EMERGING INFECTIOUS DISEASES®

November 2011

Cholera

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On the Cover

Georges Desarmes (b. 1950)
The Bathers (2006)
Acrylic on canvas
(61 cm × 50.8 cm)

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Conference Summary

Deaths Associated with Pandemic (H1N1) 2009 among Children, Japan, 2009–2010

Akihisa Okumura, Satoshi Nakagawa, Hisashi Kawashima, Takashi Muguruma, Osamu Saito, Jun-ichi Fujimoto, Chiaki Toida, Shuji Kuga, Toshihiro Imamura, Toshiaki Shimizu, Naomi Kondo, and Tsuneo Morishima

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Release date: October 21, 2011; Expiration date: October 21, 2012

Learning Objectives

Upon completion of this activity, participants will be able to:

- Distinguish the most common presenting symptom in fatal cases of pandemic (H1N1) 2009 infection among children
- Assess the most common causes of death among children with pandemic (H1N1) 2009 infection
- Analyze the causes of death in fatal cases of pandemic (H1N1) 2009 infection among children.

Editor

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To clarify the cause of deaths associated with pandemic (H1N1) 2009 among children in Japan, we retrospectively studied 41 patients <20 years of age who had died of pandemic (H1N1) 2009 through March 31, 2010. Data were collected through interviews with attending physicians and chart reviews. Median age of patients was 59 months; one third had a preexisting condition. Cause of death was categorized as unexpected cardiopulmonary arrest for 15 patients, encephalopathy for 15, and respiratory failure for 6. Preexisting respiratory or neurologic disorders were more frequent in patients with respiratory failure and less frequent in patients with unexpected cardiopulmonary arrest. The leading causes of death among children with pandemic (H1N1) 2009 in Japan were encephalopathy and unexpected cardiopulmonary arrest. Deaths associated with

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respiratory failure were infrequent and occurred primarily among children with preexisting conditions. Vaccine use and public education are necessary for reducing influenza-associated illness and death.

A novel reassortant strain of influenza A (H1N1) virus containing swine, avian, and human elements (*1*) emerged in Mexico in March 2009. The virus initially spread within North America, causing severe respiratory illnesses in Mexico (*2*) and the United States (*3*,*4*), and then began to spread rapidly worldwide. On June 11, 2009, the World Health Organization confirmed an influenza pandemic.

In Japan, the first case of pandemic (H1N1) 2009 was confirmed on May 16, 2009. The first outbreak occurred in western Japan, where the number of cases increased then decreased quickly. The second outbreak started in early June and quickly spread to all parts of Japan. The first death associated with pandemic (H1N1) 2009 in Japan was confirmed August 15, and the first death of a child occurred September 17. As of March 31, 2010, the Ministry of Health, Labour, and Welfare (MHLW) reported on its website (www.mhlw.go.jp/kinkyu/kenkou/influenza/houdou.html) that 198 patients in Japan with pandemic (H1N1) 2009 had died, of whom 41 were children <20 years of age.

Several authors have reported that respiratory diseases associated with pandemic (H1N1) 2009, including viral pneumonia and acute lung injury, that required intensive care occurred most often in children (*5*–*16*). In Japan, hospitalizations of children because of severe pneumonia or other respiratory complications increased (*17*). Concerns were raised regarding deaths among children from acute encephalopathy in association with pandemic (H1N1) 2009 because acute encephalopathy has been associated with death from seasonal influenza in Japan (*18*,*19*). Neurologic complications associated with pandemic (H1N1) 2009, including acute encephalopathy, altered mental status, and status epilepticus, also have been reported from other countries (*20*–*23*).

Accurate data on the causes of death associated with pandemic (H1N1) 2009 among children are necessary for making a counterplan against future pandemic influenza. We investigated detailed clinical data collected by MHLW for children whose deaths were associated with pandemic (H1N1) 2009. We focused on the direct cause of death and clinical differences by cause of death.

Materials and Methods

After the first patient was identified in May 2009, all medical professionals were required to report deaths associated with pandemic (H1N1) 2009 to MHLW. Press releases on patient deaths were provided on MHLW's website. As of March 31, 2010, a total of 41 patients <20 years of age were listed.

Infection with pandemic (H1N1) 2009 virus was confirmed with nasal swab specimens or aspirates from the nose, throat, or tracheal tube by using real-time reverse transcription PCR (RT-PCR) at local public health laboratories or the National Institute of Infectious Diseases in Japan, according to the institute's recommended protocol. Samples for RT-PCR could not be obtained for 3 patients; however, rapid antigen tests were positive for influenza A for all 41 patients. Because influenza A viruses other than pandemic (H1N1) 2009 virus were rarely isolated in Japan during the study, these 3 patients were included in our analysis.

Two research groups collaborated to collect detailed data on deaths associated with pandemic (H1N1) 2009 among children. The collaborative study group comprised 3 chief members (A.O., S.N., and H.K.) and 6 assistant members (T.M., O.S., J.F., C.T., S.K., and T.I.). During February–June 2010, members of the collaborative study group contacted the attending physician of each child who died and visited the hospital to obtain detailed information. We abstracted data from medical records by using a structured report form and obtained demographic, clinical, laboratory, and radiologic data from interviews with attending physicians and chart reviews. Onset of influenza was considered the time at which a temperature >38°C was first recorded. The chief members of the study group reevaluated chest radiographs; computed tomography (CT) scan of the head, chest, and abdomen; and magnetic resonance images of the head, including those obtained at autopsy.

Cause of death (Table 1) for each patient was categorized after the 3 chief members reviewed the detailed clinical course and laboratory and radiologic data. At first, each chief member independently presumed the cause of death for each patient. When they agreed on the presumed cause of death, it was adopted as a cause of death. When the chief members disagreed, they reached a consensus on the cause of death after discussion.

Because the study was considered to be a public health activity entailing surveillance of deceased persons, approval from an ethics committee or institutional review boards at participating hospitals and informed consent were not required. Anonymous data were collected retrospectively and were kept confidential.

Statistical analyses were performed to identify differences among patients by cause of death. Because the number of patients who died of myocarditis and viral sepsis was small, these cases were excluded from statistical analyses. We also excluded 1 patient who died of presumed incidental intracranial hemorrhage. The Kruskal-Wallis test was used to compare numerical variables. When a p value

Table 1. Causes of death for 41 patients <20 years of age with pandemic (H1N1) 2009, Japan, May 2009–March 2010

Cause	Definition
Unexpected cardiopulmonary arrest	Cardiopulmonary arrest without clear findings of respiratory failure, cardiomyopathy, or encephalopathy
Respiratory failure	Desaturation, need for oxygen supplementation or mechanical ventilation or both, associated with radiologic findings of pneumonia or acute lung injury
Myocarditis	Markedly reduced cardiac output, severe and refractory arrhythmia, or severe circulatory collapse
Viral sepsis	Refractory hypotension and rapidly progressing multiorgan failure associated with at least 2 of the following: tachypnea; leukopenia <4000 cells/μL or leukocytosis >12,000 cells/μL; tachycardia; body temperature >38.0°C or <36.0°C; cold extremities; and increased capillary refill time
Encephalopathy	At least 1 of the following: altered mental state without profound respiratory and cardiac failure or neuroimaging findings consistent with encephalopathy such as marked brain edema, focal lesions, and blurred gray-white matter junction
Incidental	Other findings that are not directly attributable to influenza infection

<0.05 was obtained by Kruskal-Wallis test, post hoc testing was performed by using the Tukey test. We compared categorical variables by using the χ^2 test. When the χ^2 test gave a p value <0.05, adjustment residual analysis was performed. An absolute value of the adjustment residual >2 was considered significant.

Results

Study Population

Deaths included in the study were distributed almost evenly throughout Japan. The timing of infection with pandemic (H1N1) 2009 virus was concentrated primarily during October 2009–January 2010 (Figure 1).

Median age of children was 59 months (range 7–206 months); 20 (49%) patients were 0–4 years of age and 12 (29%) were 5–9 years of age (Figure 1). Twenty-five (61%) patients were boys. Fourteen (34%) patients had ≥1 preexisting conditions. Respiratory disorders (at least 1 of asthma, chronic lung disease, or a disorder necessitating tracheostomy) were present in 9 patients, none of whom were receiving systemic corticosteroids. Neurologic disorders (at least 1 of cerebral palsy, mental retardation, epilepsy, or neuromuscular disease) were present in 11 patients, 9 of whom had ≥2 neurologic disorders, and 7 had concurrent respiratory disorders. No patients had endocrine or immunologic disorders or obesity. History of febrile seizures was noted for 6 (15%) patients. One patient had been vaccinated against pandemic (H1N1) 2009 virus and another 2 against seasonal influenza virus. The other 38 had not been vaccinated against pandemic (H1N1) 2009 virus or seasonal influenza virus. Close contact with a person who had influenza within a few days before symptom onset was reported for 15 (44%) of 34 patients for whom this information was available.

Information about clinical signs and symptoms of infection with pandemic (H1N1) 2009 virus was available for all but 1 patient. Clinical signs included temperature >38°C (40 [100%] patients), cough (20 [50%]), rhinorrhea (12 [30%]), tachypnea (10 [25%]), dyspnea (12 [30%]), and wheezing (6 [15%]). Vomiting was observed in 8 (20%) patients; diarrhea (3 patients), tachycardia (3), headache (1), and myalgia (2) were rare.

Influenza was diagnosed by rapid antigen test within 2 days after onset of fever for 39 (95%) patients. Before the life-threatening event, 19 (46%) patients received oseltamivir and 5 (12%) received zanamivir. These antiviral drugs were prescribed soon after diagnosis of influenza by rapid antigen test. Acetaminophen was administered to 13 (39%) of 33 patients for whom this information was available.

