Cholera in the Americas: Foodborne Aspects

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ABSTRACT

Over 100 serotypes of Vibrio cholerae exist, but generally the toxigenic strains of the serogroup O1 cause cholera and possess documented epidemic potential. The main symptom of cholera is a profuse diarrhea resulting in dehydration, that if untreated, leads to death. Seven pandemics of this contagious disease have been recorded during the last 200 years. A sick person secrets in his stool billions of organisms daily, and water and food contaminated with such a stool are the primary sources of infection during the epidemics. With the increase of the international food trade, food is often shipped from countries with endemic or epidemic cholera. With one exception, no documented cases of cholera have been reported, as a result of the internationally regulated food trade. However, during the present Latin American epidemic, inadequately cooked seafood has been implicated as a source of cholera. As a result of the epidemic, over 100 cases of cholera have occurred in the United States related to seafood consumed during a visit to Latin America or after its noncommercial transport into the country. Furthermore, V. cholerae persists as a free-living organism in environmental reservoirs in Australia and the U.S. Gulf Coast; there have been 65 domestically acquired cases of cholera in the United States since 1973. Molecular typing methods have enabled us to identify and characterize endemic and epidemic strains, and to document transmission of cholera when food was implicated epidemiologically as a vehicle of transmission.

Cholera an acute dehydrating diarrheal disease caused by toxigenic Vibrio cholerae O1. Severe cases of cholera are characterized by profuse diarrhea and vomiting, muscular cramps, and collapse; the fluid loss is so dramatic that a healthy person can die within hours. Cholera is also a very contagious disease, and large epidemics with high mortality have been reported throughout historic time. Although the honor of discovering the Vibrio bacillus went to Robert Koch in 1883, long before then it was known that the disease was transmissible, and hygienic implications like quarantine were used to prevent its spread. As early as 1855, John Snow’s epidemiologic surveillance study documented waterborne transmission of cholera in London. In this review we present epidemiologic and laboratory data implicating food as a defined vehicle of cholera transmission, emphasizing case-control studies conducted during the last two decades.

Historical background of cholera in the Americas

Cholera reached the Americas for the first time in recorded history during the spread of the second pandemic (1829-1850); it arrived by ships in the port of Quebec in 1832, despite a quarantine in the area (76). It spread from the city of Quebec into the interior of the country. At the same time, cholera appeared in New York, and in 1834, it crossed the Rocky Mountains and spread west to the Pacific Coast. Mexico, Cuba, Guatemala, Nicaragua, Panama, Colombia, and Jamaica were also affected. During the third (1852-1860) and fourth (1863-1875) pandemics, cholera appeared in 29 countries in North and South Americas. During and after the Civil War, its spread within the United States was thought to be enhanced by troop movements, and in 1873, cholera cases were reported in 19 states (108). Serious outbreaks were reported in New York in 1886. Cholera spread to the Midwest by rail and by ships to Texas, Louisiana, and other southern states. During the fifth pandemic (1881-1896), cholera once again reached New York. In 1887 and 1888, V. cholerae was isolated from immigrants at the Marine Hospital, Staten Island, NY, by Dr. Kinyoun, who had previously studied cholera at the Pasteur Institute with Robert Koch in 1881 (101). This time it was successfully controlled, and it did not spread. Again in 1892, 10 cases of cholera occurred in immigrants arriving by ships in New York, but no spread was reported. Toward the end of the century, cholera began to recede from the Americas, and the last cases of cholera were recorded in Brazil, Uruguay, and Argentina in 1895.

The present seventh pandemic

Bengal, the principal focus of cholera, has not been free from the disease in recent historic times, and the first six cholera pandemics are thought to have originated in that area (34). However, in 1937, a new focus appeared in Sulavesi (Celebes), Indonesia (54). It was caused by the El Tor biotype of V. cholerae O1, which was widely considered, at that time, to be a "paracholera" organism and not a cause of true cholera. The World Health Organization (WHO) Assembly decided in 1958 that the disease caused by V. cholerae El Tor should not be considered cholera and consequently not be subject to international sanitary regulations. However, in 1961, the present, or seventh cholera
pandemic began; it was caused by the El Tor biotype of *V. cholerae* O1, and subsequently, the WHO Assembly decision was overruled (34). In this present pandemic, cholera extended across Asia into the Middle East during the 1960s and through Africa, southern Europe, and the Pacific Islands during the 1970s (80). Over the past 30 years, cholera has been reported in over 100 countries worldwide (110) (Fig. 1).

The Latin American epidemic

In late January 1991, cholera appeared in Peru, its first appearance in South America in the 20th century (15). Cholera spread rapidly within Peru and to other countries at the rate of approximately one new country per month (99,109,111). By August 26, 1992, 19 countries had reported 640,000 cholera cases and 5,600 cholera deaths, more cases than were reported for the entire world over the past 5 years (21,110). Subsequently, cholera was introduced into the United States by travelers who either ate contaminated food during their stay in the Latin American countries or brought contaminated food into the United States. However, no secondary spread from the index cases has been documented.

**Taxonomy and Classification**

*Vibrio cholerae* is a gram-negative, curved rod with a single polar flagellum. It is just one of more than 30 currently recognized *Vibrio* species (57). This species is divided into more than 100 serogroups, based upon agglutination in antiserum directed against the cell-wall O antigen. Strains belonging to serogroup O1 that are capable of producing cholera toxin are associated with epidemic cholera (10), (Fig. 2). In addition to toxin production, strains in serogroup O1 are further characterized by biotype and serotype. There are two biotypes, classical and El Tor, which generally differ by hemagglutination of chicken erythrocytes, hemolysis, Voges-Proskauer reaction, phage susceptibility, and sensitivity to polymyxin B (4,37,39,57). The classical biotype is believed to be the cause of the cholera pandemics occurring before 1950. The present seventh pandemic is caused by the El Tor biotype of *V. cholerae* O1, and strains of the classical biotype are now found only in Bangladesh (23,85). Each biotype can be further classified as Inaba or Ogawa serotype, based on agglutination in specific O1 antisera. The O1 antigen is composed of A, B, and C factors; the Inaba serotype consists of A and C, while Ogawa is A and B (80). Serotype conversion from Ogawa to Inaba and Inaba to Ogawa has been documented, and the genetic basis for such a conversion has been established (94). Until recently, toxigenic strains that belong to serogroups other than O1, formerly referred to as nonagglutinable or noncholera vibrios, have not been associated with epidemic cholera (50,70,71). However, since November 1992, over 10,000 cases of clinical cholera have been reported from India and Bangladesh; the great majority of cases belong to a newly established serogroup O139.

