Cholera in Piura, Peru: A Modern Urban Epidemic

Allen A. Ries, Duc J. Vugia, Luis Beingolea,
Anna Maria Palacios, Esther Vasquez, Joy G. Wells,
Noe Garcia Baca, David L. Swerdlow,
Marjorie Pollack, Nancy H. Bean, Luis Seminario,
and Robert V. Tauxe

In late January 1991, epidemic cholera appeared in Peru. Within 2 months, 7922 cases and 17 deaths occurred in Piura, a Peruvian city of 361,868. A hospital-based culture survey showed that 79%–86% of diarrhea cases were cholera. High vibriocidal antibody titers were detected in 34% of the asymptomatic population. A study of 50 case-patients and 100 matched controls demonstrated that cholera was associated with drinking unboiled water (odds ratio [OR], 3.9; 95% confidence interval [CI], 1.7–8.9), drinking beverages from street vendors (OR, 14.6; CI, 4.2–51.2), and eating food from street vendors (OR, 24.0; CI, 3.0–191). In a second study, patients were more likely than controls to consume beverages with ice (OR, 4.0; CI, 1.1–18.3). Ice was produced from municipal water. Municipal water samples revealed no or insufficient chlorination, and fecal coliform bacteria were detected in samples from 6 of 10 wells tested. With epidemic cholera spreading throughout Latin America, these findings emphasize the importance of safe municipal drinking water.

Cholera was last reported in Peru in 1867 and in South America in 1895 [1]. The continent remained cholera-free until late January 1991, when epidemic cholera emerged explosively in several large urban centers [2]. By 28 December 1991, Peru had reported 321,334 cases and 2906 deaths due to cholera. Surveillance demonstrated an abrupt increase in the number of reported diarrhea cases in several cities, including Piura, a city of 361,868 persons in northern Peru (figure 1).

After the introduction of cholera, extensive mass-media efforts urged the public to boil water, avoid raw fish and shellfish, and seek medical treatment for diarrhea. Most patients with severe diarrhea in Piura appeared at one of the five major hospitals or nine government health centers for treatment.

Studies of cholera transmission during the last two decades had incriminated a wide variety of vehicles of transmission, including raw or undercooked shellfish, contaminated ground water [3–5], moist grains held at ambient temperature [6, 7], and raw or partially dried fish [3, 8]. However, the vehicles of transmission in Peru were unknown, and initial control measures were directed at many possible vehicles simultaneously. We conducted an epidemiologic investigation to determine the magnitude of the epidemic and the major vehicles of transmission and to help prioritize prevention measures.

Methods

Surveillance records of persons presenting for treatment of diarrhea were reviewed. Age-specific population estimates for 1991 were used to calculate attack rates.

To determine the proportion of diarrhea cases attributable to cholera in Piura, rectal swabs were obtained from three groups of persons seeking medical care at the Hospital Regional Cayetano Heredia (HRCH) between 6 and 13 March 1991: adults with severe diarrhea requiring intravenous rehydration, adults with mild diarrhea treated only with oral rehydration solution in an observation area, and children age ≤5 years admitted for treatment of diarrhea. Swabs were placed in Cary-Blair transport medium and held at ambient temperature for <48 h until they were plated on thiosulfate–citrate–bile salts–sucrose agar (TCBS). Colonies typical of Vibrio cholerae on TCBS were subcultured and tested for agglutination with V. cholerae O1 polyvalent and monovalent antisera. Duplicate specimens were cultured at the clinical laboratory at HRCH and at the National Institutes of Health (Lima, Peru). Suspected V. cholerae isolates were sent to the Centers for Disease Control (CDC, Atlanta), where the isolates were serologically confirmed and tested for cholera toxin production by ELISA [9].

Selected V. cholerae O1 isolates from patients and water were biochemically identified, biotyped, and tested for hemolysin production [10]. One patient and 1 water isolate were tested for susceptibility to a routine panel of antimicrobials [11]. BglI digests of chromosomal DNA from 1 human and 1 water isolate of V. cholerae O1 were analyzed by Southern blot assay and probed for 16s and 23s rRNA genes [12].

Serum samples were tested at CDC for vibriocidal antibodies by the microtiter technique [13]. In our study, a vibriocidal antibody titer of ≥160 indicated possible recent infection with V. cholerae O1.
**Cases**

<table>
<thead>
<tr>
<th>January</th>
<th>February</th>
<th>March</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>29</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>16</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>25</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>12</td>
</tr>
</tbody>
</table>

**Figure 1.** Cases of cholera reported in Piura by day of presentation for treatment, 20 January through 13 March 1991.

*cholerae* O1; a titer of ≤40 indicated no evidence of recent infection.