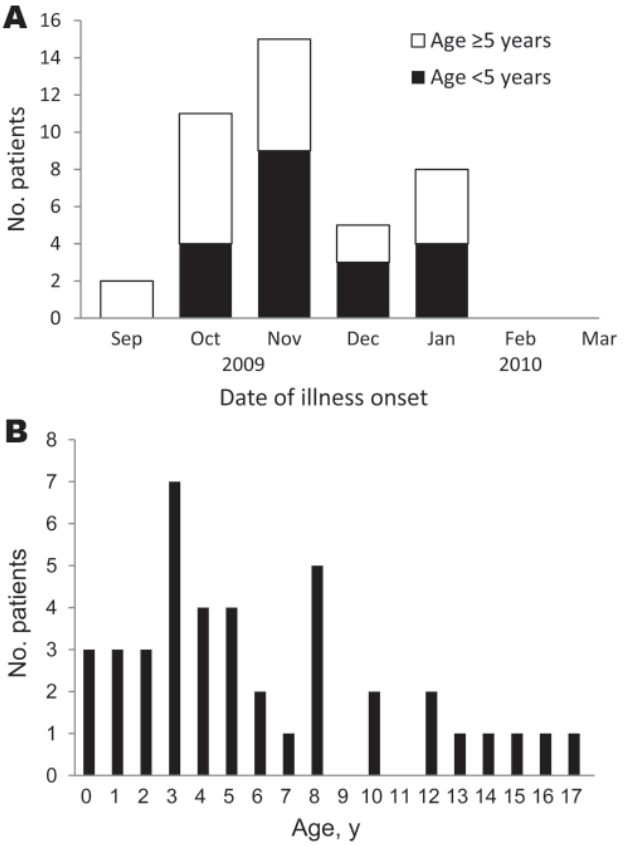

Figure 1. Timing of onset of pandemic (H1N1) 2009 in children and patient age, Japan, May 2009–March 2010. A) Date of illness onset for children ≥15 years of age compared with those <15 years of age. B) No. patients at each age at time of illness onset.

For 34 (83%) children, a life-threatening event occurred within 2 days after influenza onset (Figure 2). Twenty-nine (71%) children died within 4 days after influenza onset (Figure 2). Patient age, interval between onset of fever and life-threatening event, or interval between onset of fever and death did not differ by presence or absence of preexisting conditions. Death was confirmed in an emergency department for 14 patients, intensive care unit for 13, inpatient ward for 12, outside of a hospital for 1, and outpatient clinic for 1.

Blood culture test results were positive for only 1 of 21 patients who had at least 1 blood culture; this patient had had pneumonia associated with methicillin-resistant *Staphylococcus aureus* (MRSA) before infection with pandemic (H1N1) 2009 virus. Bacterial cultures from respiratory tract samples were positive for 2 of 16 patients (1 with MRSA and 1 with *Streptococcus pneumoniae* infection). Information about pathologic findings was not available for any of the 6 patients for whom postmortem examinations were conducted.

Causes of Death

Cause of death was categorized as unexpected cardiopulmonary arrest (CPA) for 15 patients, encephalopathy for 15, respiratory failure for 6, myocarditis for 2, viral sepsis for 2, and incidental for 1. Median age of patients who died of unexpected CPA was 43 months. Only 1 of these patients had a preexisting condition. For 13 patients, unexpected CPA occurred outside the hospital; most patients were presumed to have been found several hours after CPA. Two patients experienced unexpected CPA in the hospital, 1 in the outpatient clinic and 1 during hospitalization. Chest radiographs and CT scans of the head and chest were unremarkable for all children examined.

Encephalopathy was considered the cause of death for 15 patients (median age 62 months). Five of these patients had a preexisting condition, and 3 had preexisting neurologic disorders. All 15 patients had altered mental state or convulsions or both and marked brain edema according to head CT scan or magnetic resonance images or both, which suggests increased intracranial pressure. Nine patients also had low-density areas in the bilateral thalami or brainstem or both. Most patients had clinical brain death within several hours after onset of encephalopathy in association with multiple organ failure. For some patients, mild infiltration was seen on chest radiograph, but pulmonary involvement was not likely the cause of death.

Six patients were judged to have died of respiratory failure; their median age was 78 months. Five of these patients had preexisting neurologic conditions and had radiologic findings consistent with severe pneumonia. Two had been hospitalized because of pneumonia attributable to other pathogens (MRSA for 1 and undetermined for the other) before infection with pandemic (H1N1) 2009 virus; their respiratory state markedly worsened after infection. Nosocomial transmission was strongly suspected, and influenza was diagnosed for both patients on the day after fever onset. One previously healthy patient had severe and

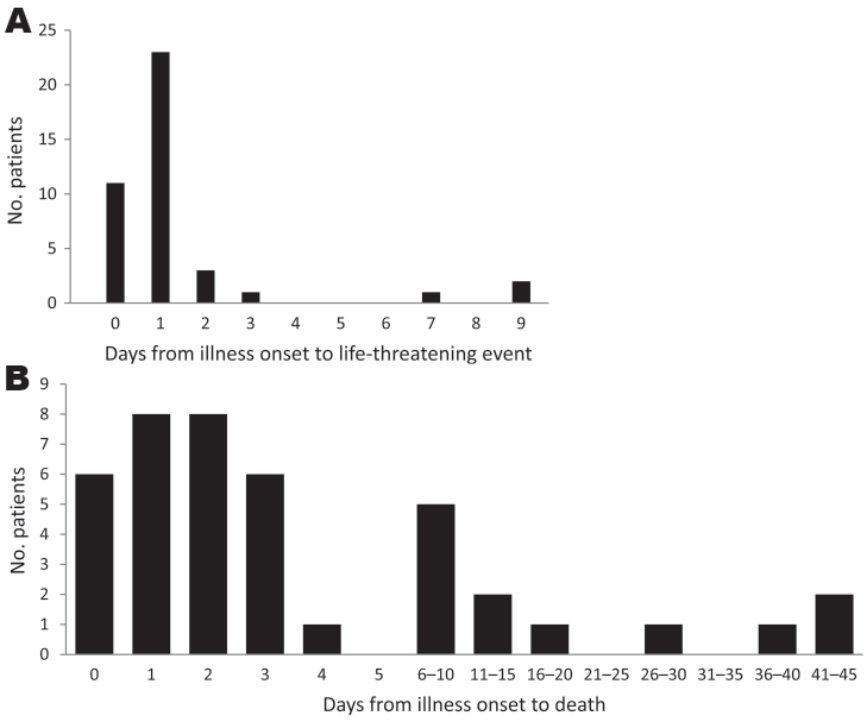

Figure 2. Days from onset of pandemic (H1N1) 2009 illness to A) life-threatening event or B) death among patients <20 years of age, Japan, May 2009–March 2010.

rapidly progressive dyspnea and hypoxemia. Chest CT scan performed at autopsy indicated severe infiltration in the entire lungs, corresponding to acute lung injury.

Two patients died of myocarditis; both were ≥12 years of age and previously healthy. One had unexpected circulatory collapse in a local pediatric clinic; the other was found lying on the floor at home without preceding respiratory or neurologic symptoms. At admission, both patients had markedly elevated creatine kinase (>9,000 IU/L) and markedly reduced cardiac output on cardiac ultrasonography; chest radiographs were unremarkable. In the clinic patient, intensive resuscitation, including intra-aortic balloon pumping and continuous hemodiafiltration, was performed but was ineffective.

Viral sepsis resulting from pandemic (H1N1) 2009 virus developed in 2 patients; 1 was severely disabled. Tachypnea, cold extremities, and lethargy were noted for both patients at the local pediatric clinic; shock was diagnosed, and they were immediately transferred to tertiary emergency hospitals. Both had rapidly progressive multiple organ failure with refractory hypotension. For both patients, chest radiographs were unremarkable.

Cause of death was presumed to be incidental to pandemic (H1N1) 2009 virus infection for 1 patient. This patient was hospitalized because of intracranial hemorrhage, which neuroimaging suggested resulted from rupture of an arteriovenous malformation. On day 12 of illness, infection with pandemic (H1N1) 2009 virus was confirmed by RT-PCR.

Comparisons by Cause of Death

We compared demographic and laboratory data of 36 patients by cause of death (Table 2). Patients who died of myocarditis, viral sepsis, or incidental intracranial hemorrhage were excluded. Patients with unexpected CPA were younger than other patients, although these differences were not significant ($p = 0.053$). Respiratory or neurologic disorders occurred significantly more often in patients with respiratory failure and significantly less often in patients with unexpected CPA. The interval between influenza onset and life-threatening event did not differ by cause of death. Most life-threatening events occurred on the day of or 1 day after influenza onset. Although the percentage of clinical signs and symptoms did not differ by cause of death, tachypnea/dyspnea or wheezing were frequent in patients with respiratory failure. Drugs taken before a life-threatening event did not differ by cause of death. Leukocyte and platelet counts did not differ by cause of death. Alanine transaminase and creatine kinase levels were significantly higher in patients with unexpected CPA than in those with respiratory failure. Blood urea nitrogen concentration was significantly higher in patients with encephalopathy than in those with unexpected CPA or respiratory failure. Levels of aspartate aminotransferase, lactate dehydrogenase, and creatinine did not differ by cause of death.

Discussion

We investigated the causes of death associated with pandemic (H1N1) 2009 among children in Japan. Most cases were in young, previously healthy children who died after a brief fulminant illness. Unexpected CPA and acute encephalopathy were the leading causes of death. Children who died of respiratory failure often had preexisting conditions, whereas unexpected CPA occurred among younger children without preexisting conditions.

Our finding that encephalopathy was a leading cause of death associated with pandemic (H1N1) 2009 among children in Japan differs from reports from other countries that few children have died of neurologic complications (*5*,*24*). Children with acute encephalopathy or encephalitis associated with pandemic (H1N1) 2009 have been reported outside Japan (*20*–*23*), but most survived with no or mild neurologic sequelae. Most children with acute encephalopathy, such as acute necrotizing encephalopathy (*25*) and acute encephalopathy with biphasic seizures and late reduced diffusion (*26*), were of Japanese or east Asian descent. Children in Japan are presumed to have an underlying genetic predisposition for development of acute encephalopathy (*26*). The median age of children who died of encephalopathy (62 months) was older than that of patients with encephalopathy associated with seasonal influenza (median 2–3 years) (*19*,*27*). This difference in age may be related to the age of infected patients; in Japan, more patients 5–9 years or 10–14 years of age were infected with pandemic (H1N1) 2009 than were those 0–4 years (*28*). A fulminant clinical course and marked brain edema were characteristic and common in the encephalopathy patients in our study, irrespective of age, presence or absence of preexisting conditions, and neuroradiologic findings.

