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WATERBORNE TRANSMISSION OF EPIDEMIC CHOLERA IN *TRUJILLO*, PERU: LESSONS FOR A CONTINENT AT RISK

Contents

The epidemic of cholera that began in Peru in January, 1991, marked the first such epidemic in South America this century. Subsequently, over 533 000 cases and 4700 deaths have been reported from nineteen countries in that hemisphere. We investigated the epidemic in ***Trujillo***, the second largest city in Peru. ***Trujillo's*** water supply was unchlorinated and water contamination was common.

Suspect cholera cases were defined as persons presenting to a health facility with acute diarrhoea between Feb 1, and March 31, 1991. We studied a cohort of 150 patients who had been admitted to hospital and conducted a matched case-control study with 46 cases and 65 symptom-free and serologically uninfected controls; we also carried out a water quality study.

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By March 31, 1991, 16 400 cases of suspected cholera (attack rate 2.6%), 6673 hospital admissions, and 71 deaths (case-fatality rate 0.4%) had been reported in the province of ***Trujillo***. 79% of stool cultures of patients with diarrhoea presenting to a single hospital yielded Vibrio cholerae O1. In the case-control study, drinking unboiled water (odds ratio [OR] 3.1, 95% confidence interval [CI] 1.3-7.3), drinking water from a household water storage container in which hands had been introduced into the water (4.2, 1.2-14.9), and going to a fiesta (social event) (3.6, 1.1-11.1) were associated with illness. The water quality study showed progressive contamination during distribution and storage in the home: faecal coliform counts were highest in water from household storage containers and lowest in city well water. V cholerae O1, biotype El Tor, serotype Inaba, was isolated from three city water samples.

Cholera control measures in ***Trujillo*** should focus on treatment of water and prevention of contamination during distribution and in the home. ***Trujillo's*** water and sanitation problems are common in South America; similar control measures are needed throughout the continent to prevent spread of epidemic cholera.

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Introduction

Toxigenic Vibrio cholerae O1, the aetiological agent of cholera, causes severe watery diarrhoea, vomiting, dehydration, and sometimes death. Although the seventh cholera pandemic began in 1961 and spread rapidly through Asia, Africa, Oceania, and Europe, epidemic cholera had not been reported from South America this century. In late January, 1991, toxigenic V cholerae O1, biotype El Tor, serotype Inaba, appeared almost simultaneously in several coastal Peruvian cities.(n1,n2) over 533 000 cases and 4700 deaths have now been reported from nineteen countries in South America. The epidemic in Peru continued in many coastal cities and extended into rural areas along the coast and highlands. By April, 1992, Peru had reported over 426 000 probable cholera cases and over 3300 deaths to the Pan American Health organization; the epidemic had spread into all but three Latin American countries; and 101 associated cases have been reported in the United States (ref 3, and Centers for Disease Control [CDC] unpublished data).

In early February, 1991, the Peruvian Ministry of Health advised that all drinking water should be boiled and that fruits and vegetables should be washed with boiled water. They also recommended the avoidance of raw seafood, including ceviche made with raw fish, one of the country's most popular foods. Treatment guidelines and materials were widely disseminated and nationwide surveillance was established.

Background

In early February, 1991, cases of severe watery diarrhoea were reported in ***Trujillo*** (population 322 944), the second largest city in Peru (fig 1); within 2 weeks, several hundred patients presented daily to health facilities. Cases were initially concentrated in poorer neighbourhoods and the epidemic spread from inner city areas to peripheral and rural regions. In mid-March, epidemic activity was especially severe in a newly affected city neighbourhood, Victor Larco (population 31 930), resulting in 20-30 patient visits a day to Belen Hospital, the largest hospital in ***Trujillo***.

The city of ***Trujillo*** is supplied with water from sixty-one wells throughout the city. Although the water distribution system is interconnected, neighbourhoods are usually supplied with water from one or two wells. There was no routine chlorination of water and ample opportunity existed for contamination of municipal water. In many neighbourhoods, illegal connections to major water lines break the integrity of the distribution system. Low and intermittent water pressure facilitates back-siphonage of contaminants into the system. Because running water is available for only 1-2 h a day in many neighbourhoods, most families store water in household containers.