**Pathophysiology and Clinical Features**

The cholera toxin catalyzes the massive outpouring of fluid that characterizes the severe form of cholera. The toxin is a heat-labile 85-kDa protein that has two functional units (67). The B subunit consists of five identical 11.6-kDa peptides which bind to the GM1 ganglioside receptors on the surface of the mucus epithelial cells of the small intestine. The smaller A1 segment of the 27-kDa A subunit is the active part. It enters the cell through

Figure 1. Countries reporting cholera since 1959.

☐ 7th pandemic;
☐ Latin American epidemic (1991-1992);
☑ Documented environmental reservoirs (U.S. Gulf Coast and northeast Australia).

* Excluding imported cases.
**Vibrio cholerae**

![Diagram of Vibrio cholerae]

Figure 2. V. cholerae: Interrelationship of O1 serogroup, ability to produce cholera toxin, and ability to cause epidemic cholera.

the hole formed by the five B subunits and stimulates adenylate cyclase activity. This causes active secretion of chloride and blocks normal absorptive functions of the cells. Water, potassium, and bicarbonate follow chloride into the lumen of the intestine, producing profuse secretory diarrhea (36). V. cholerae may contain other virulence factors such as adherence and motility, in addition to cholera toxin (47). Recently, two new toxigenic factors, zonula occludens toxin and accessory cholera enterotoxin, have been described (6,38).

Dehydration and vomiting, painful muscle cramps, and clouded mental status are typical clinical symptoms associated with severe cholera (44). However, cholera has a wide clinical spectrum, and only 2% of persons infected with El Tor biotype develop such severe symptoms or “cholera gravis”; about one-fourth of infected persons have mild to moderate diarrhea, and 75% are asymptomatic (44). Successful treatment of cholera depends on rapid oral or intravenous replacement of the fluid and electrolyte losses (44). As much as 12-24 L a day must be administered to patients with cholera gravis during the first several days (112). A package form containing the optimum mix of electrolytes and sugar for oral treatment is called oral rehydration salts. Antibiotics may be useful because they shorten the volume and duration of diarrhea and the duration of excretion of V. cholerae O1, thereby reducing the dissemination of the organism into the environment (44,86). However, rapid development of multiresistance has been reported in Tanzania (7), Bangladesh (41), and recently in Latin America (106).

**SOURCES AND TRANSMISSION**

The primary source of infection in cholera epidemics is human feces. Cholera patients excrete $10^6-10^9$ organisms per ml of stool (7). A patient producing 10 L of stool per day could theoretically produce $10^{14}$ organisms. Since the infectious dose is about $10^8$ organisms, the number of organisms excreted by one patient in one day is sufficient to infect 1-10 million people (33). Feces from cholera patients are therefore the primary source of potentially massive environmental pollution. Chronic carriers are rare and are not known to play any role in cholera persistence (33). Cholera is exclusively a human disease, and no animal species has been found to be consistently infected (7). There is strong evidence for long-term persistence of V. cholerae O1 in environmental reservoirs (9,11,30,32,69). Two such reservoirs are documented in remote rivers in North Australia and in the U.S. Gulf Coast waters (12,28,55,56,90). Cholera is primarily transmitted through ingestion of water or food contaminated by human feces. Because the infectious dose is very high, cholera does not spread through direct person-to-person contact, such as shaking hands or touching (44).

Waterborne transmission was first recognized in Europe in the mid-19th century. Investigations by John Snow and others showed that municipal water supplies contaminated with sewage were the principal route of cholera transmission at that time (87,92). Therefore, even before the causative agent was described in 1883 by R. Koch, “sanitary reform” improved water systems, sewage disposal, and disease surveillance in the industrialized world (108). Cholera was one of the first epidemic diseases to be controlled by public health measures. Water continues to play an important part in cholera transmission (24). Commercially bottled water caused a large number of cases of cholera in Portugal in 1974 (8). More recently, water has been a major vehicle of cholera transmission in the present Latin American epidemic (97,99). One report described large municipal systems distributing unchlorinated and unfiltered water at low pressure through often defective water piping. In that investigation, individuals had “tapped” into the city water supply, and these holes could provide an entry for V. cholerae O1, especially when the water pressure in the pipes dropped. Storage of home water supplies in wide-mouth open containers allowed for further contamination (97) (Fig. 3).

**OCCURRENCE AND SURVIVAL OF V. CHOLERAE O1 IN FOODS**

**General considerations**

Factors enhancing the survival and growth of V. cholerae O1 in food are lower temperatures, high organic content, near neutral pH, absence of competing flora, and high moisture (25,76,77,91). If exposed to temperatures of 80-100°C, V. cholerae O1 dies within a few seconds; at 65°C it dies within 10 min. Freezing does not necessarily kill V. cholerae O1, and it can survive for long periods in the frozen state (76). More organisms are destroyed at -2 to -10°C than at -70°C. Drying and sunlight affect V. cholerae O1 unfavorably (57,76).