Unexpected CPA was another leading cause of death associated with pandemic (H1N1) 2009 among children in Japan. Most cases of unexpected CPA occurred in previously healthy children <5 years of age. The elevated alanine transaminase and creatine kinase levels in these children could be attributable to postmortem changes. The direct cause of unexpected CPA is difficult to determine. One possible explanation is severe brain damage resulting in CPA; however, none of the patients in our study had obvious neurologic signs or symptoms until CPA, nor did they have any evidence of brain herniation. Abrupt onset of CPA suggests a cardiogenic origin such as fatal arrhythmia from undetected myocarditis (*29*,*30*). Myocarditis associated with pandemic (H1N1) 2009 has been reported (*31*,*32*). Gdynia et al. reported an unexpected death of a young adult caused by pandemic (H1N1) 2009–associated

Table 2. Comparisons by cause of death among patients <20 years of age, Japan, May 2009–March 2010*

Characteristic	Unexpected CPA, n = 15†	Encephalopathy, n = 15†	Respiratory failure, n = 6†	p value
Median age, mo (range)	43 (7–164)	62 (17–200)	78 (45–206)	0.053
Male sex, no. (%) patients	9 (60)	10 (67)	2 (33)	NS
Preexisting condition, no. (%) patients				
Any	1 (7)‡	5 (33)	5 (83)§	<0.05
Respiratory disorders	0‡	3 (20)	4 (67)§	<0.05
Neurologic disorders	1 (7)‡	3 (20)	5 (83)§	<0.05
Previous history of febrile seizures	2 (13)	3 (20)	0	NS
Days from influenza onset to life-threatening event (range)	1 (0–9)	1 (0–2)	1 (0–9)	NS
Days from influenza onset to death (range)	1 (0–9)	3 (0–45)	1.5 (1–11)	<0.05¶
Clinical signs, no. (%) patients				
Cough	5 (36), n = 14	11 (73)	3 (50)	NS
Rhinorrhea	4 (29), n = 14	7 (47)	1 (17)	NS
Tachypnea or dyspnea	1 (7), n = 14	4 (27)	3 (50)	NS
Wheezing	1 (7), n = 14	1 (7)	2 (33)	NS
Vomiting or diarrhea	2 (14), n = 14	5 (33)	0	NS
Drugs received before life-threatening event, no. (%) patients				
Oseltamivir	7 (47)	6 (40)	5 (83)	NS
Zanamivir	1 (7)	2 (13)	2 (33)	NS
Acetaminophen	3 (30), n = 10	7 (50), n = 14	2 (40), n = 5	NS
Leukocyte count, cells/μL, median (range)	6,600 (4,200–11,100), n = 11	9,350 (3,100–28,730), n = 14	10,500 (8,650–101,200), n = 5	NS
Platelet count, $\times 10^4$ cells/μL, median (range)	20.5 (11.4–45.8), n = 11	15.1 (6.2–32.2)	11.5 (8.4–49.0), n = 5	NS
Aspartate aminotransferase, IU/L, median (range)	248 (55–1,981), n = 12	233 (18–1,760)	52 (34–73), n = 4	0.060
Alanine transaminase, IU/L, median (range)	157 (32–845), n = 12	70 (9–1,058)	26 (16–57), n = 4	<0.05¶
Lactate dehydrogenase, IU/L, median (range)	704 (215–4,801), n = 12	899 (160–3,610), n = 14	535 (222–1,022), n = 4	NS
Creatine kinase, IU/L, median (range)	302 (136–10,612), n = 12	190 (63–1,026), n = 14	64 (16–211), n = 4	<0.01#
Blood urea nitrogen, mg/dL, median (range)	11.2 (7.0–31.0), n = 12	22.5 (11.7–40.0), n = 14	10.0 (4.0–15.0), n = 4	<0.01**
Creatinine, mg/dL, median (range)	0.65 (0.17–1.40), n = 12	1.01 (0.62–1.39)	0.25 (0.08–1.00), n = 4	<0.05**

*CPA, cardiopulmonary arrest; NS, not significant.
†n is for all values unless indicated otherwise.
‡Percentage significantly lower than for the other groups.
§Percentage significantly higher than for the other groups.
¶p<0.05 unexpected CPA vs. encephalopathy.
#p<0.05 unexpected CPA vs. respiratory failure.
**p<0.01 unexpected CPA vs. encephalopathy; p<0.05 respiratory failure vs. encephalopathy.

myocarditis (*31*). The clinical course in this patient was characterized by sudden collapse at home followed by fatal arrhythmia. Viral sepsis may also be related to unexpected CPA. Clinical signs of viral sepsis are nonspecific and may be missed. Considering that most cases of unexpected CPA occurred outside the hospital, rapid progression of viral sepsis may have occurred. Unexpected CPA has also been reported in some case series (*5–7*,*24*,*33*). Cardiac arrest outside the hospital was observed for 67 of 270 children who died in the United States (*24*). In a report from England, 16 of 70 children who died were in CPA when seen in an emergency department (*7*). Detailed postmortem examinations are necessary to clarify the mechanism of unexpected CPA.

Respiratory failure was an uncommon cause of death among children in Japan. In other countries, diffuse viral pneumonia or pneumonitis with severe hypoxemia were strongly associated with intensive care unit admission associated with pandemic (H1N1) 2009 (*8–13*). Several reports on cases of pandemic (H1N1) 2009 in children also showed that respiratory distress is most common among hospitalized children (*5–7*,*14–16*,*24*,*34*,*35*). In a study of children in Argentina, refractory hypoxemia caused 62% of all deaths (*14*). A report from England described predominantly respiratory symptoms when care was sought in 53 of 70 children who died (*7*). Most children who died of respiratory failure in Japan had preexisting neurologic or respiratory disorders or both; this finding is similar to

reports from other countries (*5–7*,*10*,*12*,*14–16*,*24*,*34*). Children with preexisting neurologic or respiratory disorders are at increased risk for severe illness or death with influenza, and influenza vaccination should be a priority for these children.

The infrequency of preexisting conditions appears to be a feature of deaths associated with pandemic (H1N1) 2009 among children in Japan. Only one third of the participants in our study had ≥1 preexisting conditions. In contrast, research in Argentina showed that 9 of 13 patients who died of pandemic (H1N1) 2009 had a preexisting condition, including neurologic disorders and chronic lung disease (*14*). In England, preexisting severe or incapacitating systemic diseases were recognized in all deaths in children <5 years of age and in most of those 5–14 years of age (*15*). According to the US Centers for Disease Control and Prevention, 205 of 301 children in the United States whose deaths occurred in association with pandemic (H1N1) 2009 had high-risk medical conditions as defined by the Advisory Committee on Immunization Practices (*24*).

The strength of our study is use of the detailed and precise information obtained during interviews with attending clinicians. Clinical course and demographic data were accurate and detailed, and laboratory data, chest radiographs, and other radiologic data were directly assessed by the study team. Data were standardized by use of a structured report form. In addition, cause of death was determined on the basis of the consensus of the chief study members rather than by the attending clinicians. We thus consider that the data from our study are objective.

Nevertheless, our study has some limitations. First, in some cases, infection might not have been confirmed by PCR; thus, the number of deaths associated with pandemic (H1N1) 2009 among children might have been underestimated. Second, complete data on the number of all children infected with pandemic (H1N1) 2009 virus were not available. In Japan, data on the number of patients with influenza-like symptoms are collected from ≈3,000 sentinel pediatric physicians and 2,000 sentinel internal medicine physicians participating in the surveillance system. Because an accurate number of all infected children could not be obtained, the case-fatality rate could not be determined.

Several authors have suggested that neuraminidase inhibitors will be effective for preventing severe illness in patients with pandemic (H1N1) 2009 virus infection (*36*,*37*), and the usefulness of early treatment with neuraminidase inhibitors has been emphasized. However, neuraminidase inhibitors did not appear to be effective in our patients, even though the drugs had been used without delay, which indicates the difficulty of improving the outcome for children with the most severe illness. Prevention and control of influenza with vaccine use and public education is necessary for reducing illness and deaths associated with influenza not only in high-risk children but also in previously healthy ones.

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Global Distribution and Epidemiologic Associations of *Escherichia coli* Clonal Group A, 1998–2007

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Analyze the prevalence of trimethoprim/sulfamethoxazole resistance among *Escherichia coli* clonal group A (CGA) isolates
- Distinguish geographic locations with the highest prevalence of CGA
- Assess variables that significantly affect the prevalence of CGA
- Evaluate temporal trends in the prevalence of CGA

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Escherichia coli clonal group A (CGA) was first reported in 2001 as an emerging multidrug-resistant extraintestinal pathogen. Because CGA has considerable implications for public health, we examined the trends of its global distribution, clinical associations, and temporal prevalence for the years 1998–2007. We characterized 2,210 *E. coli* extraintestinal clinical isolates from 32 centers on 6 continents by CGA status for comparison with trimethoprim/sulfamethoxazole (TMP/SMZ) phenotype, specimen type, inpatient/outpatient source, and adult/child host; we adjusted for clustering by center. CGA prevalence varied greatly by center and continent, was strongly associated with TMP/SMZ resistance but not with other epidemiologic variables, and exhibited no temporal prevalence trend. Our findings indicate that CGA is a prominent, primarily TMP/SMZ-resistant extraintestinal pathogen concentrated within the Western world, with considerable pathogenic versatility. The stable prevalence of CGA over time suggests full emergence by the late 1990s, followed by variable endemicity worldwide as an antimicrobial drug–resistant public health threat.

Extraintestinal infections caused by *Escherichia coli* are a substantial source of illness, death, and increased health care costs and have become increasingly challenging to manage because of the rising prevalence of resistance to first-line antimicrobial drugs (*1*). The resistance problem is now recognized as having a prominent clonal component attributable in large part to the emergence and dissemination of specific antimicrobial drug–resistant clonal groups of extraintestinal pathogenic *E. coli* (*2*–*6*).

One such emergent antimicrobial drug–resistant extraintestinal pathogenic *E. coli* clonal group is clonal group A (CGA) (*2*). Most traditionally recognized extraintestinal pathogenic *E. coli* clonal groups derive from *E. coli* phylogenetic group B2; however, CGA derives from phylogenetic group D (*7*), and, according to multilocus sequence typing (MLST), CGA corresponds with clonal complex 69 (*8*,*9*).

CGA first came to attention during the late 1990s as a prominent cause of trimethoprim/sulfamethoxazole (TMP/SMZ)–resistant urinary tract infections among otherwise healthy women across the United States (*2*,*10*). Isolates of CGA typically exhibit a fairly conserved virulence genotype that includes P fimbriae (with the F16 structural subunit variant), group 2 capsule (with the K52 capsular antigen), and the aerobactin and yersiniabactin siderophore systems. They also commonly exhibit resistance to multiple antimicrobial agents other than TMP/SMZ, including tetracycline, chloramphenicol, streptomycin, and spectinomycin, with the corresponding resistance genes carried either on a large conjugative plasmid (*2*) or within a genomic resistance module (*11*).

CGA has been recognized primarily as a cause of community-acquired cystitis and pyelonephritis in adult women mainly in the United States (*2*,*10*,*12*,*13*). It is largely unknown to what extent CGA might have broader pathogenic capabilities with respect to anatomic site of infection (urine vs. nonurine), site of acquisition (hospital vs. community), and host age (adult vs. child). Likewise, although a global survey of *E. coli* clinical isolates from 2001 found CGA was significantly associated with the United States (*14*), assessment of its distribution beyond the United States has been limited (*5*). Furthermore, no recent data are available regarding whether the overall prevalence of CGA is rising, stable, or waning, as can occur on a local level for CGA and other extraintestinal pathogenic *E. coli* clonal groups (*3*,*13*). Therefore, because of the major public health implications of CGA, we assessed the prevalence of this *E. coli* clonal group during 1998–2007 in multiple locales in the United States and internationally, paying specific attention to specimen type, inpatient versus outpatient status of host, and host age.