The National University of ***Trujillo*** and the Municipal Water Services, ***Trujillo***, documented bacteriological contamination of water in a study conducted in February, 1991. of fifty water samples from the distribution system, 60% contained coliform bacteria, 14% had faecal coliforms with counts of up to 1800 faecal coliforms/ 100 ml, and three samples (6%) yielded V cholerae O1. of fifty-two samples from city wells, coliforms were detected in 19% and faecal coliforms in 13% (unpublished data). In some parts of the city, untreated sewage obtained through clandestine diversion from sewer lines is used to irrigate crops including cabbage, lettuce, carrots, and watermelon.

Materials and methods

Surveillance

For surveillance, suspect cholera was defined as acute diarrhoea in a person presenting for treatment to a health facility in the province of Trujillo between Feb 1 and March 31. All hospitals and health centres participated in reporting.

Hospital cohort

To determine clinical characteristics of suspect cases at time of presentation, all residents of Victor Larco

presenting to Belen Hospital with diarrhoea between March 9 and 16, 1991, were interviewed on arrival at hospital. For infants and children, parents were questioned. Symptoms and prehospital treatment were characterised. Rectal swabs were obtained and placed in Cary-Blair transport medium for culture at Belen Hospital.

Case-control study

A matched case-control study was conducted between March 9 and 16 to determine modes of transmission. Cases were persons from the hospital cohort described above, selected because they had severe dehydrating diarrhoea. only patients who had the first recognised case of cholera in their household or who became ill within 24 h of the initial household case were eligible. Within 24 h of arrival at hospital, case-patients were interviewed by a study investigator by use of a standard questionnaire which referred to exposures in the 3 days before onset of illness. Questions concerned sources of water for drinking, ice, and beverage making, water storage and boiling practices, and the preparation, storage, and eating of specific foods including seafood, pasta, rice,.fruits, and vegetables. Questionnaire format included "Yes", "No", and "Don't know" responses; "Don't know" responses were excluded.

For each case-patient, we selected 2 neighbourhood controls, matched for sex and age, from households without diarrhoea since Feb 1, by going door to door systematically from the matched case-patient's home. Within 72 h of the case interview, matched controls were interviewed by use of the standardised questionnaire about food and drink exposures during the 3-day interval before onset of illness in the patient. A single rectal swab and a 10 ml blood sample were requested from each control. Rectal swabs were transported in Cary-Blair medium. Controls whose rectal swabs yielded V cholerae O1, those who had vibriocidal antibody titres > 40, and those from whom no blood or stool specimens were collected were excluded.

Water quality study

To determine whether water quality worsened during distribution and storage, we measured indicators of water contamination in a section of Victor Larco neighbourhood. First, water was sampled from the two municipal wells that supplied water to this neighbourhood. Then, in randomly selected households, paired samples were taken from water taps and from household drinking water storage containers. Total coliform and faecal coliform counts were determined with membrane filtration(n4) and the "most probable number" method.(n4) At the same sites, 1-litre water samples were cultured for V cholerae O1 with membrane filtration, and 10-litre water samples were tested with the Spira jar technique.(n5)

Laboratory investigation

Rectal swabs from the hospital cohort were examined at Belen Hospital with thiosulphate-citrate/bile-salts-sucrose (TCBS) agar. Rectal swabs from controls in the case-control study were transported in Cary-Blair medium to CDC and placed in alkaline peptone broth for 6 h at 37 Celsius for enrichment before plating onto TCBS agar. In both laboratories, colonies typical of V cholerae O1 on TCBS were subcultured and tested for agglutination with V cholerae O1 polyvalent and monovalent antisera.

All suspect V cholerae O1 isolates from patients in ***Trujillo*** and three strains isolated from water by the National University of ***Trujillo*** were transported to CDC for serological confirmation. Representative V cholerae O1 isolates were biochemically identified, biotyped, and tested for haemolysin production,(n6) cholera toxin production by enzyme-linked immunosorbent assay (ELISA),(n7) and antimicrobial susceptiblity by the modified Kirby Bauer technique.(n8) Bg/I digests of chromosomal DNA from representative human and water isolates of V cholerae O1 were analysed by Southern blot assay and probed for 16s and 23s rRNA genes.(n2) Serum samples from controls in the case control study were tested for vibriocidal antibody levels by the microtitre technique.(n9)

Statistical analysis

We used CHI^2 analysis with the Mantel-Haenszel correction to compare proportions between groups; Robins, Greenland, and Breslow 95% confidence intervals (CI) on odds ratios (OR) for matched samples;

(n10) conditional logistic regression to identify independent risk factors in the case-control study; and the t test for dependent samples to compare the geometric means of water samples. All reported p values are two-tailed.

