CHAPTER 3

Cholera for a Dime

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INTRODUCTION

Listening to the radio late one night in Boston in May, 1974 while taking a break from studying for my master's degree in public health finals from the Harvard School of Public Health, I was riveted by the news that cholera had broken out in Portugal. Might I be sent to Portugal? I would soon be an epidemiologist in the enteric diseases branch of the Centers for Disease Control (CDC) and would be an obvious candidate for an investigation in Portugal because I could speak Portuguese, having lived as a child in a Portuguese colony, Angola. On the other hand, my epidemiologic skills were weak. I had joined the Epidemic Intelligence Service (EIS) at the CDC because of my international public health interests and to avoid military service in Vietnam* and had been sent to Puerto Rico. My 2 years there had been rich in public health experience but devoid of on-the-job supervision in traditional CDC "shoe-leather epidemiology." In those days, communication with my supervisors at the CDC in Atlanta required hours, even days, of struggles with the much-loathed Federal Telecommunications System. I was buffing up my fledgling epidemiologic expertise with a master's in public health, but I still felt inadequate. Within days, however, the CDC called to ask whether I was interested in going to Portugal, and I was indeed. My wife, who would be left with two small boys in a new neighborhood in Atlanta, was less enthused.

* We occasionally referred to ourselves as the "Yellow Berets" (in contrast to the Green Berets, elite troops who fought in Vietnam), although in truth our work could be dangerous, and one of my classmates died in the line of duty when his plane crashed in Africa.

Cholera is a diarrheal disease caused by toxigenic *Vibrio cholerae* O-group 1 or O-group 139. The infection is often mild or subclinical, but in the worst cases, severe diarrhea and vomiting can cause death within 24 hours. The incubation period ranges from a few hours to 5 days. In the Northern Hemisphere, cholera usually peaks in August to September. The main source of infection is human feces. The infectious dose is very high, requiring about 1 million organisms in food and even more in water. The organisms are very sensitive to acid, and persons with low gastric acid are at greater risk for cholera. Back in 1974, few analytic studies of cholera transmission had been performed. The disease was thought to be caused largely by polluted drinking water, with food playing a minor role. Fish and shellfish had been reported to cause cholera, but the evidence was circumstantial until 1973, when studies in Italy showed that cholera was associated with eating mussels thought to be contaminated after harvest by "freshening" with polluted harbor water.[1]

Portugal had been free of cholera for many decades until 1971, when it reported 89 cases caused by *V. cholerae* O-group 1 serotype Ogawa, mostly in the Lisbon area. Neither the source of introduction nor the vehicles of transmission were determined; however, the outbreak ended, and no cases were detected in 1972 and 1973.

Throughout the summer of 1974 I was kept on alert, and the epidemic grew while the CDC worked with officials in Washington, DC to secure an invitation from Portugal. Most countries understandably are reluctant to have foreigners document their public health failures, and few invitations materialize. The situation was complicated by uncertainty after Portugal's virtually bloodless leftist military coup (the "Carnation Revolution") in April 1974 against the right-wing dictatorship of President Américo Thomaz and Prime Minister Marcelo Caetano, successor to António Salazar. There was ongoing infighting in the government and military. Remarkably, an invitation arrived on Friday, September 6, perhaps prompted by the escalating epidemic, which peaked in late August. My departure was delayed until Monday so that I could fly to Washington to be briefed on Portuguese politics at the State Department's "Portugal Desk"; however, the briefer was taking a 3-hour lunch break, and I proceeded unbriefed.

My CDC supervisors had instructed me thoroughly on cholera, and I was crammed with advice and laden with reference material. Most useful was Bill Baine's CDC report on his investigation of cholera in Italy the year before,[1] when his matched-pair case control studies incriminated ingestion

of raw shellfish. The matched-pair case control technique had been used in chronic disease investigations, but to our knowledge, Bill was the first to use it in an infectious disease investigation outside of a hospital. It was particularly useful in investigating scattered, apparently unrelated cases because each case was matched to an age- and gender-matched neighborhood control subject (rather than a hospital control—Bill's innovation), and the matching was maintained in the analysis; thus, the results would not be distorted by age, gender, or socioeconomic (as reflected by neighborhood) status. My supervisors expected me to have a study of new Lisbon cases using Bill's technique underway by the end of the week. My objectives were to learn how cholera transmission was occurring to guide prevention and control measures in Portugal and to gain a better understanding of cholera transmission that would help cholera control worldwide.

FIRST INVESTIGATION—LISBON

I arrived in Lisbon at dawn on Tuesday, September 10, with little sleep, a headache, and no luggage (it arrived 36 hours later), but fearing the worst, I had my papers in a carry-on bag. Black and green taxis drumming along cobblestone streets, streetcars, double-decker buses, red tile roofs, colorfully tiled facades, palm trees, cascading bougainvilleas, Portuguese voices, and the smell of grilling sardines and diesel exhaust in the air—despite my fatigue, it was exhilarating to be in Lisbon! I checked into my hotel and hurried to the U.S. Embassy; immediately, however, I faced the first of many delays as I discovered that not everyone shared my sense of urgency. I had to wait all day to see the deputy ambassador and used the time to work with consular officials to get statistics for Portugal, newspaper clippings on the cholera epidemic, a desk, and access to a mimeograph machine and a massive mechanical calculator that used metal parts rather than electronics to add and subtract (these were the olden days). For division and multiplication, I had a slide rule.