**Contamination of food by V. cholerae O1**

Generally, food can be contaminated with V. cholerae O1 in three ways:

i. By the feces of an acutely ill person or an asymptomatic transiently infected person who is handling and preparing food. V. cholerae O1 can survive in feces up to 5 d, and in soil 4-10 d in tropical and subtropical climates (32).

ii. By another vehicle, usually water, which has previously been contaminated with V. cholerae O1 from human feces, for example, when sewage water is used for watering crops, when food is contaminated with water during washing and in preparing

**Figure 3. The major transmission routes of cholera.**
meals, and when seafood is harvested from areas contaminated by sewage. In surface waters and sewage, *V. cholerae* O1 survives up to 20 d (31).

iii. By exposure to environmental reservoirs where *V. cholerae* O1 occurs naturally. There is strong evidence that *V. cholerae* O1 exists and multiplies in those reservoirs, independent of human fecal contamination (9,12,28,30,32,56,69) (Fig. 3).

The survival of *V. cholerae* O1 under various conditions in food has been extensively investigated. The most rapid growth of *V. cholerae* O1 occurs in moist and alkaline foods. On most food, *V. cholerae* O1 can survive from 2 to 14 d, better at 5-10°C than at 30-31°C (77). *V. cholerae* O1 seems to survive better on cooked than on raw food (60). Cooking eliminates competing microorganisms and destroys some heat-labile growth inhibitors that are present in the food. Both classical and El Tor biotypes grow well on cooked food, although the El Tor biotype usually reaches higher numbers (60). Normal reheating temperatures may be too low to entirely eliminate *V. cholerae* O1 (64,76). The risk of transmission of *V. cholerae* O1 by foods may be especially high in the present pandemic because it is caused by the El Tor biotype. In studies in which food has been intentionally contaminated, this biotype survives better in certain foods than the classical biotype (60).

**Specific food items**

**Seafood.** Seafood may be contaminated if it is harvested from water polluted with sewage or from environments where *V. cholerae* O1 occurs naturally. *V. cholerae* O1 produces chitinase, an enzyme that dissolves chitin, the skeletal structure of copepods (zooplankton). Zooplankton are ingested and filtered from seawater by molluscs and crustaceans from seawater (52). Once ingested by crabs and molluscs, *V. cholerae* O1 can persist for many weeks and is not spontaneously cleared. Oysters and clams that have been sterilized or boiled before being artificially contaminated can support survival of *V. cholerae* O1 for more than 3 weeks when refrigerated (25). Crabs boiled for less than 10 min or steamed for less than 30 min may still harbor viable *V. cholerae* O1 organisms (9). The bacteria can then multiply to high numbers if crabs are left at ambient temperature for several hours.

**Meat.** Warm-blooded animals are generally free of *V. cholerae* O1, but food of animal origin may be contaminated by human manipulation. An increase from 10⁴ to 10⁹ organisms within 16 h has been observed on cooked chicken (60).

**Fruit and vegetables.** Vegetables and fruits with a pH above 4.5 are also risk factors because of potential contact with fecally contaminated soil and surface water. Contaminated water may be used for cleaning, and it may be injected into larger fruits to increase their weight or improve turgor (37). Fruit concentrates and pulps are not a risk for cholera transmission if their pH is below 4.5. On acidic fruit, such as lemons or oranges, *V. cholerae* O1 dies within hours; however it may live as long as 2-3 d on onions and garlic (34,76).

**Milk and milk products.** Milk and milk products, soft desserts, and sweets containing eggs and sugar permit survival of *V. cholerae* O1 for as long as 2 weeks. Milk does not support multiplication of *V. cholerae* but will support persistence of the organisms for 2-4 weeks, especially if refrigerated (34). Standard pasteurization procedures kill *V. cholerae* O1.

**Other food.** On cooked rice, pasta, eggs, and potatoes *V. cholerae* O1 survives for 2-5 d. Even on spices, such as cinnamon or pepper, *V. cholerae* O1 can survive for several days (76).

**ISOLATION AND CHARACTERIZATION OF *V. CHOLERAE* FROM FOODS**

Although simply isolating *V. cholerae* O1 from a food does not incriminate that food as a vehicle of transmission, the laboratory plays a vital role in the investigation of foodborne cholera outbreaks. It can often determine whether or not statistically implicated sources and vehicles are biologically plausible. The elements of the laboratory activities in foodborne cholera outbreaks include the following:

i) Use of appropriate methods and media to isolate the organism.

ii) Use of appropriate assays to identify *V. cholerae* O1 strains reliably (based on phenotypic characteristics of the organism, such as colony morphology on selective media, biochemical tests, and serotyping (37)).

iii) Detection of cholera toxin or cholera toxin genes.

iv) Use of molecular typing methods that enable subtle differentiation within the *V. cholerae* O1 serogroups.

**Isolation**

Tolerance to alkalinity and rapid generation times are the bases of most selective media used to isolate *V. cholerae* O1, especially if the organisms are few in number (63). Enrichment broths rely upon the ability of *V. cholerae* O1 to grow at alkaline pH. The enrichment broth is usually streaked within 6-8 h onto a selective medium that has a high pH and contains bile salt, since other competing microflora may overgrow *V. cholerae* O1 during an extended enrichment period. Food samples should be held at +4°C until cultured because less die-off of *V. cholerae* O1 occurs at this temperature than when frozen.