Results

Surveillance

In the province of ***Trujillo*** (population 626 456), during February and March, 1991, there were 16400 cases of suspected cholera (attack rate 2.6%), 6623 hospital admissions, and 71 deaths (case-fatality rate 0.4%) (fig 2). Attack rates were similar in all age groups during the month of February: 0.5% for children less than 1 year old, 0.5% for children aged 1-4 years, and 0.6% for older children and adults.

Hospital cohort study

150 consecutive patients were interviewed. Age range was 2 months to 86 years (mean 32 years); 73 (49%) were male. The interval from onset of symptoms to hospital arrival varied from < 1 to 109 h (median 12 h). Before arriving at the hospital, 84 patients (56%) were treated with oral rehydration salts and 20 (13%) with homemade rehydration solutions.(n13) patients (9%) had taken an antimicrobial drug before arriving at the hospital. V cholerae O1, serotype Inaba, was isolated from 98 (79%) (95% CI 72-86%) of 124 patients from whom stool samples were obtained and who had not taken an antimicrobial drug before admission. 83 patients (55%) were admitted to an observation ward for treatment with oral and/or intravenous rehydration, 54 (36%) were admitted to hospital for at least 24 h, and 12 (8%) were treated with oral rehydration as outpatients. There were no deaths.

Case-control study

50 case-patients from the hospital cohort with dehydrating diarrhoea and 99 controls were interviewed. Analysis was restricted to controls without evidence of V cholerae O1, infection. 34 of 99 controls were excluded: 26 whose vibriocidal antibody titres were > 40 (29% of those tested), 2 others whose rectal swab cultures yielded V cholerae O1, and 6 from whom sera and rectal swab specimens were not collected. Exclusion of controls left 4 case-patients (3 females and 1 male, age range 14-35 years) with no matched controls; these case-patients were excluded from further analysis.

Case-patients ranged in age from 2 to 78 years (mean 35 years); 25 (54%) were male. 38 (83%) were admitted to hospital and 8 were treated in the observation ward for less than 24 h and then released. Symptoms included liquid diarrhoea (100%), vomiting (94%), and muscle cramps (67%). 43 (93%) had the first recognised case in their household, and 3 (7%) became ill within 24 h of the initial case in their household.

Case-patients were significantly more likely than controls to have drunk unboiled water: 26 (58%) of 45 case-patients drank unboiled water within 3 days of becoming ill vs 18 (28%) of 65 controls (OR for matched samples 3.1, CI 1.3-3.7) (table I). Case-patients were more likely than controls to have drunk water from a household water storage container in which hands had been introduced into the water: 13 (32%) of 41 case-patients vs 5 (9%) of 58 controls (OR 4.2, CI 1.2-14.9). Case-patients were also more likely to have attended a fiesta: 14 (30%) of 46 case-patients vs 10 (15%) of 65 controls (OR 3.6, CI 1.1-11.1). Case-patients tended to eat cabbage more than controls (OR 2.7, CI 0.97-7.6) and fewer case-patients ate bananas (OR 0.4, CI 0.2-1.04) but neither of these associations reached statistical significance (CI overlapped unity). only 1 case-patient and 1 control reported eating raw seafood or ceviche made with raw seafood. No other fruits, vegetables (including lettuce and carrots), fish, pasta, beverages, or other food or drink exposures were significantly associated with illness.

We constructed a conditional logistic regression model that included the three significant risk factors from the univariate analysis. When all three factors were included in the model, drinking unboiled water and drinking water from a household storage container in which hands had been introduced into the water were not independency associated with illness. However, each of these factors was independently significant ($p < 0.05$) when placed separately in a model with attending a fiesta.

Water quality study

Between March 25 and April 29, we collected nine samples from the wells that supplied water to Victor Larco and twenty-one paired samples from water taps and household storage containers in randomly selected homes within the neighbourhood. Total and faecal coliform counts were lowest in water from the wells that supplied this neighbourhood, were higher in tap water, and were highest in water from the household water containers (table II). Geometric means of coliforms and faecal coliforms per 100 ml were higher in household water containers than in tap water in both water quality tests; in three of the four comparisons, the difference was statistically significant ($p < 0.05$; t-test for dependent samples). V cholerae O1 was not isolated during the water quality study.

Laboratory results

Isolates were identified as toxigenic V cholerae O1, biotype El Tor, serotype Inaba. Strains were susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, co-trimoxazole, sulfisoxazole, tetracycline, and trimethoprim and exhibited intermediate susceptibility to streptomycin. All isolates were non-haemolytic. Ribosomal hybridisation patterns indicated that the patient isolates were indistinguishable from the strains of V cholerae O1 isolated from water by the National University of ***Trujillo*** in February, 1991, but were distinct from the US Gulf Coast clone of V cholerae O1.