The embassy arranged for me to meet with three national Portuguese officials, including Portugal's director general of health and the national epidemiologist, on Wednesday afternoon. They had only descriptive information. The first known cholera case had onset of illness on April 24 in Tavira on Portugal's southern coast. The 33-year-old man had diarrhea and dehydration so severe that he suffered a cardiac arrest, and the national laboratory isolated *V. cholerae* O-group 1 biotype El Tor serotype Inaba

from his stool. The disease spread 300 km to Lisbon within 16 days and 600 km to Porto in the far north within 20 days and eventually was reported from 17 of 18 districts (Figure 3-1). When I arrived in Portugal in early September, approximately 2,000 laboratory-confirmed cases and

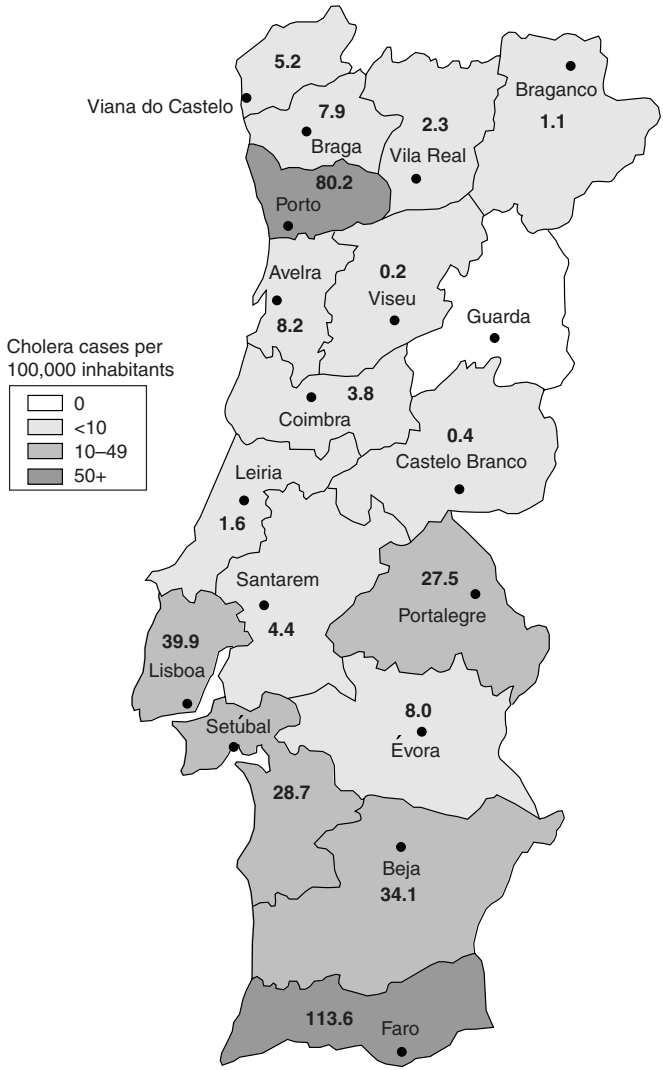

FIGURE 3-1 Hospitalized cholera patients in Portugal, by district of residence, April–October, 1974.

Reprinted with permission from Blake P et al. Cholera in Portugal 1974. II Transmission by bottled mineral water. *Am J Epidemiol* 1977;105:344–348.

several dozen deaths had been reported. The epidemic had peaked the last week in August and was now declining rapidly (Figure 3-2), but a few new widely scattered cases were still occurring in Lisbon. I began to worry that while an investigation of cases that were part of the peak of the epidemic might incriminate one or more important vehicles that caused the bulk of the cases, the last few scattered cases at the tail end of the epidemic might be caused by many different exposures (e.g., food contaminated by an infected household member), making successful incrimination of any one vehicle unlikely; however, I had arrived primed to concentrate on new cases and did not yet have the self-confidence or experience to deviate from the plan.

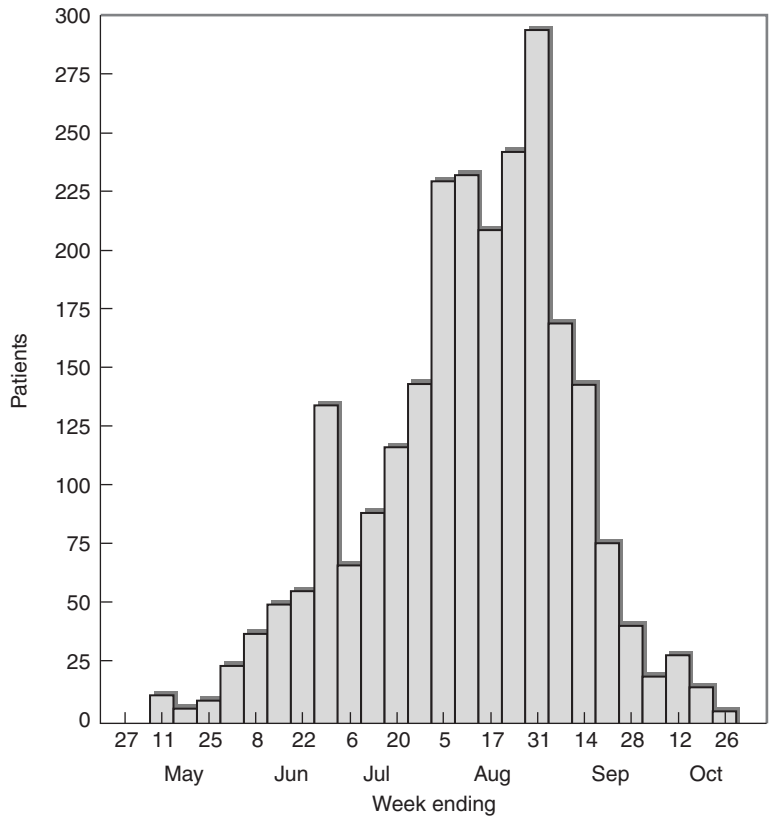

FIGURE 3-2 Patients with cholera, by date of hospitalization, Portugal, 1974.
Reprinted with permission from Blake P et al. Cholera in Portugal 1974. II Transmission by bottled mineral water. *Am J Epidemiol* 1977;105:344–348.

From anecdotes, cultures of food and the environment, and educated guesses, the Portuguese officials suspected several vehicles—cooked snails collected from sewage-contaminated gullies, lettuce irrigated with human sewage during the dry summer, watercress, Lupini beans sold by street vendors, raw shellfish, and well water. Later I learned that spring water and commercially bottled mineral water were also suspected, but were not mentioned initially because they involved an important company and thus were politically sensitive.