A matched case-control study was done to identify risk factors for illness. A case was defined as diarrhea in a person ≥5 years old residing in Piura and admitted to HRCH for intravenous rehydration between 19 and 24 February 1991 and who was the first person in the household with diarrhea that year. Two age- and sex-matched controls who had not had diarrhea since 20 January 1992 were selected by systematic search in the case-patient’s neighborhood. Patients and controls were interviewed about potential exposures in the 3 days before onset of illness in the patient. Rectal swabs were obtained from patients, and serum specimens for vibriocidal antibody tests were obtained from controls to exclude those with possible infection. The initial matched analysis included all controls. A second matched analysis included only seronegative controls and excluded controls from whom no blood sample was obtained.

In a second case-control study, undertaken to define further the role of street vendors in transmitting cholera, a case was defined as before with the added requirement of the person having consumed food or beverages from street vendors during the 3 days before illness. Neighborhood controls who had not had diarrhea since 20 January 1992 were matched by age range and had consumed food or beverages from street vendors in the 3 days before the interview.

Street vendors selling beverages in the central market were interviewed over a 3-day period using a standardized questionnaire. The pH of the beverages was measured using 1–14 pH paper (Mikro Insta-chek 0-13; Micro Essential Laboratory, Brooklyn, NY).

Records of fecal coliform counts and chlorine tests of the municipal water supply done by the Piura Unidad Departamental de Salud Office of Environmental Health were reviewed. *V. cholerae* isolates recovered from water storage cisterns were sent to CDC for further testing. Two ice factories in the city were inspected. Water samples were tested for free and total chlorine by using a colorimetric chlorimeter (2231-01; Hach, Loveland, CO).

Robins, Greenland, and Breslow 95% confidence intervals (CI) were calculated for univariate matched odds ratios (OR) [14]. Conditional logistic regression analysis was used to identify independent risk factors in the first case-control study. All *P* values are two-tailed.

**Results**

At the time of the investigation, 7807 cases of diarrhea had been treated in Piura between 20 January and 13 March 1991, giving an overall mid-epidemic attack rate of 2.2% of the population of Piura. Cases were reported from all areas of the city. Age-specific attack rates were 0–4 years, 4.1%; 5–14 years, 1.3%; and ≥15 years, 1.8%. The overall death-to-case ratio was 0.22%. Age-specific case-fatality rates were as follows: 0–4 years, 0.04%; 5–14 years, 0.33%; and ≥15 years, 0.32%. The mean age of the 17 persons who died was 38.3 years (range, 1–90).

The hospital-based stool culture survey showed that 37 (79%) of 47 adults hospitalized with dehydration, 23 (79%) of 29 adults with mild diarrhea, and 43 (86%) of 50 children <5 years old hospitalized with diarrhea had toxigenic *V. cholerae* O1 serotype Inaba detected in their stools.

In the initial case-control study, 50 patients (age range, 10–71 years; mean, 31.3; 46% male) and 100 controls (age range, 9–82 years; mean, 31.8) were interviewed. Sera were obtained from 77 controls and tested for vibriocidal antibodies. Of the 77 controls, 26 (34%) had vibriocidal antibody
Table 1. Comparison of exposures of 50 cholera patients with 100 healthy neighborhood controls and 47 seronegative controls by matched univariate analysis.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Patients, no. (%)</th>
<th>All controls, no. (%)</th>
<th>Matched odds ratio (95% confidence interval*)</th>
<th>Seronegative controls,† no. (%)</th>
<th>Matched odds ratio (95% confidence interval*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eating rice &gt; 3 h old without reheating</td>
<td>16 (32)</td>
<td>17 (17)</td>
<td>3.1 (1.2-8.4)</td>
<td>8 (18)</td>
<td>6.2 (1.3-30.2)</td>
</tr>
<tr>
<td>Eating food from street vendor</td>
<td>13 (26)</td>
<td>3 (3)</td>
<td>24.0 (3.3-191)</td>
<td>2 (4)</td>
<td>17.5 (3.3-138)</td>
</tr>
<tr>
<td>Drinking beverage from street vendor</td>
<td>28 (56)</td>
<td>15 (15)</td>
<td>14.6 (4.2-51)</td>
<td>7 (15)</td>
<td>U (47.4-inf)</td>
</tr>
<tr>
<td>Always drink boiled water</td>
<td>28 (56)</td>
<td>83 (83)</td>
<td>0.3 (0.1-0.6)</td>
<td>43 (91)</td>
<td>0.1 (0.02-0.5)</td>
</tr>
<tr>
<td>Drank unboiled water</td>
<td>31 (62)</td>
<td>33 (33)</td>
<td>3.9 (1.7-8.9)</td>
<td>14 (30)</td>
<td>6.2 (1.8-20.8)</td>
</tr>
<tr>
<td>Put hands in drinking water storage vessel</td>
<td>22 (44)</td>
<td>26 (26)</td>
<td>2.6 (1.2-5.9)</td>
<td>10 (21)</td>
<td>4.9 (1.0-7.6)</td>
</tr>
</tbody>
</table>

NOTE. U, undefined; inf, infinity.
* Confidence intervals that exclude 1 are statistically significant.
† Controls with vibriocidal antibody titer <;:40.