Discussion

The Latin American cholera epidemic, perhaps the largest this century, has spread through most of South and Central America. In the province of ***Trujillo***, over 16 000 cases of suspected cholera (attack rate 2.6%) were reported during the first 2 months of the outbreak. Although it has been suggested that the high attack rate in Peru might have been due to over-reporting of cases, most suspected cholera cases were probably true V cholerae O1 infections because this organism was recovered from 79% of the cohort of patients presenting to Belen Hospital with diarrhoea. Inapparent infections were also numerous: 29% of controls from the case-control study, who denied diarrhoea in themselves or their families, had raised vibriocidal antibody titres which suggested recent infection. High attack rates accord with community-wide exposure in a population with no prior immunity.(n11) The extraordinarily low case-fatality rate in ***Trujillo*** reflects the availability of health care and appropriate health education of the population. over half the patients in the hospital cohort had received oral rehydration solutions before arrival at hospital, some before seeing any health professional.

The source of the epidemic remains unknown, but it is not related to the previously recognised endemic focus of cholera of the US Gulf Coast.(n12) V cholerae O1 isolates from the epidemic in Peru are non-haemolytic and resemble most seventh pandemic isolates, not Gulf Coast isolates;(n2) they also differ from the Gulf Coast strain by ribosomal gene analysis.

Several factors were associated with cholera transmission. Drinking unboiled water accounted for the largest proportion of cases. The epidemic strain of V cholerae O1 from Peru is inactivated in water after one minute of boiling,(n13) so persons who drank boiled water were protected. Faecal contamination of municipal water was common and the epidemic strain of V cholerae O1 was isolated from city water. The ribosomal hybridisation patterns of the water isolates and the patient isolates were indistinguishable. "Clandestine connections" that broke the integrity of water lines, low water pressure, frequent cut-offs, and lack of chlorination contributed to widespread water contamination within the distribution system. Municipal water had not been chlorinated because of cost, lack of chlorinators and chlorine, and the belief that deep well water would not require disinfection.

Drinking water also became contaminated during storage. The practice of storing drinking water in the home was universal in poorer neighbourhoods. The water quality study showed progressive contamination of water during distribution and storage, with the highest coliform counts in household storage containers and the lowest in city well water. In the case-control study, drinking water into which others had introduced their hands was strongly associated with illness. Hands introduced into water storage containers during washing or scooping of water may have been the means by which stored water

became contaminated with V cholerae O1 and coliforms. In a study in Calcutta, contamination of water in household storage containers was associated with transmission of V cholerae O1,(n14) and in an intervention study, in which narrow-necked water containers were used in households, a reduction in cholera attack rates was shown.(n15) The narrow opening prevented hands from entering the water container and contaminating the contents. Drinking unboiled water and drinking water from household water storage containers in which hands had been introduced into the water were correlated in the multivariate analysis. It is possible that persons who drink unboiled water (and thus have lower hygiene standards) may also be more likely to dip their hands into the water of household storage containers.

Going to a fiesta was likewise associated with illness. Foods and beverages at social events may be more likely than home-prepared foods to have come from many sources, be prepared by multiple food-handlers, and remain unrefrigerated for lengthy periods. El Tor V cholerae O1 grows rapidly in many cooked foods, including rice and seafoods.(n16,n17)

Eating cabbage tended to be associated with illness and persons who ate bananas were less likely to become ill. Many farmers in this region use untreated sewage to irrigate crops. V cholerae O1 can remain viable on vegetables for 1-2 days at room temperature (longer if cooler or moist),(n18) and transmission of V cholerae O1 by contaminated crops was suspected in another outbreak.(n19) In ***Trujillo***, cabbage was typically eaten raw or cooked briefly, perhaps allowing V cholerae O1 organisms to survive. We do not know why other crops eaten raw and grown with sewage such as lettuce were not significantly associated with illness. Bananas, grown high above the ground and peeled before eating, may be less likely to be contaminated than ground crops and may be safer to eat.