The national officials made it clear that they were too understaffed and overburdened to find staff to work with me, but they referred me to the Lisbon District Health Department. I went there Thursday morning; the Director was on vacation until Friday, but I met with an elderly physician who specialized in waterborne disease.* While waiting to meet the Director, I worked on a draft questionnaire and included the suspect foods, other plausible foods, and various sources of water, as well as possible risk factors such as gastric surgery and the use of antacids. I planned to ask the cases about exposures during the 5 days before onset and to ask age- and gender-matched neighbor controls about the 5 days before interview. The Portuguese had been doing a good job of culturing suspect cases, and in this and all subsequent investigations, we were able to define cases as persons with *V. cholerae* O1 isolated from their stools. In this investigation, we defined our cases as any culture-confirmed case from Lisbon or the adjacent city of Oeiras diagnosed on or after September 13.

Writing the questionnaires was doubly difficult because although I could speak Portuguese, I had never learned to read or write the language; I had to write the questions phonetically and get help from Portuguese staff in the embassy. I then struggled until nearly midnight to type stencils and mimeograph questionnaires. I returned to my hotel with inky hands and clothes but enough questionnaires to get started.

The next morning the director told me about the Lisbon District cholera activities. Eight nurses in four teams worked on cholera. One team interviewed new cases in hospitals, whereas the other three visited recent cases and their families. World Health Organization (WHO) epidemiolo-

* Dr. Leopoldo de Figueiredo gave me his publications on water and sewer systems in Portugal. My mother later told me (and he confirmed) that he was our family doctor in 1947 when I was 4 and my parents were in Lisbon learning Portuguese—a small world!

gists had visited Portugal several months earlier. At their recommendation, the Lisbon District had begun to complete a new cholera case-investigation form for all cholera patients in July. It included questions about exposures, including recent travel and sources of drinking water. The questionnaires lay unanalyzed in stacks destined, as is so often the case, for the archives rather than for analysis and use in disease control. They were to prove useful, however, in the weeks ahead.

I went out with a team the same day and completed questionnaires on three cases and two controls. On Saturday, the work went more smoothly as the nurses (and I) gained experience and our team interviewed three case control pairs in 6 hours. Being naturally diffident, it was stressful for me to knock on the doors of complete strangers, try to explain why I was there, and ask them personal questions in a language that I had hardly used in 17 years. Each interview was easier than the last, however, and the experience of going into private homes all over Lisbon was vastly more interesting than being a tourist. The case and control subjects were cooperative, and I enjoyed talking with them. One woman control looked at me quizzically as I stumbled through questions in my rusty Portuguese and finally said, "Ah! You are from Mozambique!" She recognized the African colonial accent but had the wrong colony.

Despite the seemingly interminable delays, the case control study was underway on schedule. Over the weekend I revised the questionnaires to fix problems turned up by the interviews and retyped and mimeographed them. I decided that the same person should interview both subjects in each case control pair so that the questions would be asked similarly. I worried that we needed more rigorous methods to select neighbor controls because investigators might unknowingly introduce bias if left to their own devices. Thus, I improved on the Italian studies, which selected neighbor controls from passers-by or other conveniently accessible neighbors, by adapting methods learned in a chronic disease course to create a scheme that I used in all subsequent investigations. The investigators would start at the case's house and go door to door following a printed schematic map (go right until the corner, then return to the case's house and go left until the corner, etc.) until they located a person of the same gender and within the same age range. After I amended the schematic map to include apartments, it failed only once, when the patient was a railroad crossing operator who lived in a hut by the rails—his residence was not part of a block.

I intended to train and enlist all three field teams, but although most of the nurses quickly learned proper techniques, one was overenthusiastic; she pressed patients to admit that they had eaten suspect foods and suggested to controls that they had *not* eaten those foods. Also, her suicidal driving caused a minor crash, and thus, we dropped her team from the investigation.

These were politically turbulent times in Portugal. Early one Sunday morning as I was walking up an empty cobblestone street, President (and General) António Spínola swept past in a small white car surrounded by National Republican Guards—impressive solidly built, middle-aged men on eerily quiet motorcycles. Shortly afterward, there were mass demonstrations in Lisbon and an attempted coup, and President Spínola was forced to resign on September 30. Despite the unrest, I never felt threatened, even though an American consular official chilled me by saying that as a Portuguese-speaking American I would be suspected of being a Central Intelligence Agency operative.

Each week brought fewer new cases in Lisbon; they were widely scattered and difficult to locate in the labyrinthine streets. We visited the addresses of many subjects repeatedly and at odd hours before we caught them at home. Over 3 weeks our strenuous efforts interviewed just 34 case control pairs, 59% of the 58 reported new cases. On analysis of the data, I had my worst fears realized. My effort for almost 4 weeks had failed to associate cholera with any exposure. My CDC supervisors were dissatisfied. Portuguese officials were losing interest, and some nurses returned to their precholera duties. I was dejected and wanted to go home; however, I was learning how to operate in Portugal. My Portuguese was improving daily, and I was learning the limitations of case control studies. I wanted to try again with cases that had occurred earlier in the epidemic when single vehicles might have been important.

On September 20, in the midst of the Lisbon investigation, I was joined by Mark Rosenberg, an Afro-coifed, Earth Shoe-shod, first-year EIS officer from my branch (this was the 1970s, after all—I sported a bushy C. Everett Koop beard) (Figure 3-3). We quickly adapted to each other's work styles, and although he did not know Portuguese, he could communicate with many Portuguese professionals in French. He plunged into the work but helped the most by being an epidemiologist with whom I could discuss the details of our investigations face to face; he was the quintessential devil's advocate, sometimes to a fault.

FIGURE 3-3 Paul Blake and Mark Rosenberg in the Algarve, October 1974.

As the Lisbon case control study of current cases limped to a close, Mark and I explored possibilities for other studies. The Lisbon cholera nurses told us in late September that back in August they began to see cases in the upper and upper-middle classes for the first time. Many of these patients reported recent travel to Vimeiro Thermal Springs, a spa in Lisbon District but 50 km north of Lisbon in Torres Vedras County, and others had drunk Agua do Vimeiro, commercially bottled water from the same springs. At about the same time, prompted by two cholera cases in a nearby village, a sanitarian cultured water from the springs as part of a sanitation inspection of the area. On August 22, *V. cholerae* was isolated from the spring water samples. On the 23rd, the springs and the bottling plant were closed, and the bottled water was recalled. A press release was issued on August 24.