The U.S. Food and Drug Administration, Department of Public Health, recommends that a 25-g food test sample be aseptically weighed in a sterile blender jar (100). Larger pieces should be cut up before blending, and 225 ml of alkaline peptone water should be added (10³ dilution). To culture oysters, a 25-g composite sample from 10-12 blended animals, including shell liquor, should be added to 225 ml alkaline peptone water. Duplicate samples are recommended for incubation at both 35 and 42°C; the latter temperature has been associated with improved recovery (26). Isolating *V. cholerae* O1 from vegetable samples containing high concentrations of enteric bacteria may be difficult because of overgrowth of *Enterobacteriaceae*. Therefore, blended samples should be serially diluted to a final concentration of 10⁴ to 10⁶, and then the procedure should be carried out as usual. At least two tenfold dilutions (10² and 10³) of the blended food samples in alkaline peptone water should be prepared, but further dilutions (10⁴ to 10⁸) enhance the probability of isolation of *V. cholerae* O1. Alkaline peptone water should be incubated at 35°C (and 42°C if duplicate samples have been prepared) for 6-8 h (frozen food for 18-24 h). Then, the surface growth (pellicle) should be streaked onto thiosulphate-citrate-bile salts-sucrose agar (TCBS) and an other selective medium such as modified cellobiose-polymyxin B colistin. The plates should incubate 18-24 h at 35-37°C.

**Biochemical tests and serologic identification**

Because of the epidemic potential of cholera, it is critical to quickly determine whether a vibrio-like isolate is actually *V. cholerae* O1. Typical yellow colonies from TCBS should, therefore, be subcultured to blood agar, heart-infusion agar, or other nonselective media and incubated at 35-37°C for 6-16 h. When the growth is sufficient, the organism should be tested for oxidase and for agglutination with polyvalent *V. cholerae* O1 antisera. If positive in both tests, the strain can be further tested by slide agglutination with specific Inaba and Ogawa antisera (57). Arginine dihydrolase activity is another effective screening procedure. Salt tolerance should also be tested at this time (1% tryptone without NaCl and 1% tryptone with 3% NaCl [100]). *V. cholerae* O1 is capable of growth without added NaCl, while other *Vibrio* species are not. Specific tests to differentiate between the classical and El Tor biotypes include hemagglutination of sheep red blood...
cells, polymyxin B sensitivity testing, the Voges-Proskauer test, and susceptibility to specific bacteriophage (4,59,63).

Detection of cholera toxin
Since only cholera toxin (CT)-producing strains cause epidemic cholera, it is essential to establish the toxin-producing capability of V. cholerae O1 strains from areas not known to have cholera. Nontoxigenic V. cholerae O1 strains have been found in both South and North America (11,22,55,56). Cholera toxin can be detected by several methods.

Bioassay. The tissue culture technique using adrenal V1 cells and Chinese hamster ovary cells reveals distinct morphologic changes after exposure to CT (45,84).

Immunoassay. Two of the most commonly used immunoassays are the GM1 enzyme-linked immunosorbent assay (ELISA) and coagglutination to detect CT in culture supernatants (1). The GM1 ELISA is performed in microtiter wells coated with ganglioside GM1 to which CT binds. Rabbit anti-CT antibodies are used to make a sandwich ELISA that is detected by goat anti-rabbit immunoglobulin conjugated with alkaline phosphatase. Latex agglutination assays employ polystyrene latex particles coated with purified CT antiserum.

Probes. Both natural DNA fragments (23,73) and synthetic oligonucleotide probes (2) that detect V. cholerae toxin genes have been developed. Polymerase chain reaction (PCR)-generated amplicons from the CT gene labeled with dUTP have been used as probes as well (103).

Polymerase chain reaction (PCR). Unlike traditional methods, which are limited to detecting viable organisms, the PCR can detect DNA from dead and lysed organisms (79). This assay does not require that DNA be denatured or made single-stranded by heating. A defined segment of DNA can be detected by hybridization of two specific DNA oligonucleotides (primers) to the single-stranded test DNA, thus designating the beginning and the end of the gene segment which will be duplicated by the enzyme, DNA polymerase. The process is repeated automatically, amplifying the target DNA segment. A PCR that employs primers that can recognize the A subunit of the cholera toxin gene was developed and evaluated at the Centers for Disease Control, Atlanta, GA (35,75). The PCR generated amplicon from the CT gene A subunit is visualized on an agarose gel (Fig. 4).

Figure 4. Polymerase chain amplification of a 564-bp DNA product from the cholera toxin A subunit gene for detection of toxigenic V. cholerae O1.

Lane A: V. cholerae O1, H 23337, human, Bangladesh, 1970
Lane B: V. cholerae O1, 2164-78, human, Louisiana, 1978
Lane C: V. cholerae O1, E 9956, human, Philippines, 1963
Lane D: V. cholerae O1, C 6706, human, Peru, 1991
Lane E: V. cholerae O1, C 8034, human, Ecuador, 1992
Lane F: V. cholerae O1, E 2432-88, human, Rwanda, 1988
Lane G: V. cholerae O1, C 7724, water, El Salvador, 1991
Lane H: Campylobacter jejuni, D 121, human, Canada
Lane I: V. cholerae O1, C 8129, human, Ecuador, 1992, positive control
Lane J: Nontoxigenic V. cholerae O1, C 8155, human, Japan, 1992, negative control
Lane K: Molecular weight ladder

Molecular epidemiologic markers
In investigations of outbreaks, phenotypic characteristics are often insufficient to differentiate the epidemic strain from other strains or to trace the source and vehicles of transmission. These standard or traditional tests analyze gene products from a relatively small proportion of the ~6 x 10^9 basepair bacterial genome. Molecular techniques, on the other hand, examine the DNA or RNA of a strain directly and provide additional information. Some of them have already been successfully used to trace foodborne V. cholerae outbreaks.

Restriction fragment length polymorphisms (RFLPs). Restriction enzymes cleave (digest) DNA molecules at specific 4-8-basepair sequences; this results in hundreds of different restriction fragments of the genomic DNA. Differences in the sizes of such fragments between strains are called restriction fragment length polymorphisms (RFLPs). Although the identification of genomic RFLP patterns can be quite cumbersome because of their large number, that obstacle can be overcome by two different approaches. The restriction fragments can be transferred from the agarose gel to a membrane and then hybridized with a specific probe. The probe can be either a DNA or RNA sequence that will hybridize to only those restriction fragments that contain DNA homologous to the probe (48). The result is a characteristic banding pattern with a limited number of bands. The ability of this approach to detect differences between isolates depends primarily on the choice of restriction enzyme since the enzymes generate fragments of different sizes and cut DNA at different locations.