This study was conducted after the population had been told to avoid raw seafood, and only 1 case-patient and 1 control reported eating raw seafood. The risk associated with these foods during the early stages of the epidemic while they were still consumed could not be determined. The scarcity of persons eating raw seafood, a major food source before the epidemic, exemplifies the effectiveness of public health education in Peru. In the USA and elsewhere, undercooked seafood (crabs and oysters) has been an important source of transmission of V cholerae O1,(n12,n20) and crab transported from Ecuador in travellers' suitcases lately caused two outbreaks of cholera in the USA. (n21,n22)

Street vendors were banned from ***Trujillo*** in January, 1991, after an outbreak of typhoid fever. As a result, consumption of beverages or foods purchased from street vendors, a suspected source of cholera in some settings,(n23) could not be evaluated in ***Trujillo***.

We used positive stool cultures and raised vibriocidal antibody titres to define recent infection with V cholerae O1. Persons infected with V cholerae O1 mount a vibriocidal antibody response 1-2 weeks after exposure and the antibody level remains raised for 1-2 months.(n9,n24,n25)

We controlled for neighbourhood in the case-control study (in addition to age and sex) to try to eliminate the confounding effects of socioeconomic factors. Since cases in ***Trujillo*** clustered in poorer neighbourhoods, without neighbourhood matching, controls may have been wealthier than their cases and any factor associated with being poor (eg, not owning a stove, eating certain foods, eating from street vendors) may have been incorrectly found to be associated with illness. Conversely, some of these factors may actually have caused illness, but all persons in the neighbourhood were exposed and therefore these factors would not have been identified in the study.

The epidemic in ***Trujillo*** was largely associated with drinking contaminated water from the municipal water system. Long-term control measures to decrease transmission of cholera should focus on providing safe water. Eliminating cross-connections, providing continuous supplies of water at high pressure, and improving the integrity of the sewage collection system will decrease contamination, but these measures will take many years to implement. Central "in line" chlorination at the wells by use of simple and inexpensive drip chlorinators is appropriate. However, chlorination at the wells alone may not provide adequate residual chlorine concentration in peripheral locations because of progressive heavy contamination during water distribution. Meanwhile, boiling or disinfection of water in the home is a practical short-term measure to ensure adequate disinfection. The use of narrow-necked storage vessels and covered storage containers, and instruction of household members about keeping their hands out of water containers may decrease water contamination during storage. Until long-term projects to ensure

adequate treatment of sewage can be implemented, proper washing and cooking of fruits and vegetables are suitable control measures. The safety of raw seafood and ceviche remains to be documented.

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TABLE I--UNIVARIATE ANALYSIS OF RISK FACTORS FOR CHOLERA AMONG PATIENTS AND CONTROLS, TRUJILLO, PERU, 1991

Risk factor	Proportion exposed: Patients (%)	Controls (%)	Matched OR
Drank unboiled water	26/45 (58)	18/65 (28)	3.1
Drank water from container in which hands had been introduced into water	13/41 (32)	05/58 (9)	4.2
Went to a fiesta	14/46 (30)	10/65 (15)	3.6
Ate cabbage	14/45 (31)	11/65 (17)	2.7
Ate bananas	24/46 (52)	46/65 (71)	0.4
Ate raw seafood	01/46 (2)	01/65 (2)	1.0

Risk factor	95% CI
Drank unboiled water	1.3-7.3
Drank water from container in which hands had been introduced into water	1.2-14.9
Went to a fiesta	1.1-1.11
Ate cabbage	0.97-7.6
Ate bananas	0.2-1.04
Ate raw seafood	0.06-16.0

TABLE II--RESULTS OF WATER SAMPLING IN TRUJILLO, PERU

Method	Test and source	No of samples	No of coliforms/100 ml (geometric mean, range)	p(*)
Most probable number	Total coliform			
	Municipal well	9	1, 0	
	Tap water	20	6, 0-1100	
	Water container	20	794, 20-1100	<0.05
	Faecal coliform			
	Municipal well	9	1, 0	
	Tap water	20	2, 0-240	
	Water container	20	20, 0-1100	<0.05
Membrane filtration	Total coliform			
	Municipal well	9	1.2, 0-3	
	Tap water	21	9, 0-1700	
	Water container	21	118, 5-1800	<0.05

Faecal coliform			
Municipal well	9	1.1, 0.1	
Tap water	21	4, 0-75	
Water container	21	8, 0-150	NS

(*) Geometric means of water from water container vs tap water,
t-test for dependent samples.
NS = not significant.

MAP: Fig 1--Map of Peru with location of ***Trujillo*** indicated.

GRAPH: Fig 2--Cases of suspected cholera by data of presentation to health facilities, province of ***Trujillo***, Peru, February-March, 1991.

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