We painstakingly reviewed the Lisbon government cholera questionnaires for August; there was no bottled water question, but the nurses asked about it on their own initiative (smart nurses!) after they learned of the potential problem. Torres Vedras County had 16 cases in persons who worked at (4), visited (1), or lived near (11) the springs within 5 days before onset. In Lisbon District, excluding Torres Vedras County, 29 of 418 cases

reported visiting the springs, and at least 81 reported drinking Vimeiro bottled water within 5 days before onset. The peak number of cases appeared in all three groups (Torres Vedras county residents, spa visitors, and Vimeiro water drinkers) at about the same time—the last 2 weeks of August.

Our interest was piqued. We asked the Lisbon Health Director for a car and a sanitarian to visit the Vimeiro springs and bottling plant. He agreed, but for several days, there was one delay after another—car trouble, illness, and so forth. Finally, the light dawned—because a large business was involved, the situation was politically sensitive, and they did not want us to visit the springs but did not want to tell us that directly. We had been careful not to rock the political boat, but we decided it was time to take risks. Accordingly, I told the authorities that we understood how difficult it was to free up a car and a sanitarian for a day and that Mark and I would just hire a taxi and visit the plant without a health department escort. I feared that they might forbid it, but suddenly they found a car and a sanitarian to take us. Sr. João Florencia, the wiry, chain-smoking, espresso-fueled sanitarian who had collected the Vimeiro water samples, drove us sedately to the springs, giving us no hint of the driving style that he would exhibit on the ride back to Lisbon; in retrospect, he was still sizing us up.

The spa's owner gave us some statistics. In 1973, the previous year, about 20,000 people visited the spa during August, and about 70% of these were from Lisbon District. Approximately half of the bottled water was carbonated, and half was untreated. Usually about 10.5 million liters of water were bottled annually, but in 1974, production increased about 50%, apparently because people turned to bottled water for fear of cholera. The uncarbonated water was distributed in 5-gallon jugs and in smaller capped bottles (Figure 3-4) that sold for 3 escudos (10 cents). In August, the month of greatest demand, bottles could be on Lisbon store shelves within 4 hours after production. Approximately 42% of the bottled water was distributed outside of Lisbon District.

We visited the two springs, the spa, and the bottling plant. Most interesting was the Fonte Santa Isabel (Santa Isabel Spring), the source of most of the water. The Fonte lay less than 50 feet from a small river, the Ribeira de Alcabrichel, which carried sewage from upstream towns; cultures of river water samples collected on August 13 and August 26 yielded *V. cholerae* O1. The Fonte originally welled up spontaneously from the underlying limestone rocks, but subsequently, a large chamber was dug

FIGURE 3-4 Carbonated and noncarbonated Agua do Vimeiro.

into the limestone and covered with concrete, creating an underground reservoir. Untreated water was pumped from this reservoir to the baths, drinking water spigots (Figure 3-5), a swimming pool, and the bottling plant. Limestone aquifers are infamous for having underground fissures and channels through which water can flow rapidly. Five of six water samples collected from the Fonte on August 13, 22, 26, and 28 yielded *V. cholerae*. The springs were closed to the public on August 23 and were still closed when we were there.

In the midst of our tour, we had soft drinks, but the spa's bartender said he had been ordered not to charge us. We insisted that we could not appear

FIGURE 3-5 Termos do Vimeiro grotto with drinking water outlets (bottom).

to be "bought," but he looked shaken and resisted. Finally we just left money on the bar.

We finished late and had a wild ride back to Lisbon through the gathering night. João careened the VW beetle at up to 90 km/h through town and country on the narrow winding roads, flashing the high beams and passing on curves. He compensated for the car's anemic acceleration by not slowing for anything other than certain catastrophe. He said he had been a paratrooper until recently and didn't know the meaning of fear, but we certainly did. Fortunately, I was in the back seat (my invariable choice), but Mark sat in the front passenger seat which, João told him with relish, the Portuguese refer to as "o lugar do morto" (the place of the dead). João stopped half a block short of our hotel—to let us out, or so we thought. Instead, we were out of gas. We pushed the car to a gas station.

We planned a case control study in Lisbon to find out whether Agua do Vimeiro was associated with cholera, but our CDC supervisors vetoed it, pointing out that bottled water was a highly unlikely vehicle because it had never been shown to cause cholera or any other disease. They directed us toward Faro District (the Algarve), Portugal's southern coast where the epidemic had begun and the incidence was highest to see whether we could implicate shellfish. Mark left for Faro on October 8 to see whether studies there were feasible, and I followed 2 days later after tying up loose ends.

My calls to Atlanta to brief and consult with my supervisors were always challenges. Public telephones were invariably in noisy public places where I found it difficult to hear and to think, and I had to watch what I said in public. At the CDC end, a crowd would gather on a bad speaker phone, making the acoustics even worse, and interruptions were frequent, breaking trains of thought. Our study's progress was slow, and I was asked by someone at CDC, "Are you working nights and weekends?" This implied that I was loafing—I could barely contain my rage. I couldn't explain all of the details and subtleties by telephone, and I was plied with advice that I thought was misguided; however, I couldn't say that to my new bosses. I felt at a great disadvantage because I was new to the branch and had no significant publications from my EIS experience, although the branch was one of the most "academic," prestigious, and publication-oriented units at the CDC. I was afraid that I would return to the CDC a failure and would have no future in the branch. Thus, I was noncommittal on the phone and once off did what I thought was best. I wrote this to my wife: "I'm going to avoid calling Atlanta—they are trying to solve the problems without understanding the situation, and I can't explain it all to them at $2 a minute ($8 in today's dollars) standing in the embassy lobby surrounded by a dozen noisy people, shouting into the telephone, and barely able to hear. It takes me a couple of hours to calm down after every call. When Bill Baine investigated cholera in Italy he called them once in 2 months, and that sounds about right to me!" Nevertheless, I kept on calling as instructed.

SECOND INVESTIGATION—TAVIRA, FARO DISTRICT

We had a warm welcome in Faro, the capital of Faro District, although we had to "waste" a lot of time building relationships by enduring well-meant distractions—for example, a 7-hour tour of the district's many hotels and

seemingly endless irrelevant (although entertaining) stories. The district health director gave us a key to the health department for after-hours access and found nurses to help us.