Methods

Strains

Sets of unpublished human clinical extraintestinal *E. coli* isolates were obtained from 32 clinical microbiology laboratories and affiliated repositories worldwide (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-0488-Techapp.pdf). Submitters were asked to provide ≈25 consecutive TMP/SMZ-resistant extraintestinal *E. coli* isolates and 25 concurrent TMP/SMZ-susceptible extraintestinal *E. coli* controls (1 per patient), or more, as available. When possible, isolates were to be distributed evenly by inpatient versus outpatient source and, within each of these categories, by specimen type (urine vs. other). Information was requested about the adult (age ≥18 years) versus child (age <18 years) status of the hosts and the local overall prevalence of TMP/SMZ-resistant *E. coli*.

Isolates were submitted to the research laboratory (J.R.J.) in agar stabs and frozen at −80ºC in 15% glycerol pending further analysis. Selected isolates underwent additional screening, by breakpoint agar dilution and disk diffusion, for confirmation of TMP/SMZ phenotype.

Molecular Analysis

Major *E. coli* phylogenetic group (A, B1, B2, D) was defined by triplex PCR (*15*). Group D isolates were assessed for CGA (i.e., clonal complex 69) status in a staged fashion. First, all were screened by PCR for CGA-associated single-nucleotide polymorphisms (SNPs) in *fumC* (*16*). All *fumC* SNP-positive isolates (which included all CGA isolates, plus any non-CGA isolates containing

the same *fumC* SNPs) then underwent pulsed-field gel electrophoresis (PFGE) analysis of *Xba*I-restricted total DNA (*17*). Those with ≥94% PFGE profile similarity to a known CGA isolate (determined on the basis of previous *fumC* and *gyrB* SNP analysis or 7-locus MLST) (*5*) were defined as CGA because this degree of PFGE similarity reliably predicts identity by MLST (J.R. Johnson, unpub. data). The *fumC* SNP-positive isolates without a PFGE profile match of ≥94% to a known CGA isolate were individually screened by PCR for CGA-associated SNPs in *gyrB*. If the isolates were positive for *gyrB* SNPs and for the CGA-associated *fumC* SNPs, which together provide highly accurate identification of CGA isolates, they were defined as CGA isolates (*5*,*6*,*16*).

Statistical Analysis

Unpaired and paired comparisons of proportions were tested by using the Fisher exact and McNemar tests, respectively. Selected variables were assessed as predictors of CGA status by using generalized linear models based on the generalized estimating equation (GEE; logistic GEE regression) to account for clustering by locale, supplemented by univariable and multivariable logistic regression analysis, as needed. Statistical analyses were conducted by using SPSS version 19.0 (IBM, Somers, NY, USA).

Results

Study Population

A total of 2,210 *E. coli* clinical isolates from 32 globally dispersed centers were studied (Table 1); the isolates differed from those used in a prior global survey (*14*). Each center provided a median of 54 isolates (range 24–320). All centers but 1 provided TMP/SMZ-susceptible and TMP/SMZ-resistant isolates in approximately equal numbers. The median year of isolation was 2002 (range 1998–2007). Seventeen centers provided isolates for the first half of the study period only (1998–2002); 12 centers provided isolates for the last half of the study period only (2003–2007); and 3 centers provided isolates for both halves of the study period.

The 32 centers were in 19 countries, each represented by a single center, except the United States, which was represented by 14 centers. The 6 inhabited continents were each represented by multiple centers, as follows: Africa, 2; Asia, 4; Australia/New Zealand, 2; Europe, 5; North America, 15; and South/Central America, 4. Of the 15 centers in North America, 1 was in Canada and 14 in the United States (5 in Minnesota and 9 in other states).

Of the 32 centers, 31 reported the age (i.e., adult vs. child) of the patients from whom the clinical specimens were obtained. All 31 centers provided isolates derived from specimens from adults (n = 1,909), and 19 centers also provided isolates derived from specimens from children (n = 250). Of the 32 centers, 31 provided urine-source isolates (n = 1,511); 28 centers also provided isolates from other (nonurine) sources (n = 653). Outpatient versus inpatient source for clinical samples was reported by 29 centers, all of which provided isolates from outpatient specimens (n = 1,135); 26 centers also provided isolates from inpatient specimens (n = 926). As reported by 31 centers, the prevalence of *E. coli* TMP/SMZ susceptibility, by center, ranged from 36% to 90% (median 78%).

Phylogenetic Group and CGA Status versus TMP/SMZ Phenotype

Within the total group of isolates, phylogenetic group distribution was significantly associated with TMP/SMZ phenotype (Table 2). Although the susceptible (n = 1,083) and resistant (n = 1,127) populations exhibited the same rank order for phylogenetic group prevalence (i.e., B2 >D >A >B1), absolute prevalences differed greatly by TMP/SMZ phenotype. That is, among susceptible isolates, group B2 predominated overwhelmingly, being nearly 3× as prevalent as group D. In contrast, among resistant isolates, phylogenetic groups B2 and D were closely matched (approximately one third of isolates each). Accordingly, group D was strongly associated with TMP/SMZ resistance.

Molecular typing identified 144 CGA isolates (Table 1), which accounted for 6.5% of all isolates and 25.2% of the group D isolates (Table 2). CGA was strongly associated with TMP/SMZ resistance, accounting for 10.1% of resistant isolates overall but for only 2.8% of susceptible isolates ($p<0.001$) (Table 2). Even within phylogenetic group D, CGA was nearly 2× as prevalent among resistant as among susceptible isolates (29.8% [114/383] vs.16.0% [30/188], $p<0.001$) (Table 2).

CGA Status in Relation to Geography

Of the 32 centers, 26 provided at least 1 CGA isolate (Table 1). The prevalence of CGA by center varied greatly, ranging from 0% to 34% (median 5%) for TMP/SMZ-resistant isolates and from 0% to 9.4% (median 1.4%) for TMP/SMZ-susceptible isolates. In all but 2 centers, CGA was at least as prevalent among TMP/SMZ-resistant as among TMP-SMZ-susceptible isolates; in 5 of the centers, the difference in prevalence was statistically significant. The 5 centers with the highest prevalence of CGA isolates (2 in the United States, 3 in other countries) had ≥20% CGA prevalence among resistant isolates, and another 5 (1 United States, 4 in other countries) had 10%–19% prevalence. At the other extreme, 6 centers (3 in the United States, 3 in other countries) had no CGA isolates.

The prevalence of CGA also varied substantially by continent, in a resistance-dependent manner (Table 3). Among TMP/SMZ-susceptible isolates the prevalence of

Table 1. Origin and epidemiologic background of 2,210 extraintestinal *Escherichia coli* isolates from 32 globally distributed centers and susceptibility to trimethoprim/sulfamethoxazole, 1998–2007*

Continent, location†	Year(s) of isolation	Total no. isolates	S, %	Source, no. isolates					No. CGA isolates/total no. (%)	
				Specimen type		Setting				
				Urine	Nonurine	In	Out	Child‡	R	S
Africa										
Ile-Ife, Nigeria	2004	41	NK	41	0	22	19	4	3/36 (8)	0/5 (0)
Lusaka, Zambia	2001	51	59	4	37	NK	NK	0	0/31 (0)	0/20 (0)
Asia										
Chandigarh, India	2006	50	60	37	13	34	16	9	0/36 (0)	0/14 (0)
Kitakyushu, Japan	2001–2005	56	80	37	19	36	20	7	1/29 (3)	0/27 (0)
Singapore	2002	50	60	43	7	NK	NK	0	1/25 (4)	0/25 (0)
Taiwan	1998–2004	320	46	256	64	66	254	73	8/161 (5)	8/159 (5)
Australia/New Zealand										
Canberra, Australia	1998–2001	121	80	50	71	0	121	5	10/60 (17)	3/61 (5)
Palmerston North, New Zealand	2006	51	78	36	15	18	33	3	1/24 (4)	0/27 (0)
Europe										
Zagreb, Croatia	2001–2002	91	81	44	47	58	33	0	8/46 (17)	1/45 (2)
Athens, Greece	2003–2005	149	66	96	53	92	57	3	15/75 (20)	1/74 (1)
Varese, Italy	2006	51	75	35	16	31	20	3	0/26 (0)	0/25 (0)
Santander, Spain	2003	53	70	35	18	19	34	9	0/26 (0)	2/27 (7)
Bellinzona, Switzerland	2006	54	75	36	18	34	20	2	3/27 (11)	0/27 (0)
North America										
Calgary, Alberta, Canada	2001	54	78	36	18	34	20	10	6/27 (22)	1/27 (4)
United States										
Denver, CO	2001	100	78	50	50	50	50	0	17/50 (34)	3/50 (6)
West Haven, CT	2006	34	76	24	10	17	17	0	0/16 (0)	0/18 (0)
Chicago, Il	2001	60	74	40	20	37	23	0	0/30 (0)	0/30 (0)
Lexington, KY	2001	60	80	40	20	7	53	5	3/30 (10)	1/30 (3)
Petoskey, MI	2001	45	89	NK.	NK	NK	NK	0	5/21 (24)	0/24 (0)
Duluth, MN	2001	50	90	39	11	8	42	0	4/26 (15)	0/24 (0)
Minneapolis, MN†	2001	66	87	56	10	15	51	0	1/26 (4)	1/40 (3)
Minneapolis, MN†	2001	46	90	38	8	21	25	0	0/18 (0)	0/28 (0)
Northfield, MN	2001	24	95	24	0	0	24	0	1/12 (8)	0/12 (0)
St. Louis Park, MN	2001	64	83	64	0	0	64	7	9/32 (28)	3/32 (9)
Fargo, ND	2001	54	90	49	5	5	49	11	1/27 (4)	1/27 (4)
Philadelphia, PA	2006	94	78	87	7	13	81	0	2/22 (9)	0/72 (0)
Houston, TX	2001	60	65	40	20	35	25	9	1/30 (3)	1/30 (3)
Salt Lake City, UT	2001	47	85	31	16	24	23	4	1/21 (5)	1/26 (4)
South/Central America										
Concepción, Chile	2006	51	57	36	15	33	18	NK	5/24 (21)	1/27 (4)
Cali, Columbia	2005–2006	51	52	36	15	27	24	16	3/24 (13)	1/27 (4)
Panama City, Panama	2007	54	36	36	18	19	35	52	1/27 (4)	1/27 (4)
Lima, Peru	2002–2006	58	82	39	19	30	28	18	4/58 (7)	Not done

*S, susceptible to trimethoprim/sulfamethoxazole; in, inpatient; out, outpatient; CGA, clonal group A; R, resistant to trimethoprim/sulfamethoxazole; NK, not known.
†A list of the 32 centers is provided in the online Technical Appendix (wwwnc.cdc.gov/EID/pdfs/11-0488-Techapp.pdf). Note that 2 centers were located in Minneapolis.
‡<18 y of age.