When RNA is used as a probe, different patterns or ribotypes among the strains belonging to the same serotype can be established because the number of rRNA gene copies and their location within the V. cholerae O1 genome varies (59,81,103). Figure 5 shows different ribotyping patterns obtained after Bgl digestion of whole chromosomal DNA of V. cholerae O1 strains, followed by hybridization with a digoxigenin-labeled cDNA probe obtained by reverse transcription from 16S + 23S rRNA from Escherichia coli. Specific probes for the A or B subunit genes of the cholera toxin can detect those genes in the V. cholerae O1 genome. This approach has been extremely useful in differentiating the U.S. Gulf Coast V. cholerae O1 strains from imported seventh pandemic strains (56,103,113). Probes to Vca-3 bacteriophage, which is sometimes present in the bacterial chromosome, have also been used to characterize V. cholerae O1 strains from the U.S. Gulf Coast (2).

The other approach is to apply pulsed-field gel electrophoresis in conjunction with restriction enzymes recognizing 8-basepair targets to generate a small number (10-50) of large-molecular-weight chromosomal DNA fragments (88). This results in banding patterns that are more suitable for epidemiologic comparison. Therefore, RFLPs can be analyzed and type patterns identified without the further use of the specific probe. This method has been already successfully applied as a typing method for some other species (83,114), and recently for V. cholerae O1 as well (5).

Figure 5.

Restriction fragment length polymorphisms (RFLPs) of whole chromosomal DNA of V. cholerae O1 strains from the U.S. Gulf Coast (2).

strains following hybridization with digoxigen-labeled cDNA obtained by reverse transcription from E. coli 16S + 23S rRNA.

Lane A: V. cholerae O1, H 23337, human, Bangladesh, 1970
Lane B: V. cholerae O1, 2164-78, human, Louisiana, 1978
Lane C: V. cholerae O1, E 9956, human, Philippines, 1963
Lane D: V. cholerae O1, C 6706, human, Peru, 1991
Lane E: V. cholerae O1, 2432-88, human, Rwanda, 1988
Lane F: V. cholerae O1, C 7724, water, El Salvador, 1991

Multilocus enzyme electrophoresis. The principle of this technique is to separate different allelic forms of enzymes involved in standard metabolic processes of the bacterial cell by electrophoresis. Different enzymes are visualized in a gel matrix by adding color-developing substances. Generally, 15 to 20 enzymes are assayed, and a profile of the enzyme alleles is used to define the electrophoretic type of different strains (89). This method has been used to show that most nontoxigenic strains of V. cholerae O1, which do not cause cholera, are not related to toxigenic pandemic strains (22). More recent data indicate that all toxigenic strains from the current Latin American epidemic belong to one unique electrophoretic type (105).

Plasmid profiles. Plasmid profiles have been particularly useful in differentiating Salmonella strains involved in outbreaks from other strains (102,104). However, this technique is not particularly suitable for differentiating between strains of V. cholerae O1. Some plasmids have been identified in classical strains, but V. cholerae O1 of the El Tor biotype seldom have plasmids other than those associated with acquired antibiotic resistance (23).

DNA sequencing. DNA sequencing has recently been automated, and gene sequencing information could be useful for epidemiologic purposes. The cholera toxin B subunit gene has been sequenced and three different types of the toxin (genotypes) have been discovered. Genotype 1 contains strains that belong to the classical biotype of V. cholerae O1 and the El Tor strains originating from the Gulf Coast area. Genotype 2 contains strains from Australian environmental reservoirs, and genotype 3 contains strains of the El Tor biotype from the seventh pandemic isolated over the past 30 years (74).

**EPIDEMIOLOGY OF FOODBORNE CHOLERA OUTBREAKS**

Traditionally, water has been considered to be the most important vehicle of cholera transmission, but already in the 19th century it was recognized that there were other ways of transmitting cholera (92,108). Various foods have been incriminated in investigations of cholera outbreaks over the past 30 years. Seafoods commonly have been implicated in cholera outbreaks throughout the world. A particularly important vehicle has been shellfish harvested from sewage-contaminated beds or from environments where V. cholerae O1 occurs naturally, and then is eaten raw or undercooked (58,78). Crabs eaten undercooked or cross-contaminated after cooking have also been a repeated source of cholera in the United States and elsewhere (13,40,61,62). A variety of other foods have also been shown to be vehicles for cholera transmission. Raw fruits and vegetables contaminated with polluted water have been suspected, but the evidence is uncertain (32). Cholera has been traced to foods initially contaminated by acutely injected foodhandlers (93).

Cholera outbreaks associated with food during the seventh pandemic

In August 1971, cholera was introduced into Africa, which had been free of cholera for more than 70 years (42). It spread rapidly and has affected at least 30 of the 46 African countries (109). Cholera in Africa has been characterized by its rapid dissemination throughout the continent, its 20-year persistence, and its association not only with water but with a variety of foods. In Conakry, Guinea, a case-control study associated cholera with eating leftover unheated rice with peanut sauce. However, leftover rice served with acid tomato sauce was actually protective because of the low pH of the sauce (93). Investigations of two cholera outbreaks after funerals for cholera victims showed that they were caused by rice served at the funerals. Following their usual practice, women cleaned the deceased body and evacuated the bowel contents. The same women then prepared large quantities of food well before the arrival of the funeral attendees, probably contaminating the rice with their hands. The rice remained at ambient temperature for several hours before being eaten, allowing V. cholerae O1 to multiply (93). Cholera was associated with eating leftover millet gruel in a drought-affected village in a case-control study of cholera patients in Mali in 1984. Millet gruel, a staple of the Sahel diet, is generally prepared once a day; it may be held at ambient temperature for many hours and is then eaten communally without reheating (98).