We discussed the cholera epidemic with local health officials and pored over their lists of cases to chart the course of the epidemic in the various municipalities. The first case had been detected in Tavira, a coastal town in Faro District near the Spanish border. Founded by the Phoenicians over 2,700 years ago, Tavira is known for its "Roman" bridge (actually Moorish from the 12th century) over the Gilão River. The river flows through the town into the Ria de Faro, a coastal strip of mud flats and islands 50 km long and up to 5 km wide that separates Tavira and Faro from the open sea and supplied most shellfish consumed in Portugal. Raw sewage from coastal towns emptied into the Ria, where water and shellfish had been known to have high coliform bacteria counts for at least a decade. After anecdotal reports of shellfish causing cholera, the Maritime Biology Institute in Faro isolated *V. cholerae* from 24% of seawater and 42% of shellfish samples from the Ria between May and August 1974.

We went to Tavira to try to find out how the epidemic began. Local health officials pointed out elements that might have contributed to the outbreak—raw sewage flowing into the tidal river, people gathering shellfish near the sewage outlets ('where the cockles are fattest"), sewage and water lines under repair, and two closed springs. Although chlorinated, Tavira's municipal water supply was suspect because the water lines were old, ruptured frequently, and ran beside leaking sewage lines. Water and sewer system renovation began in 1973, and we found excavated streets and wooden plugs in exposed pipes. We were told that when cholera first occurred heavy rain filled the excavations with sewage-contaminated water, enhancing the potential for sewage to contaminate potable water. Two suspect springs within the town were closed on May 10 and May 11.

The first detected case in Tavira (this was also the first case detected in Portugal, as described previously) had onset of illness on April 24. No other cases were identified for 13 days, but then a cluster of 14 cases in Tavira had onset between May 6 and May 15, followed by other clusters within the town over the succeeding months. Review of Tavira hospitalization records revealed an increase in diarrheal illnesses the second half of April; thus, there may have been some undetected cholera in April, and there may have been cases before the first detected case. We decided to focus on the first 15 cul-

ture-proven cases in Tavira, hoping that our findings would help us to understand how the Portuguese epidemic began.

In planning an investigation, we worried that recall of specific exposures 6 months before would be difficult for cases and worse for control subjects who had no illness as a reference point; however, we guessed (correctly, as we found out) that subjects would be able to recall their usual practices and unusual experiences like travel. Our questionnaire asked about demographic data; travel; frequency of eating raw vegetables, fruits, and seven varieties of shellfish; shellfish cooking methods; and drinking water sources. We asked all subjects about exposures during April and May and also asked the cholera patients about exposures during the 5 days before the onset.

Working with two nurses, we located and interviewed 14 of the 15 initial cases and matched controls in 2 days (October 14 and 15). The work went quickly because of short distances and relative ease in locating the patients. Our excitement mounted as case after case said that they liked the flavor of the water from one of the two local springs, the Fonte do Bispo, so much that they regularly walked across town to fill their jugs. Furthermore, they were angry that it was closed because decades of drinking that water had never made them sick. Eleven of 14 cases and none of 14 control subjects recalled drinking water from the Fonte do Bispo. We constructed a table that shows how matched-pair case control data are analyzed (Table 3-1). It maintains the matching, and the numbers refer to case control pairs of individuals rather than just to individuals. The probability that the result of our interviews occurred by chance is calculated using just two cells: pairs in which the case drank but the control did not

Table 3-1 Distribution of 14 Case Control Pairs by History of Drinking Fonte do Bispo Water During April and May, 1974, Tavira, Portugal

	Control		
Case	**Drank**	**Did Not Drink**	**Total Pairs**
Drank	0	11	11
Did not drink	0	3	3
Total pairs	0	14	14

The other local spring was not implicated.

(11) and pairs in which the control drank but the case did not (0). The two-tailed exact test for matched pairs testing our hypothesis that having cholera was associated with drinking water from the Fonte do Bispo yielded a *P* value of 0.001, and the relative risk (11/0) was infinite. More than a month after arriving in Portugal, we had a significant *P* value!

Our epidemiologic analysis failed to explain the index case in which the person did not drink water from the Fonte do Bispo or travel outside Portugal in 1974. Although our analysis had not demonstrated that having cholera was statistically associated with eating raw or partially cooked shellfish, the story from the index case suggested that they played a role. Three days before onset of illness, he gathered cockles from the Ria near the mouth of the Gilão and heated them only until they opened, and then he and two others ate them. Only the patient, who took antacids, developed diarrhea. There was no suggestion that any cases were related to drinking municipal water, and thus, the broken pipes appeared to be a red herring.

How might *V. cholerae* O1 El Tor serotype Inaba, the epidemic strain, have been introduced into Portugal? Soldiers traveled back and forth from a military training base 120 meters uphill from the Fonte do Bispo to the wars in Portugal's three African colonies—Angola, Mozambique, and Portuguese Guinea—where El Tor Inaba cholera was endemic. Sewage from the base emptied into the Gilão and flowed to the Ria. Thus, vibrios from an infected soldier could be taken up by filter-feeding shellfish in the Gilão and the Ria. Then people infected by eating contaminated shellfish would discharge more vibrios down the river, and the epidemic would be underway. Even though they were thousands of miles away, the African colonies were a much more likely source than nearby North Africa, where only El Tor Ogawa cholera was being reported. Subsequently, phage typing, a more sensitive method than serotyping to detect differences between cholera strains, showed that the 1974 Portuguese Inaba strains were indistinguishable from Angolan Inaba strains. Angola is a south-central African country that was a Portuguese colony until 1975.

Health officials had suspected that the Fonte do Bispo (a pipe emerging from the side of a hill through a concrete wall on a street corner) (Figure 3-6) caused a typhoid outbreak long before the advent of cholera; however, the public would not let them close it because they did not believe that it had caused the outbreak and they liked the flavor of the water. The officials said the spring produced clear water until September 1973, when, after construction blasting of the rock behind the spring followed by a

FIGURE 3-6 Fonte do Bispo, Tavira, Portugal, October 1974.

heavy rain, the emerging water was muddy for a few days. A sewer line running down the hill beside the spring could have been damaged during the blasting. The sewer line was renovated in 1973, but it was unclear whether that occurred before or after the blasting. Perhaps damage from the blasting allowed sewage from persons infected by shellfish or from troops up the hill to pollute the spring. Unfortunately, dye testing was not politically feasible.