CGA was uniformly low, regardless of continent (median prevalence 3.0%, range 0%–3.8%), whereas among TMP/SMZ-resistant isolates it was substantially higher in the Western world (Australia/New Zealand, Europe, North America, and South/Central America; median prevalence 13.1%, range 9.7%–13.2%), compared with Africa and Asia (4.2% and 4.0%, respectively) (Table 3).

Accordingly, data for Africa and Asia were combined for comparison with data from other regions. Among TMP/SMZ-susceptible isolates, CGA was similarly prevalent among the isolates from Africa and Asia combined and the isolates from other areas (3.3% vs. 2.8%, $p>0.10$). In contrast, among TMP/SMZ-resistant isolates, CGA was significantly more prevalent among isolates from areas other than Africa and Asia than it was among isolates from Africa and Asia (12.5% vs. 4.0%, $p<0.001$) (Table 3). Likewise, CGA was significantly associated with TMP/SMZ resistance among the isolates from areas other than

Table 2. Phylogenetic group distribution and clonal group A status of extraintestinal *Escherichia coli* isolates from 32 globally distributed centers, 1998–2007*

Phylogenetic group and clonal group A status	No. clonal group A isolates/total no. isolates (%)			p value†
	Total, n = 2,210	TMP/SMZ susceptible, n = 1,083	TMP/SMZ resistant, n = 1,127	
E. coli phylogenetic group				
A	345 (15.6)	141 (13.0)	204 (18.1)	0.001
B1	223 (10.1)	121 (11.2)	102 (9.1)	
B2	1,071 (48.5)	633 (58.4)	438 (38.9)	<0.001
D	571 (25.8)	188 (17.4)	383 (34.0)	<0.001
Clonal group A	144 (6.5)	30 (2.8)	114 (10.1)	<0.001

*TMP/SMZ, trimethoprim/sulfamethoxazole.
†p values, by Fisher exact test, for TMP/SMZ-susceptible vs. -resistant isolates are shown where p<0.05; otherwise, p>0.10.

Africa and Asia (p<0.001) but not among the isolates from Africa and Asia (p>0.10) (Table 3).

CGA Status versus Other Variables

We also examined the prevalence of CGA in relation to other variables, after stratification for TMP/SMZ phenotype (Table 4). For each variable (i.e., specimen type, host age group, host inpatient/outpatient status, and year isolate was obtained from patient specimen), CGA was significantly more prevalent among TMP/SMZ-resistant than TMP/SMZ-susceptible isolates. In contrast, for a given TMP/SMZ phenotype, the prevalence of CGA varied minimally in relation to the other variables. Specifically, CGA was similarly (and, in some instances, slightly more) prevalent among isolates from nonurine versus urine specimens, children versus adults, inpatients versus outpatients, the first half versus the second half of the study period (Table 4), and centers with isolates with a below-median versus above-median prevalence of TMP/SMZ resistance (not shown). This finding suggested that continent and TMP/SMZ status were closely associated with CGA status, whereas other study variables were not.

Logistic GEE Models and Multivariable Analysis

To account for possible confounding of these associations because of clustering by center, we used logistic GEE regression models to assess associations of CGA with TMP/SMZ phenotype, continent (as Africa/Asia vs. other), and the other nongeographic variables. Univariable analyses identified the same significant associations (or lack thereof) with CGA as noted initially; only TMP/SMZ phenotype and continent were confirmed as significant correlates of CGA status (Table 5).

Accordingly, we constructed a multivariable logistic GEE regression model based on TMP/SMZ phenotype and continent to assess the independent association of these 2 variables with CGA status. However, the model did not run to completion, possibly because of small numbers in certain cells (not shown). Univariable logistic regression analysis yielded results for these 2 variables separately that were similar to those obtained with the (univariable) generalized linear models (Table 5), which provided evidence that clustering by center had little effect on the associations. Therefore, a multivariable logistic regression model was constructed with TMP/SMZ phenotype and continent as the candidate predictor variables. This model yielded results similar to those of the univariable models, which provided evidence that the associations of CGA with TMP/SMZ phenotype and continent are largely independent of each other (Table 5).

Table 3. Prevalence of clonal group A, by region and TMP/SMZ phenotype, among 2,210 extraintestinal *Escherichia coli* isolates from 32 globally distributed centers, 1998–2007*

Region	No. clonal group A isolates/total no. (%)			p value†
	Total	TMP/SMZ susceptible	TMP/SMZ resistant	
Overall	144/2,210 (6.5)	30/1,083 (2.8)	114/1,127 (10.1)	<0.001
Africa	3/92 (3.3)	0/21 (0)	3/71 (4.2)	
Asia	18/476 (3.8)	8/225 (3.6)	10/251 (4.0)	
Australia/New Zealand	14/172 (8.1)	3/88 (3.4)	11/84 (13.1)	0.025
Europe	30/398 (7.5)	4/198 (2.0)	26/200 (13.0)	<0.001
North America	63/858 (7.3)	12/471 (2.5)	51/387 (13.2)	<0.001
South/Central America	16/214 (7.5)	3/80 (3.8)	13/134 (9.7)	
Africa/Asia combined	21/568 (3.7)	8/246 (3.3)‡	13/322 (4.0)§	
Not Africa/Asia	123/1,642 (7.5)	22/837 (2.6)‡	101/805 (12.5)§	<0.001

*TMP/SMZ, trimethoprim/sulfamethoxazole.
†p values, by Fisher exact test, for TMP/SMZ-susceptible vs. -resistant isolates are shown where p<0.05; otherwise, p>0.10.
‡For Africa/Asia vs. other, p>0.10.
§For Africa/Asia vs. other, p<0.001.

Table 4. Prevalence of clonal group A, by clinical/host variables, among 2,210 extraintestinal *Escherichia coli* isolates from 32 globally distributed centers, 1998–2007*

Clinical/host variable and year isolate obtained from patient clinical specimen	No. clonal group A isolates/total no. isolates (%)†			p value‡
	Total	TMP/SMZ susceptible	TMP/SMZ resistant	
Specimen type				
Nonurine	48/653 (7.4)	9/318 (2.8)	39/335 (11.6)	<0.001
Urine	88/1,470 (6.0)	21/739 (2.8)	67/731 (9.2)	<0.001
Host age group, y				
<18	16/250 (6.4)	4/118 (3.4)	12/132 (9.1)	0.08
≥18	122/1,909 (6.4)	25/938 (2.7)	97/971 (10.0)	<0.001
Host hospital status				
Outpatient	84/1,135 (7.4)	17/573 (3.0)	67/562 (11.9)	<0.001
Inpatient	54/926 (5.8)	13/434 (3.0)	41/488 (8.4)	<0.001
Year isolated				
1998–2002	95/1,330 (7.1)	21/661 (3.2)	74/669 (11.1)	<0.001
2003–2007	49/880 (5.6)	9/422 (2.1)	40/458 (8.7)	<0.001

*TMP/SMZ, trimethoprim/sulfamethoxazole.
†Data for each clinical variable include only isolates for which status with respect to the particular variable was known.
‡p values, by Fisher exact test, for comparisons of TMP/SMZ-susceptible vs. -resistant isolates within each subgroup. For all comparisons between subgroups within a given category (whether overall or by TMP/SMZ phenotype), p>0.10.

Discussion

In this global survey for the recently recognized *E. coli* lineage CGA among extraintestinal clinical isolates from humans during 1998–2007, we identified strong associations of CGA with TMP/SMZ resistance and with regions other than Africa and Asia; this evidence indicates that CGA is primarily a TMP/SMZ-resistant pathogen concentrated within the Western world. In contrast, we found no association of CGA with other epidemiologic variables, which suggests that CGA is a similarly prominent pathogen among children and adults, among inpatients and outpatients, and within and outside the urinary tract. Finally, the fairly stable prevalence of CGA throughout the study period suggests that CGA had fully emerged by the late 1990s and now is an endemic public health threat in many centers worldwide.

The observed overall association of CGA with TMP/SMZ resistance is consistent with the findings of multiple studies (*2*,*10*,*12*–*14*,*18*–*20*). However, we did not find this association in Africa and Asia. Overall prevalence of CGA was also lowest in these regions. Taken together, these findings suggest that the TMP/SMZ-resistant variants of CGA had a selective advantage in the Western world but not in Asia and Africa, which led to the lineage's expansion in Europe, the Americas, and Australia but not in Asia and Africa. Why such an expansion seemingly has not occurred in Africa and Asia is unclear. One possibility is that TMP/SMZ-resistant CGA isolates emerged first in the Western world and have had insufficient time to diffuse to and expand within Africa and Asia. Alternatively, Africa and Asia may already have had an abundance of successful endemic TMP/SMZ-resistant clones competing with CGA for the same niche, effectively excluding it, or conditions in Africa and Asia may be somehow less permissive to the dispersal and expansion of this clonal group. Further comparisons of the TMP/SMZ-resistant populations from Africa and Asia versus other locales could be informative in this regard.

Clear-cut variation in the prevalence of CGA was evident at the continent level. However, marked differences also were apparent even among closely located centers, as noted in our smaller global survey (*14*). For example, whereas 1 Minneapolis center had no CGA isolates, another center had a high prevalence of CGA. To what extent these differences are real, rather than a reflection of the inherent imprecision of small samples, is unclear. However, because different hospitals in the same locale often serve different patient populations and may draw from different catchment areas, the possibility of true variation by hospital is plausible. The determinants of this local variation, if real, would be potentially useful to discover as a step toward developing preventive measures. The center with the highest prevalence was in Denver, Colorado, USA, which also was the site of a previous survey with a high CGA prevalence; that survey involved different isolates than those included here (*12*). This consistency across studies suggests that Denver may be a focus of high-level endemicity for CGA.