Not associated with consumption of a specific beverage. However, 27 (87%) of 31 case-patients but only 27 (63%) of 43 controls consumed a beverage containing ice in the 3 days before illness began (OR, 4.0; CI, 1.1-18.3).

When the 50 patients were compared with all 100 controls, several exposures were significantly associated with illness (table 1): eating rice that was left out for >;:3 h after cooking without reheating it, eating food from a street vendor, drinking a beverage from a street vendor, drinking unboiled water, and having a household drinking water storage vessel into which someone had put their hands. Always drinking boiled water was protective. Analysis restricted to seronegative controls showed the same associations, except that hand contact with water in the household water vessel and eating cold leftover rice were no longer significant. The OR for drinking beverages from street vendors, although undefined, was extremely high, since the lower 95% confidence limit was 47.4.

Multiple linear logistic regression was done to examine the relationship between illness and the factors described above. In the analysis including all controls, drinking unboiled water, drinking a beverage from a street vendor, and putting hands in a drinking water storage vessel were independently associated with illness (P < .05). When analysis was restricted to seronegative controls, drinking unboiled water, eating food from a street vendor, and eating cold, leftover rice were independently associated with illness. Drinking a beverage from a street vendor could not be included in the latter multivariate analysis because the OR is undefined.

For the second case-control study, data were collected from 32 case-patients and 43 controls. Both groups consumed a similar number of beverages from street vendors per week (mean, 3.7 and 4.1, respectively; P = .6). Illness was not associated with consumption of a specific beverage. However, 27 (87%) of 31 case-patients but only 27 (63%) of 43 controls consumed a beverage containing ice in the 3 days before illness began (OR, 4.0; CI, 1.1-18.3).

The 31 street vendors surveyed sold beverages made by boiling grain in water, cooling it, and adding sugar. Ice was added by 89% of vendors. Vendors purchased ice from local ice factories. The mean pH for one beverage sold by 90% of vendors was 5.7 (range, 5-6. 27 samples). Only 2 vendors (6%) reported washing the glasses with soap between uses.

Water samples for chlorine testing were taken at the well head after water had flowed past the entry port for chlorine. Specimens collected on 4-5 February from 11 (69%) of Piura’s 16 municipal wells had no residual chlorine. Specimens collected on 8-10 March from 7 (64%) of 11 wells had no residual chlorine; the 5 other wells with nonfunctional chlorination apparatus were not tested. Of 17 water samples collected from home and public taps during the investigation, 15 (88%) had no residual chlorine.

Well depths were 120-140 m. Fecal coliform bacteria were detected in well-head water samples of 6 of 10 wells, with counts ranging from 1 to “too numerous to count.” Fecal coliform bacteria were also isolated from five cisterns used to store municipal water, including two cisterns located at ice factories. Toxigenic V. cholerae O1 Inaba biotype El Tor was isolated from two cisterns from municipal water located in government facilities and from a tap connected to municipal water.

The patient and water isolates of V. cholerae O1 were susceptible to all antimicrobials tested except streptomycin, to which they exhibited intermediate susceptibility. All isolates were nonhemolytic. Ribosomal hybridization patterns indi-
cated that the patient isolate was indistinguishable from the water isolate, they resembled other recent Latin American isolates, and they were distinct from the US Gulf Coast clone of *V. cholerae* O1.

Two ice factories were inspected. Ice was made from municipal water that received no further treatment at the plant. Ice was produced under poor hygienic conditions: Open ice casings were submerged in large tanks full of discolored water with grossly visible particles. Ice plant managers reported that the ice was not intended for human consumption but rather for keeping fish and seafood fresh.

**Discussion**

In late January 1991, Piura, like other cities in Peru, experienced an explosive increase in cases of diarrhea due to cholera. Between 79% and 86% of patients seeking treatment for diarrhea had cholera. Even among persons without diarrhea, 34% had serologic evidence suggesting recent *V. cholerae* O1 infection. On multivariate analysis, three risk factors were independently associated with illness: drinking unboiled water, eating food from street vendors, and drinking beverages from street vendors. Epidemiologic and bacteriologic evidence indicated that the organism was spread throughout the city principally through the municipal water system and by ice used by street vendors, as well as by foods they sold. The water and patient isolates were microbiologically indistinguishable.