THIRD INVESTIGATION—FARO

There is nothing like a significant *P* value to raise epidemiologists' spirits. Now that we knew how the epidemic began, we wanted to examine the vehicles of transmission during the rest of the epidemic. We decided to try to continue our investigations in Faro District because we had good working relationships there and it had the highest incidence of cholera in Portugal. We immersed ourselves in analyses of Faro District data to pick our next target. We chose as our subjects the 59 cases identified in the city of Faro during the 5 months of May through September. Only eight cases occurred during May through July, but there were 51 cases during August

through September. Compelling anecdotes pointing to shellfish abounded. In one instance, four small boys found a pile of cockles by the shore, heated them on a flattened tin can over a small fire until they opened, and ate them. All four developed diarrhea, and stool from one was cultured and yielded *V. cholerae*. Our questionnaire asked cases and individually matched controls about exposures during a 2-month period—the month of onset of illness and the nearest adjacent month. On October 18, as the study began, Mark was recalled to the CDC because the branch was so shorthanded that our supervisors feared (horrors!) that they would have to investigate the next outbreak themselves. Two nurses and I interviewed and matched 53 cases over the next several days and showed that eating raw or semicooked cockles was significantly associated with cholera. I was ecstatic. These findings added credence to the theory that distribution of contaminated live shellfish from the Ria throughout Portugal could explain the rapid spread of cholera nationwide (Figure 3-7).

Now I had been in Portugal for over 6 weeks, and I ached to go home; however, on my next call to Atlanta (the worst yet, from a bar packed with rowdy tourists), my supervisors changed their minds about the plausibility of bottled water as a vehicle for cholera. Now, after Mark briefed them

FIGURE 3-7 Live cockles (above) and clams in a Lisbon bar.

in person, they wanted me to conduct a case control study of Agua do Vimeiro in Lisbon. I finished up in Faro, flew back to Lisbon on October 26, and plunged into planning the investigation.

FOURTH INVESTIGATION—BOTTLED WATER

I was able to use the available data from the Lisbon Health Department's cholera case investigation forms in a retrospective cohort approach to show that visiting the springs was associated with cholera. During August, 36 (2.57/1,000) of the estimated 14,000 visitors to the springs from Lisbon District, excluding Torres Vedras County, had cholera, but only 382 (0.25/1,000) of 1,530,831 who did not visit the springs had cholera. The cholera risk was 10.3 times greater for visitors than for nonvisitors. The big question, however, was whether bottled Agua do Vimeiro had caused cholera.

I decided to study Lisbon District cases with onset during the week ending August 24 for several reasons: The government's cholera questionnaires showed the number of new cases in persons who recalled drinking Agua do Vimeiro in the 5 days before onset peaked during that week; it was the last week when bottled Agua do Vimeiro was available in stores (it was recalled on August 23). A news release on August 24 said that the bottled water was suspect, and thus, after that date, the public would be less likely to drink bottled water they bought before the recall. Also, water collected from the Fonte Santa Isabel on August 22 was positive for *V. cholerae* O1. When I reviewed the government's cholera case investigation forms more carefully to identify the cases for study, I found that some cases had date of positive culture but not date of onset. Allowing for delay between onset and positive culture, I included cases with no recorded onset date if the patient's positive culture was between August 22 and 28. That gave me 47 symptomatic cases. I then excluded six who visited the springs (they might have been infected by drinking the water directly from the springs), three less than 10 years of age (their recall might be inaccurate), one who was not the first case of cholera in the family (cholera can spread through multiple vehicles within households), and two nonresidents who were ill before arriving in Lisbon (they were not infected in Lisbon District), leaving 35 for the investigation. Planning the study was the easy part, however; now I had to get help.

When I approached the Lisbon District Health Director, it was clear that I had worn out my welcome. Lisbon had been cholera-free for 8 days, and cholera was old news. Even though 4 days earlier he had told me by telephone that he would provide nurses to investigate Agua do Vimeiro, he now said rather brusquely that he could not. I didn't know if he really could not, if he just wanted to get rid of me, or if cholera from bottled water was so politically sensitive that he had been told to not let me touch it. I suspected the last. One official told me confidentially that under the dictatorship, before the Carnation Revolution, the public would never have known about the contamination at Termos do Vimeiro because it was a big business—it would have been hushed up. Although the revolutionary government had recalled the water and issued a press release in late August, now more than 2 months had passed, and there was reluctance to bring fresh attention to the problem through an epidemiologic investigation by a foreigner.

I visited the national epidemiologist with all of the results to date and made the case for the investigation. I told him that all I needed was a car and driver—no nurses—so that I could track down cases nights and weekends when they were most likely to be home and that when it was done I would stop bothering him and go back to Atlanta. Somehow he was able to get me the best help possible—the sanitarian João Florencia and a car. We began the study the next day.

Investigating cases with João was a revelation. With no previous experience in epidemiology, he quickly grasped the investigation's logic and techniques and worked enthusiastically far into the night, over the weekend, and on All Saints Day even though he was not paid for overtime. Finding cases in Lisbon was often exceedingly difficult. Addresses were incomplete. There were multiple streets with the same name, and some streets were only a few houses long; however, with the aid of a detailed street guide in tiny print and his experience as a sanitarian, João found almost all of them. He also proved to be an excellent interviewer, maintaining rapport and eliciting information without "leading" the interviewees. Throughout my career, I was to find that one of the pleasures of working in the field with local coworkers was serendipitous encounters with extraordinary people.

Interviewing cases at night led to awkward situations. At 10:30 one night we sat in our VW on a dark street waiting for a 17-year-old schoolgirl to return home. A person with high thick-heeled shoes, long hair, and bell-bottoms came clopping down the street and approached the door with

a young man, so I got out to interview her. At the door I asked, "Are you Constância Engrácia?" Unfortunately, the person was a young man, and I asked the question while looking him full in the face. His friend exploded with laughter while I tried to blame the darkness.