CGA has been reported primarily as a urine pathogen among ambulatory women (*2*,*10*,*13*,*19*,*20*), which might be interpreted as indicating that urine is the favored niche or context of the clonal group. However, CGA has been reported in other clinical contexts, including, for example, as a cause of community-acquired pneumonia in a male renal transplant recipient (*21*). We found no association of CGA with urine versus nonurine (extraintestinal) source, inpatient versus outpatient host status, and host age (child vs. adult). This absence of discernible niche specialization suggests that CGA is a generalist, able to cause different types of infection in diverse host populations, in the hospital

Table 5. Generalized linear modeling and logistic regression analysis of TMP/SMZ phenotype and region as predictors of clonal group A status among extraintestinal *Escherichia coli* isolates from 32 globally distributed centers, 1998–2007*

Method, type of model, variable†	OR (95% CI)	p value
GEE‡		
Univariable		
TMP/SMZ resistance	3.90 (2.04–7.46)	<0.001
Africa/Asia	0.39 (0.18–0.89)	0.02
Logistic regression		
Univariable		
TMP/SMZ resistance	4.14 (2.74–6.26)	<0.001
Africa/Asia	0.43 (0.26–0.69)	<0.001
Multivariable		
TMP/SMZ resistance	3.95 (2.62–5.96)	<0.001
Africa/Asia	0.47 (0.30–0.76)	0.002

*TMP/SMZ, trimethoprim/sulfamethoxazole; OR, odds ratio; CI, confidence interval; GEE, generalized estimating equation.
†Univariable models, but not multivariable models, included the following as candidate predictor variables, each of which yielded a p value >0.10: specimen type (urine vs. nonurine), host age group (<18 vs. ≥18), host hospital status (inpatient vs. outpatient), local prevalence of TMP/SMZ resistance, and year isolate obtained.
‡Because the multivariable GEE model that used TMP/SMZ phenotype and Africa/Asia as candidate predictor variables could not run to completion, logistic regression analysis was used instead.

and community alike. In terms of niche specialization, CGA is analogous to *E. coli* O18:K1:H7, which, although best known as an agent of neonatal meningitis, is also a prominent cause of acute cystitis in women (*22*,*23*). The pathogenic versatility of CGA has no doubt contributed to its epidemiologic success.

We found no evidence of a time trend for the prevalence of CGA, even with locale taken into account, which suggests that CGA had already emerged and established widespread endemicity by 1998, the start of the study period. Overall, CGA was not as prevalent in this study as it was in the initial reports from the mid- to late 1990s (*2*,*10*). This discrepancy could reflect selection bias rather than a true prevalence decrease by the time of the present study (i.e., our study included all patient specimens sent to clinical microbiology laboratories; the early studies included specimens from women with acute cystitis and uncomplicated pyelonephritis).

Even in regions in which prevalence was highest, CGA accounted for only a minority of TMP/SMZ-resistant isolates. This finding suggests that other resistant clonal groups are likely present, some of which could be similarly or more prominent compared with CGA (*3*,*5*,*6*,*13*,*20*,*24*,*25*). Identification of such clonal groups and investigation of their epidemiology could help clarify the basis for the non-CGA component of TMP/SMZ resistance in *E. coli*, which represents a major ongoing public health threat.

Study limitations must be acknowledged. First, lack of information regarding the infected host (e.g., clinical symptoms, underlying health status, and sex) limits our understanding of the study population and precludes assessment of these variables in relation to CGA status. Second, variability by center in the completeness of epidemiologic data reporting reduced power for analyses involving those variables and may have introduced unrecognized bias. Third, limited sampling of certain geographic regions (especially Africa and Australia/New Zealand) and host groups (children) constrained the inferences that could be drawn about those variables. Fourth, uncertainty regarding how closely each site followed the requested selection criteria allowed for possibly biased sample distribution by site.

Study strengths also must be acknowledged. First, the large sample size enhanced statistical power and permitted subgroup analyses that were not possible in previous studies. Second, the broad geographic distribution and multicenter design improved generalizability and allowed analyses by region. Third, the availability of basic epidemiologic data for most isolates enabled statistical analysis of these variables. Fourth, the predominantly prospective, consecutive sampling would be expected to provide a more broadly representative sample than a sample limited to a specific syndrome or host group. Fifth, the combined use of univariable and multivariable modeling, including adjustment for clustering, enabled optimal assessment of associations between individual variables and CGA.

In summary, our global survey for CGA during 1998–2007 identified strong associations of CGA with TMP/SMZ resistance and non-African/Asian origin but not with other epidemiologic variables—evidence that suggests CGA is a similarly prominent extraintestinal pathogen among children and adults, for inpatients and outpatients, and within and outside the urinary tract. The fairly stable prevalence of CGA through the 10-year study period suggests that CGA had fully emerged by the late 1990s and now is endemic worldwide as an antimicrobial drug–resistant public health threat.

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Group A *Streptococcus emm* Gene Types in Pharyngeal Isolates, Ontario, Canada, 2002–2010

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Group A *Streptococcus* (GAS) is a human-adapted pathogen that causes a variety of diseases, including pharyngitis and invasive infections. GAS strains are categorized by variation in the nucleotide sequence of the gene (*emm*) that encodes the M protein. To identify the *emm* types of GAS strains causing pharyngitis in Ontario, Canada, we sequenced the hypervariable region of the *emm* gene in 4,635 pharyngeal GAS isolates collected during 2002–2010. The most prevalent *emm* types varied little from year to year. In contrast, fine-scale geographic analysis identified inter-site variability in the most common *emm* types. Additionally, we observed fluctuations in yearly frequency of *emm3* strains from pharyngitis patients that coincided with peaks of *emm3* invasive infections. We also discovered a striking increase in frequency of *emm89* strains among isolates from patients with pharyngitis and invasive disease. These findings about the epidemiology of GAS are potentially useful for vaccine research.

Group A *Streptococcus* (GAS) is a gram-positive bacterial pathogen responsible for ≈600 million cases of pharyngitis each year worldwide (*1*). The widespread prevalence of this disease results in considerable costs, estimated to exceed $200 million annually in the United States alone (*2*). In addition to acute pharyngitis, GAS causes several other human diseases, ranging from relatively mild to more severe, such as necrotizing fasciitis, soft tissue infections, glomerulonephritis, acute rheumatic fever, and streptococcal toxic shock syndrome. Thus, infections caused by GAS are a major public health concern in the United States and Canada and throughout the world.

GAS strains are classified mainly on the basis of variation in a cell-surface molecule known as M protein, encoded by the *emm* gene (*3*,*4*). M protein is a critical virulence factor and a major site of the human antibody response against GAS. M type–specific immunity develops in persons recovering from some GAS infections (*5*,*6*). As a result, the portion of the *emm* gene that encodes the amino-terminal 100 residues of M protein is under strong diversifying selection pressure, and this region is hypervariable in terms of GAS types (*7*). Currently, >120 distinct *emm* types of GAS are recognized.

Despite the considerable diversity of *emm* types of GAS isolates, epidemiologic studies have found that relatively few *emm* types tend to predominate within a local population; most isolates are composed of a small number of *emm* types (*8*,*9*). In distinct geographic areas, the predominant *emm* types often vary in frequency from year to year for reasons not fully understood. In addition, sizeable outbreaks can be caused by strains of a single *emm* type or of a small number of *emm* types. Overall, this combination of factors results in a complex epidemiologic situation for GAS pharyngitis.

Recently, vaccine candidates have been identified in an effort to reduce the prevalence of GAS disease and the number of human deaths it causes (*10*). Some of these experimental vaccines are based on the amino-terminus of

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M protein because of the type-specific immunity that may develop after GAS infection. A multivalent vaccine has been developed that exploits the amino-terminus of the M protein from many different *emm* types (*11*). In principle, the effectiveness of this type of M-protein vaccine may be highly dependent on how well the M proteins selected for the vaccine match the *emm* types of locally circulating strains. Thus, a more complete understanding of geographic and temporal variation in *emm* type may be useful for vaccine design. Furthermore, the emergence of new variants of known M types has been documented. Knowledge of the rate and patterns of emergence of distinct *emm* types and their alleles may be critical for understanding how GAS may "escape" the immune response generated by a vaccine based on the amino-terminus of M protein.

We investigated the distribution of GAS *emm* types causing pharyngitis in Toronto, Ontario, Canada, during 2002–2010. We also examined the temporal change in *emm* types in pharyngitis cases and compared this distribution with data from a comprehensive population-based study of GAS *emm* types that were causing invasive infections in Ontario. Finally, we studied the *emm* types causing pharyngitis in multiple geographic locations across the province of Ontario in 2009 and 2010.

Materials and Methods

Collection of Isolates

Isolates collected from throat specimens of patients with acute pharyngitis were identified as GAS from primary media by a variety of standard methods. These GAS isolates (hereafter also referred to as pharyngeal isolates) were collected from 2002 through 2010 from multiple Ontario laboratories. GAS isolates, stripped of patient identifiers, were forwarded to Mount Sinai Hospital in Toronto for confirmation of identity and shipped to The Methodist Hospital Research Institute in Houston, Texas, for *emm* gene typing. Basic demographic information, including location where collected, age and sex of patient, and specimen collection date, was provided for isolates.

Toronto Isolates

During the summers (May through September) of 2002–2003 and 2006–2010, ≈500 consecutive isolates were collected each year from LifeLabs (formerly MDS), a large, centralized, commercial laboratory on the outskirts of Toronto. This facility primarily serves family medicine practices and outpatient clinics within the greater Toronto area but also acts as a catchment conduit from the surrounding region. The following number of isolates was obtained from this site per collection year: 523 (2002), 619 (2003), 502 (2006), 510 (2007), 522 (2008), 481 (2009), and 487 (2010). An additional ≈520 consecutive throat specimen GAS isolates from the Mount Sinai Hospital/University Health Network Clinical Microbiology Laboratory were collected during January 2008–March 2010.

Geographically Diverse Ontario Strains

Additional isolates from outlying locations of LifeLabs and Gamma Dynacare laboratory chains in London, Sudbury, and Thunder Bay, Ontario, each provided 100 consecutive GAS isolates per center during July–September 2009. The Gamma Dynacare Ottawa laboratory provided consecutive isolates up to 100 per month from July 2009 through July 2010, for a total 659 isolates. The Gamma Dynacare London branch provided 219 isolates from July through September 2009, and the distantly located North Bay and Elliot Lake branches together provided 36 GAS isolates from July through October 2009.

emm Type Assignment

GAS isolates were grown overnight at 37°C with 5% CO_2 on trypticase soy agar plates containing 5% sheep blood (TSAII; Becton Dickinson, Franklin Lakes, NJ, USA). Genomic DNA was obtained by boiling a sample obtained by streaking from multiple colonies in 0.05 mol/L NaOH for 2 min. The crude cell lysates were centrifuged for 2 min at 2,000 × *g*, and 2 μL of the lysate was used in PCRs. The hypervariable region of the *emm* gene that encodes the amino-terminus of M protein was amplified by PCR by using primers emm1 5′-TATT(C/G)GCTTAGAAAATTAA-3′ and emm2 5′-GCAAGTTCTTCAGCTTGTTT-3′. PCR products were purified by using 96-well ultrafiltration plates (EdgeBio, Gaithersburg, MD, USA), according to the manufacturer's instructions; products were suspended in 100 μL distilled water. Cycle sequencing was performed with the Big Dye version 3.1 dye-terminator kit (Applied Biosystems, Foster City, CA, USA) by using primer emm1. Unincorporated fluorescent dye terminators were removed with 96-well gel-filtration cartridges (EdgeBio). Sequencing reactions were analyzed with a 3730xl DNA sequencer (Applied Biosystems), and chromatograms were analyzed with Sequencher version 4.9 (GeneCodes, Ann Arbor, MI, USA). High quality sequences were trimmed to 220 nt in length and compared with reference sequences in the Centers for Disease Control and Prevention (CDC) *emm* database (ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/tsemm) by using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Data analysis and graphing were performed with the GraphPad software package (Prism, La Jolla, CA, USA). The invasive index of each *emm* type was calculated by dividing its frequency in invasive infections by frequency in pharyngitis infections.