In November 1972, an outbreak of cholera occurred among persons who had been economy class passengers on an aircraft en route from London to Sydney. It made a stop in Bahrain, near Saudi Arabia; Bahrain was experiencing a cholera outbreak at that time. Within several days, 47 of 331 passengers were infected with V. cholerae O1: 25 developed diarrhea, and 22 were asymptomatic. Although most patients experienced a mild infection, one person died. The implicated food was an hors d’oeuvre served on the aircraft between Bahrain and London (95).

In 1982, a foodborne cholera outbreak in Singapore affected 37 construction workers. The source of infection was traced to contaminated seafood prepared at the construction site canteen, where two food handlers were found to be infected with V. cholerae O1 (43).

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An outbreak of cholera occurred in Maryland in August 1991. Four persons had laboratory evidence of recent infection, and three of them had symptoms of cholera. Thai-style rice pudding with a topping made from frozen coconut milk was incriminated. The Food and Drug Administration isolated V. cholerae O1 from an unopened package of the same brand of coconut milk. Its chromosomal ribotyping pattern was identical to the ribotype of one of the strains currently causing the seventh pandemic in Asia and was unlike strains from Latin America and Gulf Coast. This would indicate that the contamination occurred during preparation of the food in the country of origin. Table 1 summarizes results of epidemiologic investigations in which foods were incriminated as vehicles of cholera transmission during the seventh pandemic.

Cholera cases associated with environmental reservoirs in the United States

Cholera has two documented foci of environmental reservoirs independent of the seventh pandemic: one in Northeastern Australia, and one along the U.S. Gulf Coast. Consumption of water or foods from such reservoirs is a potential risk for acquiring cholera (See Fig. 1). During the period from 1977 to 1984, five cases of cholera infections acquired from rivers in northeastern Australia were reported (12,28). The causative organism was V. cholerae O1 biotype El Tor, serotype Inaba. Identical strains have been isolated from more than a dozen brackish rivers in that area. Investigations indicated that V. cholerae O1 can survive and multiply in this environment. No route of importation or dissemination of the organism was discovered. Molecular typing methods have revealed the unique characteristics of these isolates and can distinguish the Australian strain from major epidemic strains (105).

The first naturally acquired case of cholera reported in the United States since 1911 occurred in a 51-year-old shrimp fisherman from Port Lavaca, Texas, in 1973 (107). The isolate was identified as V. cholerae O1 biotype El Tor, serotype Inaba. The source of infection was not elucidated, in spite of the extensive epidemiologic investigation. In the fall of 1978, 11 persons were infected with V. cholerae O1 of the Inaba serotype in Louisiana (9). All of them had recently eaten cooked crabs from five widely separated sites in the Louisiana coastal marsh.

Two unrelated cases of cholera associated with eating seafood from the U.S. Gulf Coast area were reported in Texas in 1981 (90). One patient recalled eating fish and turtle caught in a nearby bayou, and the other eating shrimp in a stew. A single case of cholera on an oil rig barge in Texas in the fall of 1981 was followed by 14 secondary cases of cholera and one asymptomatic infection (53). The first patient was probably infected by eating seafood from local waters. His contaminated stools were discharged into the surrounding water. Some of the water was pumped back into the salt water system, which was mistakenly cross-connected with the drinking water system in the oil rig for a short time. The next morning the cook prepared the rice and held it throughout the day in warm pans. To keep it moist he added contaminated water several times during the day. The crew members ate the rice for dinner, and 15 of them were infected with V. cholerae O1.

A case of cholera was identified in Maryland in October 1984 (61). A 72-year-old man had eaten meat from crabs harvested along the Texas coast. In 1986, two cases of cholera in Florida (38) and Georgia (78) were associated with eating raw oysters.

The largest indigenous cholera outbreak in the United States occurred in the fall of 1986 in Louisiana (62). Eighteen persons in 12 clusters were infected. For all patients, the incriminated food was seafood, crabs and shrimp harvested from widely scattered sites in Louisiana.

Six more sporadic cholera cases occurred in 1987, and seven more cases in 1988. A total of 65 cholera cases have been related to the Gulf Coast environmental reservoir in the United States since 1973 (Table 2). V. cholerae O1 strains isolated from patients who have acquired cholera in the United States since 1973 have been studied extensively (36). All are of the El Tor biotype and Inaba serotype. All strains are toxigenic and hemolytic on blood agar, carry a unique bacteriophage VcA3 (2), and have a unique multilocus enzyme electrophoresis pattern (22). Furthermore, all strains have the same HindIII restriction digest pattern, with two fragments containing the cholera toxin A subunit gene, and the same BglII ribotype pattern, which is different from patterns seen in strains isolated from other parts of the world. All these data strongly suggest that this toxigenic V. cholerae O1 strain has persisted as a free-living organism for at least 20 years in U.S. Gulf Coast waters, independent of the pandemics. Additional evidence presented by Hunt et al. (51) showed that in persons living in the Texas Gulf Coast area, significantly higher titers of vibriocidal antibody were found in those with exposure to seawater than in those without such exposure. Furthermore, antitoxin titers were significantly higher in those persons who consumed shellfish than in nonconsumers (51). Table 2 shows epidemiologic data associated with U.S. Gulf Coast cholera cases.