Another night we traced an older woman with cholera to a palatial mansion and were interrogated on the marble steps politely but suspiciously by the patient's son, an admiral. Apparently he checked us out with the authorities because the next day the national epidemiologist said with a knowing smile, "So you have been visiting admirals late at night?"

We tracked down 32 of the 35 patients (91%) and found neighborhood control subjects matched by age (within 5 years), gender, ethnic group, and approximate socioeconomic status. The cases and controls were asked whether, during August, they drank carbonated or uncarbonated bottled Agua do Vimeiro or visited the springs. As the investigation progressed, it became increasingly obvious that bottled water would be associated with cholera, and I worked in an advanced state of euphoria.

My fear of failure was gone. I knew that I would be going home soon, and I reveled in the opportunity to immerse myself in Lisbon and all things Portuguese. It was a privilege to talk with people at every social level in their homes and in their language. I found places that I dimly remembered from having lived in Lisbon for 8 months in 1947–1948 when I was 4 years old—our basement apartment at 22 Abaracamento de Peniche, a small park with a spreading tree under which I had played, and the botanical garden. I savored Portuguese food and music; I had café com leite and superbly crusty and chewy pães pequenos for breakfast, bife a Portuguesa for lunch, and concoctions of potato, onion, tomato, and fish with olive oil for dinner. I drank one brand of orange soft drink almost exclusively and then learned at the end from João that it had the worst coliform counts among the soft drinks. I continued, however, to add iodine to my drinking water, didn't have a salad in 9 weeks, and stayed well.

The results were clear-cut: 13 cholera patients, but only two control subjects had consumed bottled non-carbonated Agua do Vimeiro ($P = 0.003$) (relative risk = 12). Interestingly, cholera was not associated with drinking carbonated Agua do Vimeiro, which made sense because carbonated water is acidic and *V. cholerae* cannot tolerate a low pH. The bottled water had infected all levels of society from an admiral's mother living in a mansion to someone living under metal roofing leaning against a wall. As I pored over the national data, I began to suspect that Agua do Vimeiro caused

many cases all over Portugal because the epidemic peaked in the north (Porto), middle (Lisbon), and south (Faro) and in some other districts during the last 2 weeks of August, coinciding with contamination of the bottled water. Although 42% of the bottled water was distributed outside of Lisbon District, I wondered if vibrios could survive being trucked long distances at ambient temperature. It was too late to do more investigations, but I returned to our incompletely analyzed Faro case control data and discovered that we had implicated Agua do Vimeiro in Faro without realizing it! In Faro, nine cases and two control subjects reported having drunk Agua do Vimeiro ($P = 0.046$), and the association remained significant ($P = 0.031$) when controlling for eating cockles. Because the spa and the bottled water plant were closed on August 23 but the spring remained culture positive until at least August 28, stopping access to the spring water clearly prevented many cases of cholera. The Portuguese government did not allow the bottled water plant to reopen until the water source was changed to a deep well drilled in the same area as the Fonte Santa Isabel; however, at a higher altitude, the well water was shown to contain no pathogenic bacteria, and the plant began to treat the water with ultraviolet light before bottling.

WRAP-UP

I prepared a report for my exit interviews with Portuguese officials, and after 9 weeks, my work in Portugal was finally done. I felt, however, that I had barely scratched the surface of the possibilities that the cholera epidemic in Portugal presented for understanding cholera transmission. Once *V. cholerae* O1 is widely distributed by a vehicle of transmission (in Portugal raw shellfish), each infected person excretes enormous numbers of vibrios that can then contaminate foods (where they can multiply) and water and cause other outbreaks. Thus, the epidemic curve describing the course of an epidemic may represent the combined effects of many outbreaks, large and small, caused by a variety of vehicles, with only the largest outbreaks (such as the bottled water outbreak) having enough cases to cause marked distortion of the overall epidemic curve. Bits and pieces of information from across Portugal suggested that further investigations could have been fruitful. I mourned the lost opportunities—among others, a large inland outbreak attributed to a contaminated well in Portalegre, a sharp and massive outbreak in Porto affecting all age groups equally that may have been caused by public water, and a daycare center outbreak in

Portimão that may have been caused by the diaper washer reconstituting powdered milk.

I returned to Atlanta through Geneva at the request of the WHO. Epidemiologists often feel that the value of outbreak investigations is self-evident; however, that is not true, and it was certainly not the case at the WHO in the early 1970s. Our data, however, impressed the WHO officials, and they asked me to write a simple description of how to perform matched-pair case control studies to determine vehicles of transmission for use and publication by the WHO. Subsequently I complied, thinking it would help the WHO provide critical assistance to countries with epidemics, but in fact, it was buried in an appendix of a WHO monograph on shellfish hygiene.[2] Sic transit gloria mundi (thus passes the glory of the world). Nevertheless, at the WHO, the successful cholera investigations in Portugal and Italy lent credibility to CDC investigations and may have helped ease the way for future requests from the WHO for CDC epidemiologists to investigate outbreaks worldwide.

On November 29, 1974, Portugal was declared free of cholera. In all, 2,467 culture-confirmed cases and 48 deaths were reported to the WHO. The case-fatality ratio was 1.9%, remarkably low considering that only the more severe cases were likely to be culture confirmed. Cholera did not reappear the following year. In 1974, five European countries reported 10 cases of cholera imported from Portugal. By writing to a case's physician in England, I learned that the patient visited Vimeiro Thermal Springs in mid August and drank spring water there.

Back in Atlanta, I struggled to find time to complete the analyses and write up the results, and I quickly discovered that my work had just begun and that the "fun" part was over. Over the years, I have seen many exquisite investigations (some of them, sadly, my own) that failed to achieve their potential public health impact and faded from memory because the investigators lacked the self-discipline to publish them. I had little experience in scientific writing, and organizing the results from our multiple studies in Portugal was particularly difficult. Now any resentment I harbored against my supervisors from our difficult communications in Portugal faded as they provided superb mentoring one on one. With help from my supervisors and coworkers, I eventually produced two papers (with five Portuguese coauthors) that we thought were ready for publication, and in September 1975, I sent them to the Portuguese director general of health for approval.