Results

Overview of Pharyngitis Strains

We determined the *emm* type for 4,635 GAS isolates that were causing acute pharyngitis in the province of Ontario during 2002–2010. Of these, 3,209 isolates were collected from the greater Toronto metropolitan region, and 1,426 isolates were obtained from 5 sites located throughout Ontario (London, Ottawa, North Bay/Elliot Lake, Sudbury, and Thunder Bay). The mean age of patients was 16.1 years (range 8 months to 105 years).

Distribution of *emm* Types in Toronto GAS Pharyngitis Strains

Consistent with findings from previous surveys of GAS isolates that have caused pharyngitis in North America, Europe, and elsewhere (*8*,*9*,*12*), we found that a relatively small number of *emm* types dominated. For example, the 6 most prevalent *emm* types collected in Toronto during 2002–2010 were (in order of prevalence) *emm*12, *emm*1, *emm*4, *emm*28, *emm*2, and *emm*89 (Figure 1). These 6 *emm* types came from 68.9% of the pharyngeal isolates, whereas 29 *emm* types came from the remaining 31.1% of the isolates. Analysis of the annual change in *emm* type distribution indicated that, with few exceptions, these 6 types were consistently the most commonly collected. This finding suggests that the *emm* type population dynamic is relatively stable. However, *emm*89 strains were a key exception. The frequency of *emm*89 isolates increased 5-fold over the study period, increasing from 2.6% of isolates in 2002 to 14.7% in 2010 (Figure 2). In 2010, *emm*89 isolates were the second most common *emm* type among pharyngitis specimens in our sample. These data indicate a recent major expansion of type *emm89* strains among isolates causing pharyngitis in Toronto.

Identification of New *emm* Alleles

We identified 20 allelic variants of 8 GAS *emm* types that had not been previously described: *emm*1 (4 alleles), *emm*3 (3 alleles), *emm*5 (3 alleles), *emm*6 (4 alleles), *emm*8 (1 allele), *emm*11 (2 alleles), *emm*12 (2 alleles), and st106M (1 allele). Nucleotide sequences for these alleles have been submitted to the CDC *Streptococcus pyogenes emm* sequence database (designations listed in Table 1). Seventeen of these alleles differed from the most closely related reference sequence by 1 single-nucleotide polymorphism (SNP); 2 allelic variants differed by 2 SNPs; and 1 isolate had a 6-bp in-frame insertion, resulting in the addition of 2 amino acid residues. Of the 21 SNPs identified, all but 1 resulted in a predicted amino acid substitution in the translated M-protein sequence. This excess of nonsynonymous mutations underscores the effect of the strong diversifying selection pressure acting on the *emm* gene.

Ontario *emm* Types in Relation to an Experimental 26-Valent GAS Vaccine

Overall, 18 of 57 *emm* types found in the Toronto pharyngeal isolates are represented in an experimental 26-valent GAS vaccine described elsewhere (*11*). These 18 *emm* types included 11 of the 12 most prevalent *emm* types that represent strains causing 78.5% of the pharyngitis cases we studied, a number similar to estimates for the US population (*11*). Notably, the single most commonly observed GAS *emm* type (*emm*4) not included in the 26-valent experimental vaccine was very common in Toronto. For example, during 2002–2010, *emm*4 was the third most common *emm* type causing pharyngitis, and in 2007 and 2008, it was the most common *emm* type. This finding is not entirely unexpected because *emm*4 has been one of the most common serotypes identified by other pharyngitis surveys (*8*,*9*,*12*,*13*).

Although a multivalent GAS vaccine based on the amino-terminus of M protein has theoretical promise, a

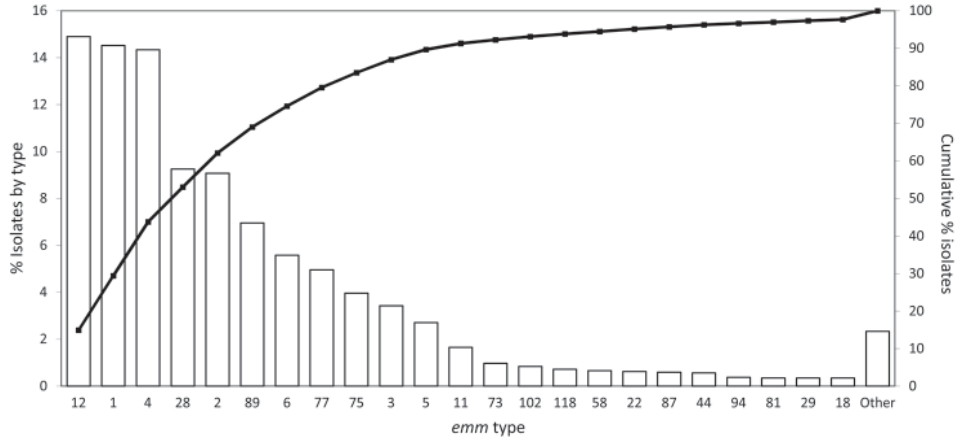

Figure 1. Distribution of group A *Streptococcus* (GAS) *emm* types collected in Toronto, Ontario, Canada, 2002–2010. Thirty-four GAS *emm* types with <10 isolates each (≈0.3% of total) comprise the "other" category. Line graph showing cumulative percentage is superimposed with percentage scale shown on right.

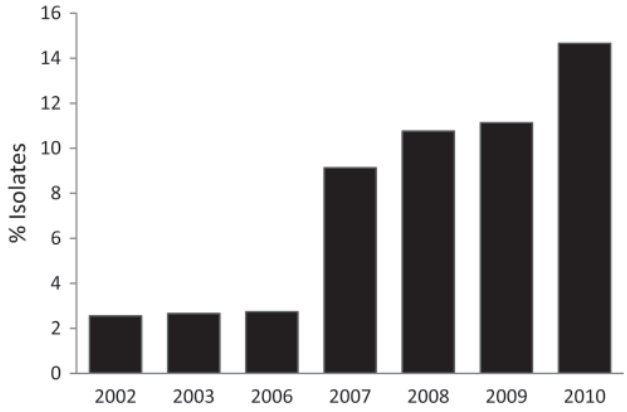

Figure 2. Annual frequency of *emm*89 isolates among patients with group A *Streptococcus* pharyngitis, Toronto, Ontario, Canada, 2002–2010.

potential concern is the detrimental effects of allelic variation on vaccine efficacy. Virtually all new and previously described *emm* alleles collected from pharyngitis patients in Toronto contained nucleotide changes that resulted in changes in amino acid sequence, with few alleles defined only by silent nuclear polymorphisms. New *emm* alleles generated by strong diversifying selection pressure acting on the *emm* gene could provide the means by which GAS strains evade a vaccine that includes only a single variant of each M-protein serotype; that is, creating vaccine-escape mutants.

To determine how common allelic variation was in the Toronto GAS population, we examined the number of alleles for each *emm* type found in specimens from pharyngitis patients in Toronto. For the top 10 serotypes, the most prevalent allele was found in 87.2% of the isolates (range 100%–42.7%). Notably, several prevalent *emm* types had an extensive number of alleles that would encode variant M proteins. For example, of the 6 most common serotypes, 4 had >6 allelic variants, and the most common *emm* type (*emm*12) had 16 different alleles.

Comparison of *emm* Type Distribution in Pharyngitis and Invasive GAS isolates

Previous studies have identified nonrandom associations between specific *emm* types and an increased risk for invasive infection (*14*–*17*) or increased severity of invasive infection (*18*,*19*). Thus, we tested the hypothesis that certain *emm* types were more prevalent in invasive disease isolates than in pharyngitis isolates in the Toronto region. Consistent with previous reports (*9*,*20*,*21*), we found that *emm*1 and *emm*3 strains each had an invasive index >1.0 (Table 2), which suggests that these *emm* types are overrepresented among invasive infections. Additionally, *emm*49 strains had an exceptionally high invasive index (16.7), largely because of the rarity of these strains among the pharyngeal isolates.

Table 1. Newly identified *emm* allelic variants from group A *Streptococcus* pharyngeal isolates, Ontario, Canada, 2002–2010

emm type	Allele designation
*emm*1	*emm*1.56, *emm*1.57, *emm*1.58, *emm*1.59
*emm*3	*emm*3.63, *emm*3.64, *emm*3.65
*emm*5	*emm*5.83, *emm*5.84, *emm*5.87
*emm*6	*emm*6.76, *emm*6.77, *emm*6.78, *emm*6.79
*emm*8	*emm*8.2
*emm*11	*emm*11.10, *emm*11.11
*emm*12	*emm*12.54, *emm*12.55
st106M	st106M.5

Comparison of the annual change in *emm* type frequencies in pharyngitis and invasive disease isolates indicated that certain *emm* types had highly variable frequencies, consistent with epidemic behavior. In particular, *emm*3 pharyngitis strains peaked in frequency in 2006 to become the fourth most common *emm* type that year. This timing corresponds with the observed peak in cases of invasive disease caused by *emm*3 strains in 2006 (Figure 3), which suggests a relationship between abundance of pharyngitis cases and invasive infections.