The unboiled water associated with illness was municipal water collected from taps inside or outside homes and kept in water storage vessels. Piura’s water system distributed the infection throughout the city, showing that the hazards posed by untreated water in a centralized distribution system identified by Snow and others in the mid-19th century still exist. Factors probably contributing to the contamination of the water include insufficiently maintained pipes, low or intermittent pressure, clandestine connections, and water taps located below ground level, providing multiple opportunities for introduction of organisms during distribution. The high fecal coliform counts in samples at several well heads suggest that the aquifer may also have been contaminated. Without chlorine in the system, contaminated water was rapidly and efficiently disseminated. The marginal association with hand contact with water in household storage vessels suggests that in some cases, water could also have been contaminated after it was brought to homes. Persons who heeded warnings to boil their drinking water were protected.

Foods and beverages sold by street vendors were another important mode of transmission. The pH of some beverages was sufficiently high to permit the survival and growth of *V. cholerae* O1. The case-control study of people consuming street-vended beverages implicated ice as the most likely source of contamination. Other possible means of introduction include unboiled water added after the beverages were prepared, vendors’ or customers’ hands, or water used to rinse glasses. Cross-contamination could have occurred among beverage containers as well.

The ice used in the street vendors’ beverages was made from unchlorinated municipal water that had been stored in open cisterns. Although we did not culture ice for vibrios, freezing does not kill vibrios, and *V. cholerae* O1 can survive in ice for 4–5 weeks [15].

Not all risk factors identified in the initial analysis remained significant in the analysis that used only seronegative controls. This analytic restriction increased the precision by reducing bias caused by the presence of infected persons in the control group, but it also decreased the overall power of the study to detect weaker associations. As with all case-control studies, recall bias is another limitation. We minimized the effect by interviewing case-patients promptly after the onset of illness and having the same interviewer question both the patients and the controls.

These investigations identified several critical measures for controlling the cholera epidemic. Central chlorination by itself, however, provides only limited protection against further heavy contamination of water during distribution or in the home. Until the water distribution systems can be repaired, prevention depends on home disinfection of water by boiling or chlorination with household bleach.

The spread of cholera by means of ice can be prevented by using disinfected water to make ice and by keeping unsafe ice out of beverages and food products. Regulations focused on street vendors would be difficult to enforce, and banning street vendors from the city may not be realistic. However, the general public could be advised to buy beverages without ice in them or to choose beverages that have low pH (i.e., that are carbonated or tart) and foods that are cooked and hot, dry and packaged, or still in the original peel.

These findings are not unique to Piura. Other cities in Peru and throughout Latin America have decaying water and sewage systems, contaminated aquifers, intermittent water pressure, inadequate chlorination, and large informal street vendor economies. The study in Piura demonstrates that the initial recommendation to boil drinking water was followed by some of the population and had measurable protective effects. However, the role of ice was unsuspected, and the investigation showed that further prevention measures were needed. Prevention efforts can be simplified by using epidemiologic investigations to focus control measures on the vehicles of transmission. The hard lessons learned in Piura need not be relearned in other Latin American cities.

**References**


Outbreak of Cholera Associated with Crab Brought from an Area with Epidemic Disease

Lyn Finelli, David Swerdlow,* Kristen Mertz,* Halina Ragazzoni, and Kenneth Spitalny

From 31 March through 3 April 1991, 8 New Jersey residents developed severe, watery diarrhea after eating crabmeat brought back in the suitcase of a traveler to Ecuador. Stool cultures yielded toxigenic *Vibrio cholerae* O1, serotype Inaba, biotype El Tor from 4 persons, and vibriocidal antibody titers were $\geq 1:640$ in 7 persons, indicating recent infection with *Vibrio cholerae* O1. Eating crab was statistically associated with illness ($P = .006$); however, no leftover crabmeat was available for testing. All 8 patients fully recovered and no cases of secondary transmission were reported. This was the first reported incident of cholera in the continental United States associated with food transported from an area with epidemic disease. Discouraging the transport of perishable souvenir seafood may prevent further outbreaks.

The seventh cholera pandemic began in 1961 and spread to Africa, Asia, Europe, and Oceania. In January 1991, epidemic cholera was reported in Peru, the first time epidemic cholera has been reported in South America in this century [1]. The epidemic has since spread to most of South and Central America [2–4]. Every Latin American country except three (Guyana, Paraguay and Uruguay) has reported cholera in 1992. Since the onset of the epidemic, more than 590,000 cases of suspect cholera and 5000 deaths have been reported (Centers for Disease Control [CDC], unpublished data, 1992). Recognized routes of *Vibrio cholerae* O1 transmission have included consumption of contaminated water and food [5]. Consuming undercooked seafood, including crabs, has been associated with acquiring cholera in several locations [6, 7]. Here we describe an outbreak of cholera in New Jersey associated with shellfish transported to the United States from Ecuador.

**Methods**

On 5 April 1991, the infection control practitioner at a community hospital in Hudson County, New Jersey, called the New