Two months dragged by with no response from Portugal, and thus, I sent the papers again stating that we planned to submit them to a journal on December 15 but could not include Portuguese coauthors without written permission. That provoked a reply. On December 11, the director general wrote that he would not agree to publication of the papers in their present form because they could harm tourism. I was crushed. With my supervisors' coaching, however, I painfully made many small changes in the papers that I should have made in the first place, trimming some place and brand names and stressing (accurately) the Portuguese government's vigorous and appropriate response to the epidemic: case investigations; tetracycline treatment of contacts; no mass vaccinations; public health education; chlorinating water; closing the Fonte do Bispo and the Vimeiro springs, spa, and bottled water plant; recalling the bottled water; monitoring bottled water quality; and accepting CDC collaboration. I sent the director general the revised papers, a detailed list of the changes, and a properly humble letter, and by May 1976, he approved publication. The papers were finally published in 1977.[3,4]

Our investigations' impact on Portugal is difficult to judge. The 1974 cholera epidemic was ending as we arrived, and thus, we could not take any credit for controlling that epidemic; however, we showed how epidemiologic investigations could systematize and quantitate the things that health officials had suspected, proving some and disproving others. Unlike 1971, when the cause of the cholera outbreak in Lisbon remained a mystery, our investigations in 1974 showed that cholera may have been imported from Angola by the military, that contamination of the Fonte do Bispo infected many people and helped amplify the number of organisms in the environment, that contaminated shellfish caused many cases in southern Portugal and could have disseminated cholera throughout Portugal, and that pollution of two springs north of Lisbon caused many cases in visitors to the springs and in people in Lisbon, Faro, and possibly throughout Portugal who drank bottled uncarbonated spring water. We hope that statistical incrimination of these vehicles helped stiffen prevention measures and thus helped prevent future epidemics.

Our investigations contributed to scientific knowledge about transmission of cholera, including the most conclusive evidence ever presented that cholera could be transmitted by shellfish contaminated before harvest, the first reports that spring water contaminated before it emerges from the ground can transmit cholera, and the first report that bottled uncarbon-

ated mineral water can transmit cholera. Because investigators sometimes focus on known vehicles and disregard possible vehicles that have not been implicated previously, publishing this information may have saved lives by alerting health authorities to these potential vehicles in prevention and control of cholera worldwide. It also had one tangible impact: The CDC changed its recommendation for international travelers to areas where chlorinated tap water is not available or where hygiene and sanitation are poor. Until 1974, the CDC recommended that one option for such travelers was to drink bottled water. After 1974, the recommendation was changed to bottled carbonated water. Carbonated mineral water is still on the list of recommended beverages for travelers.[5] Dramatic advances—many from the CDC's Enteric Diseases Branch—in understanding vehicles for cholera transmission have occurred since our investigations.[6] Foods have proven to be more important vehicles than was thought previously and include raw and cooked seafood, cooked grains and legumes, and frozen coconut milk.

This 9-week investigation shaped the rest of my career in epidemiology. It improved my epidemiologic skills and self-confidence and gave me a record of accomplishment that helped secure my career in CDC's Enteric Diseases Branch, where I worked for the next 20 years, eventually as branch chief. Cholera and other vibrio-related diseases became my special interest. The investigation made me acutely aware of the limitations of supervision by telephone and pushed me toward letting field epidemiologists use their own judgment. It enhanced my Portuguese*, leading to subsequent work in Angola, Mozambique, Brazil, and Portugal. Most important, it taught me valuable lessons—and gave me a rich source of anecdotes—that I used in mentoring EIS officers, preventive medicine residents, and other epidemiologists:

1. Matched-pair case control studies with neighborhood controls can be a powerful tool, and people with the right temperament can be trained quickly to interview subjects and select matched controls; however, supervise them carefully, and don't send them out alone until they have demonstrated competence.

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* I wrote to my wife: "For two months I thought people were telling me that they were constipated, and I tried not to listen, but now I learn that 'constipado' means 'congested,' as in stuffy nose."

2. Take pains to develop good hypotheses before plunging into an investigation.
3. Keep an open mind about possible vehicles of transmission—the fact that a vehicle seems to be unlikely (e.g., spring water) or has not been implicated before (e.g., bottled water) does not rule it out, and experts, scientific articles, and textbooks can be wrong.
4. Without conclusive evidence, don't assume that any potential source of infection, no matter how logical and likely (e.g., decrepit water pipes and sewers in Tavira), is actually a source.
5. Scattered cases at the end of an epidemic may not provide useful information; focus on the heart of the epidemic curve, any unusual peaks, and the beginning.
6. Although it is best to investigate soon after illnesses occur, you can get a history of exposures even months later if you ask about usual practices like customary sources of water or memorable one-time exposures like travel.
7. Local investigators will tire before you do—they have different motivations and their usual work is backing up. Thus, work as quickly and efficiently as possible, and treat local investigators as colleagues and coworkers rather than as errand runners.
8. Try to understand the local officials' point of view, and adapt to it as much as possible without distorting the science—they will be dealing with the consequences of the investigation (and any publications) long after you are gone.
9. Resisting the urge to cut corners and go home can really pay off; it is better to stay in the field until you have completed the studies and preliminary analyses and identified and filled gaps in knowledge such as the distribution of incriminated products.
10. Don't pack an unused jar of thiosulfate citrate bile salts sucrose agar powder, a culture medium for *V. cholerae*, with your precious papers on your trip home; when it breaks, your papers will have a sticky green crust forever.

My experience in Portugal hooked me on epidemiologic investigations for life, and even now in retirement, I get a rush when I can contribute to an investigation. To me, the greatest joy of epidemiologic investigation is trying to solve the mystery. Initially, the situation often appears chaotic, with people becoming ill for no apparent reason; however, there is always

order beneath the chaos, with everything happening for a reason. It is our fascinating job as epidemiologists to investigate, tease out the truth, and describe what happened and why it happened so that it can be stopped now and prevented in the future. The satisfaction that comes with finding out why things happen is immense.

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