### Table 1. Documented cholera outbreaks associated with consumption of food implicated by controlled studies during the seventh pandemic (1961-1991)*

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Implicated food</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>1972</td>
<td>Hors d'oeuvre (stuffed eggs)</td>
<td>95</td>
</tr>
<tr>
<td>Italy</td>
<td>1973</td>
<td>Raw shellfish (mussels)</td>
<td>3</td>
</tr>
<tr>
<td>Portugal</td>
<td>1974</td>
<td>Raw and uncooked shellfish (cockles)</td>
<td>8</td>
</tr>
<tr>
<td>Guam</td>
<td>1974</td>
<td>Home preserved padi (salted raw fish)</td>
<td>68</td>
</tr>
<tr>
<td>Gilbert Islands</td>
<td>1977</td>
<td>Raw and salt fish and clams</td>
<td>66</td>
</tr>
<tr>
<td>Bahrain</td>
<td>1978</td>
<td>Bottle feeding of infants</td>
<td>46</td>
</tr>
<tr>
<td>Singapore</td>
<td>1982</td>
<td><em>Sandal setong</em> - cooked squid</td>
<td>43</td>
</tr>
<tr>
<td>Truk</td>
<td>1982</td>
<td>Food prepared by recently ill foodhandlers</td>
<td>49</td>
</tr>
<tr>
<td>Mali</td>
<td>1984</td>
<td>Millet gruel</td>
<td>98</td>
</tr>
<tr>
<td>Guinea</td>
<td>1986</td>
<td>Leftover rice</td>
<td>93</td>
</tr>
<tr>
<td>Thailand</td>
<td>1987</td>
<td><em>Laebmoo</em> - uncooked pork</td>
<td>96</td>
</tr>
<tr>
<td>Maryland, USA</td>
<td>1991</td>
<td>Frozen coconut milk</td>
<td>19</td>
</tr>
</tbody>
</table>

* Excluding U.S. Gulf Coast and Latin American cases.
### TABLE 2. Cholera cases in the United States associated with consumption of food from Gulf Coast waters (1973-1992) *

<table>
<thead>
<tr>
<th>State</th>
<th>Year</th>
<th>No. of known cases</th>
<th>Implicated food</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texas</td>
<td>1973</td>
<td>1</td>
<td>Raw oysters</td>
<td>107</td>
</tr>
<tr>
<td>Louisiana</td>
<td>1978</td>
<td>11</td>
<td>Cooked crabs</td>
<td>9</td>
</tr>
<tr>
<td>Texas</td>
<td>1981</td>
<td>2</td>
<td>Partially cooked fish and shrimp</td>
<td>90</td>
</tr>
<tr>
<td>Texas</td>
<td>1981</td>
<td>16</td>
<td>Cooked rice</td>
<td>53</td>
</tr>
<tr>
<td>Maryland</td>
<td>1984</td>
<td>1</td>
<td>Crabs</td>
<td>61</td>
</tr>
<tr>
<td>Florida</td>
<td>1986</td>
<td>1</td>
<td>Raw oysters</td>
<td>58</td>
</tr>
<tr>
<td>Louisiana</td>
<td>1986</td>
<td>18</td>
<td>Cooked crab, cooked and raw shrimp</td>
<td>62</td>
</tr>
<tr>
<td>Georgia</td>
<td>1986</td>
<td>1</td>
<td>Raw oysters</td>
<td>78</td>
</tr>
<tr>
<td>Louisiana</td>
<td>1987</td>
<td>2</td>
<td>Partially cooked crabs</td>
<td>40</td>
</tr>
<tr>
<td>Colorado</td>
<td>1988</td>
<td>1</td>
<td>Raw oysters</td>
<td>14</td>
</tr>
</tbody>
</table>

* 11 additional cases reported, CDC, unpublished data.

### TABLE 3. U.S. cases of cholera in persons returning from Latin America or eating food from Latin America.

<table>
<thead>
<tr>
<th>State</th>
<th>Year</th>
<th>No. of known cases</th>
<th>Suspect food</th>
<th>Country of origin</th>
<th>Food origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Georgia</td>
<td>1991</td>
<td>1</td>
<td>Cooked cold crab meat</td>
<td>Peru</td>
<td>CDC data, unpublished</td>
<td>16</td>
</tr>
<tr>
<td>New Jersey</td>
<td>1991</td>
<td>8</td>
<td>Cooked crabs in cold salad</td>
<td>Ecuador</td>
<td>CDC data, unpublished</td>
<td>17</td>
</tr>
<tr>
<td>Florida</td>
<td>1991</td>
<td>1</td>
<td>Raw oysters and ceviche</td>
<td>Ecuador</td>
<td>CDC data, unpublished</td>
<td>17</td>
</tr>
<tr>
<td>New York</td>
<td>1991</td>
<td>4</td>
<td>Cooked crabs in cold salad</td>
<td>Ecuador</td>
<td>CDC data, unpublished</td>
<td>18</td>
</tr>
<tr>
<td>New Jersey</td>
<td>1991</td>
<td>1</td>
<td>Crabs</td>
<td>Ecuador</td>
<td>CDC data, unpublished</td>
<td></td>
</tr>
<tr>
<td>Connecticut</td>
<td>1992</td>
<td>2</td>
<td>Raw clams and cooked shrimp</td>
<td>Ecuador</td>
<td>CDC data, unpublished</td>
<td></td>
</tr>
<tr>
<td>New Jersey</td>
<td>1992</td>
<td>1</td>
<td>Shrimp ceviche</td>
<td>Ecuador</td>
<td>CDC data, unpublished</td>
<td></td>
</tr>
<tr>
<td>California</td>
<td>1992</td>
<td>2</td>
<td>Seafood at seastand</td>
<td>El Salvador</td>
<td>CDC data, unpublished</td>
<td>20</td>
</tr>
<tr>
<td>California</td>
<td>1992</td>
<td>76</td>
<td>Shrimp and fish in cold salad</td>
<td>Peru</td>
<td>CDC data, unpublished</td>
<td></td>
</tr>
<tr>
<td>California</td>
<td>1992</td>
<td>1</td>
<td>Raw seafood</td>
<td>El Salvador</td>
<td>CDC data, unpublished</td>
<td></td>
</tr>
</tbody>
</table>

**Cholera cases and outbreaks in the United States associated with consumption of food originating in Latin America**

Food was the vehicle of transmission of cholera from Latin America into the United States on five separate occasions in 1991. A total of 15 cholera cases were reported: five travelers returned from areas with epidemic cholera, and 10 persons developed cholera after eating seafood brought back from Latin America in the travelers’ luggage. No further spread from these cases was documented (Table 3).

