over rice, 'helados', and beverages or foods purchased from street vendors. Overlap among these categories and small numbers make it difficult to separate these foods statistically, but the results of the multivariate analysis suggest that transmission of cholera in Guatemala City is likely occurring predominantly by street vendors selling a variety of contaminated food items. Left-over rice eaten without proper reheating is a previously documented vehicle for cholera, and *V. cholerae* O1 grows rapidly in moist rice left at ambient temperatures [14, 15].

'Helados', a heterogeneous group of homemade frozen flavoured popsicle-drinks, are of particular concern. Frozen drinks have not previously been implicated in cholera transmission; they are a potential vehicle, because *V. cholerae* is not killed by freezing [16]. The finding of faecal coliforms in 40% of the helados samples suggests that unsanitary methods or unsafe water sources were used to prepare them. Because only 60% had a pH $\leq$ 4.5, the pH above which *V. cholerae* O1 survives [17], they are a plausible vehicle of transmission.

Halting the continuing spread of cholera will require further efforts to educate street vendors in proper food handling and safety measures, as well as to educate the consumer to seek safer food sources. The safety of street-vended foods and beverages may be improved if street vendors can be educated in food hygiene. Evaluation of vendor food and water handling practices may identify specific points in need of further emphasis in such courses. It would be useful to determine how the flavored ices are made and the source of water used to make them. Measuring the levels of contamination of other street-vended items may provide an index to other problematic foods. Street-vended food is a way of life in Latin America; therefore, the public should be urged to take care when selecting street-vended items, and could be encouraged to buy foods only from vendors certified as having taken a course in food hygiene.

The distinctive microbiologic characteristics of this multidrug-resistant strain signify the emergence of a new epidemic strain of *Vibrio cholerae* O1 in this hemisphere. Substantial differences in the PFGE

pattern and a unique antibiogram make it unlikely that this strain evolved from the Latin American epidemic strain. Most likely it represents a second introduction from elsewhere in the world with subsequent spread. It is unclear when and where this new strain was introduced into Latin America; similar isolates were found in Mexico, Romania, and south-east Asia as early as 1991 [8]. This study documents its presence in a second country in Latin America, where it apparently emerged as a cause of epidemic cholera in 1993. Initial isolates from Guatemala in 1991 had the same PFGE pattern as the Latin American strain and through the end of 1992, 96% of isolates tested at the Guatemalan National Laboratory were sensitive to all antibiotics tested [18].

In summary, we have documented the spread in this hemisphere of a second strain of epidemic-causing toxigenic *V. cholerae* O1, one that is distinct from the strain that was introduced and subsequently spread from Peru in 1991. The emergence of this new strain emphasizes the potential for the introduction into Latin America of *V. cholerae* O139, currently causing epidemic cholera in Asia [19, 20]. Previously implemented control measures to improve the safety of the water in Guatemala City appear to have prevented waterborne transmission there. This study illustrates the utility of rapid field investigation to indicate arenas where further interventions are necessary. *V. cholerae* is an important foodborne as well as waterborne pathogen, and successful prevention measures must be targeted at both modes of transmission. Specifically, improving the safety of the food supply in Guatemala City and elsewhere in Latin America should include education both of street vendors and of consumers who purchase street-vended foods.

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