In April 1991, a 57-year-old man had a diagnosis of cholera in New York City (18). Three of his recent contacts had laboratory evidence of infection with *V. cholerae* O1. The strains isolated from the patients in New York were identical to the strains isolated during the epidemic in Latin America with respect to serotype and biotype. An epidemiologic investigation revealed that the man with the index case had traveled to Ecuador and bought crabs from a pier in Guayaquil. The crabs had been boiled and shelled, and meat and claws were then stored in a plastic bag in a freezer. The index patient packed them in his suitcase for the return trip to the United States. Although epidemiologically implicated, the crab meat did not contain *V. cholerae* O1, when assayed by traditional cultivation methods, which are limited to detection of viable organisms only. PCR which can detect the A subunit of the cholera toxin gene in dead and lysed organisms was used to assay the frozen crabmeat (35). Three of four crab samples and the salad juice sample had no detectable *V. cholerae* O1 DNA. However, one crab sample and the strains from patients were positive. Strains isolated from the patients were further analyzed by ribotyping. The ribotyping pattern of the *V. cholerae* O1 strain isolated from one of the patients in this New York outbreak was identical to pattern typical for the strains causing the present Latin American epidemic and was clearly different from the pattern characteristic of Gulf Coast strains. In 1991 alone, there were another 11 cholera cases associated with eating seafood, either during a visit to Latin America or after the food had been brought from Latin American countries (18) (Table 3). In February 1992, an airplane from Buenos Aires, Argentina, stopped in Lima, Peru, loaded food and continued to Los Angeles. Seventy-six passengers were later shown to be infected with *V. cholerae* O1. Thirty-seven persons were ill and one person died (20). The implicated food was cold seafood salad that included shrimp and fish prepared in Lima. No other flights appeared to have been affected, and no secondary spread was detected. To date, in addition to these 76 passengers, 21 more persons have developed cholera associated with either travel to one of the countries of Latin America or with consumption of seafood brought from there. The actual risk of the spread of cholera after a traveler returns to the United States with cholera is small. Between 1961 and 1990, 41 cases of imported cholera were reported in the United States, none of them associated with secondary spread.

There has been some concern about contamination of the U.S. Gulf Coast shellfish beds with the Latin American *V. cholerae* O1 strain. In 1991, a toxigenic *V. cholerae* O1 strain was isolated from a commercial oyster bed in Mobile Bay, Alabama (27). Unexpectedly, isolates were clearly different from the previously described Gulf Coast strains and were characterized by molecular typing methods as identical to those causing the present epidemic in Latin America (103). Furthermore, samples of the ballast, bilge, and sewage from several ships arriving from Latin American ports and docking in Gulf of Mexico ports have revealed the same toxigenic strain of *V. cholerae* O1 (65). These data support the hypothesis that the initial introduction of *V. cholerae* O1 to Gulf Coast waters was by ships. Fortunately, no human illness has been associated with these environmental observations. Oyster beds have been temporarily closed, and the U.S. Coast Guard now...
requires ballast and bilge water to be flushed three times in the open ocean before entering the United States ports.

**RISKS OF CHOLERA TRANSMISSION BY FOOD IMPORTS**

Cholera is endemic in many exporting countries but, with the exception of the United States (the frozen coconut milk from Thailand [77]), to date WHO has no documented evidence of cholera outbreaks occurring as a result of commercial importation of food across international borders. There have been no cases of cholera in the United States caused by the *V. cholerae* O1 strain from Latin America from commercially imported food. The epidemic strain has not been detected in any samples of such food imported to the United States from countries of Latin America (29). Studies have shown that inflexible restrictions on goods imported from countries in which cholera is endemic are not warranted (72,82). Fresh fruits and vegetables are not a risk of cholera transmission if the time between shipping and arrival in the importing country is at least 10 d (77). Since freezing does not eliminate *V. cholerae* O1, frozen products theoretically pose a risk if they are eaten raw or are allowed to cross-contaminate other foods. Fruits with a pH lower than 4.5 generally do not pose a risk for cholera transmission. Dried and canned foods are free of *V. cholerae* provided they have been processed and handled according to recognized international standards. Since *V. cholerae* O1 appears to contaminate Marine animals in situ, it must be destroyed by cooking or other treatment of food. Proper cooking initially, or at serving, and avoidance of recontamination are necessary (77).

**CONCLUSIONS**

Cholera can easily be treated by oral rehydration therapy. Therefore, most public health efforts are presently oriented toward preventive actions to avoid the spread of the disease after its introduction into a community or country. Unlike many infectious diseases that can be effectively prevented, if not eradicated, by the use of vaccines, presently only limited optimism can be expressed for cholera. However, several vaccine trials are in progress. Prevention of the spread of cholera is best achieved through hygienic measures, such as providing safe municipal water, and decontamination of other water by boiling or chemical treatment.

We now know that food is also an important vehicle of cholera transmission, generally after it is contaminated with human feces, either directly by contact with the sick person or by previously contaminated water during the preparation or handling of food. Furthermore, several natural reservoirs of cholera are well-documented and food originating from such marine or brackish water environments poses an additional risk and requires attention in its preparation and consumption. It is not likely that cholera as a global disease can be eradicated. However, with the better understanding of the ecology of the organism, its modes of transmission, and the vehicles involved, epidemiologic and hygienic interventions can reduce the spread of the disease. Additional information on the molecular composition of the organism, its molecular epidemiology and pathogenicity will be important for the support of both public health surveillance and vaccine development.

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