Variability in Frequency Distribution of *emm* Types from Diverse Localities

Several studies have reported that *emm* type distribution can vary geographically. Generally, however, these comparisons involved localities separated by large distances. To test the hypothesis that *emm* type distribution varied over a relatively small geographic distance, we analyzed GAS isolates from pharyngitis patients at 5 additional areas across Ontario (London, Ottawa, North Bay/Elliot Lake,

Table 2. Invasive indexes for *emm* types in group A *Streptococcus* isolates from patients with invasive disease and pharyngitis, Ontario, Canada, 2002–2010

emm type	Invasive disease frequency	Pharyngitis frequency	Invasive index
1	0.298	0.142	2.09
2	0.021	0.094	0.22
3	0.051	0.037	1.38
4	0.057	0.150	0.38
5	0.028	0.025	1.12
6	0.029	0.060	0.48
11	0.031	0.017	1.82
12	0.081	0.138	0.59
22	0.005	0.007	0.71
28	0.051	0.098	0.52
49	0.036	0.002	16.7
75	0.022	0.034	0.65
77	0.024	0.058	0.41
78	0.006	0.002	3.0
89	0.058	0.058	1.0
Others	0.202	0.078	2.59

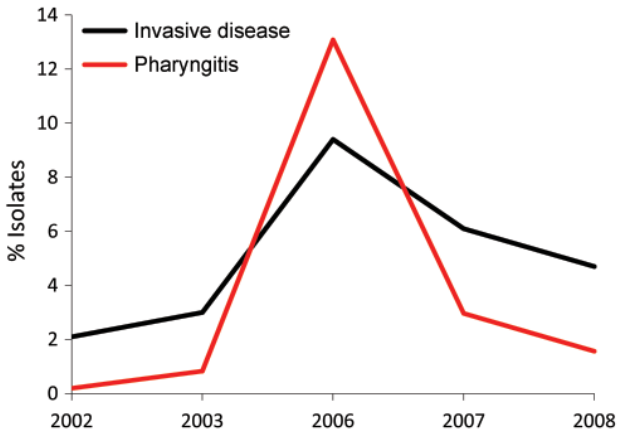

Figure 3. Frequency of *emm*3 strains among patients with group A *Streptococcus* pharyngitis and invasive disease, Ontario, Canada, 2002–2010, excluding 2004–2005. Black line indicates yearly frequency of invasive *emm*3 isolates; red line indicates *emm*3 frequency among pharyngeal isolates.

Sudbury, and Thunder Bay) during 2009–2010. In the aggregate, the *emm* types from the 5 geographically distinct collection sites closely resembled those found in Toronto in 2009 and 2010. The same 6 most prevalent *emm* types were found in 2009, and only 1 *emm* type differed in frequency in 2010. In general, *emm* types from individual collection sites were consistent from year to year (Table 3). However, we discovered striking inter-site variability in the distribution of *emm* types. For example, in 2009, *emm*89 was the most common *emm* type identified at 4 of the 6 localities, but *emm*89 strains were not among the 6 most common *emm* types found in Ottawa. Additionally, only 2 *emm* types were shared among the 5 most common organisms collected in North Bay and Sudbury, an unexpected result ($p = 0.0007$; Fisher exact test), given that these locations are separated by only ≈120 km.

We also observed apparent local outbreaks of certain *emm* types in some locations. In 2009 and 2010, *emm*3 strains were among the 6 most prevalent *emm* types in Ottawa but were rarely observed elsewhere. Most (33/43 [77%]) of the Ottawa *emm*3 strains had the *emm*3.53 allele, which differs from the *emm*3.2 allele by a single nucleotide change. Isolates with the *emm*3.2 allele are otherwise the most abundant *emm*3 strains in Ontario. We note that 1 isolate with the *emm*3.53 allele was found in Toronto in 2009, where it had not been observed in previous years, suggesting recent introduction. Subsequent studies will be required to determine whether the *emm*3.53 strain expands across Ontario and whether it has increased invasive potential.

Discussion

In this large study of *emm* type distribution among GAS pharyngitis strains in Canada, we identified a similar pattern of *emm* type distribution as reported in previous surveys and also observed that strains of a relatively few *emm* types dominate. The most abundant *emm* types were similar to those reported in previous studies of GAS pharyngitis strains from North America and Europe (*8*,*9*,*12*).

Of note, we found that *emm*89 strains have recently increased in frequency in Ontario. Specifically, over a 9-year period, *emm*89 strains increased 5-fold and in 2010 were the second most common *emm* type in the Toronto sample. The increase in *emm*89 strains among pharyngeal isolates paralleled an increase in the frequency of *emm*89 strains among invasive GAS isolates from 2003 through 2010 (Figure 4). This finding suggests that a marked expansion of *emm*89 strains has occurred in Ontario. Regional outbreaks of *emm*89 strains have been documented previously, including a clonal epidemic that occurred in northern Italy (*22*). Surveillance conducted by CDC also has reported similar increases in *emm*89 strains among invasive infections in New York and Maryland during 2007–2009, and *emm*89 was among the top 5 invasive serotypes collected by CDC in 1998, 2001–2003, and 2007–2009 (*23*). Thus, *emm*89 strains may commonly contribute to local epidemics of pharyngitis and invasive disease.

Although the fact that the frequency distribution of GAS pharyngitis *emm* types varies across localities separated by large distances has been well described, we have shown that *emm* type distributions also can vary over relatively small distances. This finding expands knowledge of GAS epidemiology. Thus, GAS pharyngitis strains circulating in Ontario are a collection of distinct populations, apparently characterized by relatively limited transmission between distant locations. Much of our understanding of GAS epidemiology has been based on the characterization of

Table 3. Five most common M types of group A *Streptococcus* from pharyngeal isolates, by location, Ontario, Canada, 2009–2010*

Year	London	Ottawa	North Bay/ Elliot Lake	Sudbury	Thunder Bay
2009	M89	M4	M89	M89	M28
	M4	M28	M75	M2	M12
	M2	M1	M4	M11	M89
	M1	M3	M1	M12	M6
	M12	M12	M77	M75	M58
2010	NA	M1	M89	NA	M28
		M3	M1		M59
		M4	M118		M1
		M28	M4		M12
		M12	M75		M89

*NA, not available.

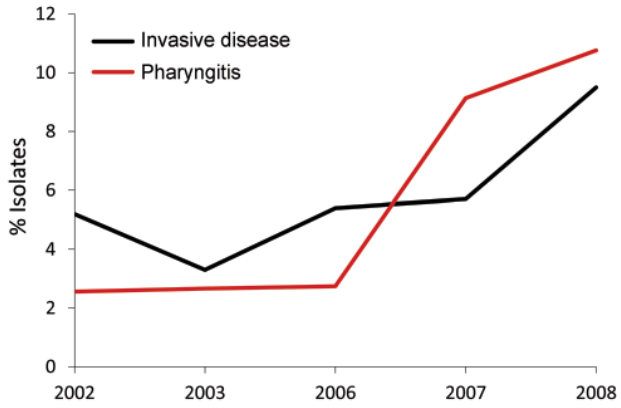

Figure 4. Frequency of *emm*89 strains among patients with group A *Streptococcus* pharyngitis and invasive disease, Ontario, Canada, 2002–2010, excluding 2004–2005. Black line indicates yearly frequency of *emm*89 among invasive disease isolates; red line indicates frequency of *emm*89 among pharyngeal isolates.

strains causing local outbreaks of invasive disease and large surveillance networks encompassing geographically expansive catchment regions. This circumstance has led to the belief that GAS exists mostly as large, homogeneous populations. Our findings suggest that GAS populations are much more complex. This conclusion is supported by our previous genomewide analysis of invasive *emm*3 isolates from Ontario, which found that genetic distance and geographic proximity were strongly correlated and that groups of clonally related isolates were frequently limited to discrete geographic locations (*24*).

Our longitudinal analysis of *emm* types in Toronto indicated that several *emm* types (including *emm*1, *emm*2, *emm*3, and *emm*77) varied substantially in annual frequency, which suggests features of epidemic behavior. Comparison of yearly frequencies of *emm* types in *emm3* isolates from patients with pharyngitis and invasive disease showed a nearly superimposable pattern, with coincident peaks of infection occurring in 2006 (Figure 3). This finding is consistent with a model in which many invasive GAS strains originate from the local pharyngitis strains and that cyclical outbreaks of invasive infection coincide or follow recent outbreaks of pharyngitis infections. A similar conclusion was reported by Hoe et al. (*25*), whose analysis of pharyngitis and invasive isolates from Finland showed that a novel streptococcal inhibitor of complement (*sic*) alleles first appeared in local pharyngitis strains before their appearance in invasive isolates. Furthermore, a GAS clone responsible for a local outbreak of invasive disease in Minnesota was common among pharyngeal isolates from school-aged children living in the outbreak area (*26*). Additional investigation into the genetic relationship between pharyngitis and invasive disease strains conducted at the full-genome level may provide useful information about the molecular events that contribute to invasive GAS.

The large size and longitudinal nature of our strain sample enabled us to obtain extensive information about the level of *emm* allelic variation and the rate of emergence of new *emm* alleles in Ontario. Previous experiments conducted by Dale et al. on 3 serotypes included in the 26-valent GAS vaccine found that slight allelic variants had little influence on bactericidal killing activity during in vitro assays, leading the researchers to conclude that variant subtypes might not affect vaccine efficacy (*27*). However, this finding contrasts with several other reports that observed a variable response to allelic variants (*28*,*29*). Whether the findings of Dale et al. are applicable to all 26 serotypes included in the vaccine is unknown (*27*). Despite its potential, albeit unproven, relevance to GAS vaccine design, we have a relatively limited understanding about this subject. We observed extensive *emm* allelic variation in Ontario, with most common *emm* types possessing ≥6 different alleles. We also found that strong selective pressure was driving the emergence of new M-protein variants, with all but one of the new alleles encoding amino acid substitutions. The observed ratio of synonymous to nonsynonymous nucleotide substitutions indicates that allelic variation most likely is shaped by selective pressure, perhaps immune mediated. Previous investigators have also reported that the N-terminal regions of M proteins possess functional domains in addition to opsonic epitopes that might constrain the amount of variability within an M type (*30*–*33*). Whether allelic variation may eventually result in escape mutants in a population with high levels of immunity, resulting from administration of an M-protein–based vaccine, is not known but should be considered. We believe this allelic variation might pose a challenge to GAS vaccine designs that rely on recombinant portions of the M-protein amino-terminus.

GAS M-protein serotypes are often regarded as genetically homogeneous populations composed of a single or relatively few clones. The remarkable level of *emm* type allelic diversity we observed in Ontario contrasts with this view. We found extensive diversity not only in the distribution of GAS serotypes but also on the allelic level and between geographic locations separated by short distances. Our recent genomewide analysis of invasive M3 isolates in Ontario revealed a strikingly complex genetic structure (*24*). Given the relationship between these populations, GAS pharyngeal isolates probably harbor an additional layer of genetic diversity that remains to be elucidated through whole-genome sequencing of a population of pharyngeal isolates.

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Close Similarity between Sequences of Hepatitis E Virus Recovered from Humans and Swine, France, 2008–2009

Jérôme Bouquet, Sophie Tessé, Aurélie Lunazzi, Marc Eloit, Nicolas Rose, Elisabeth Nicand, and Nicole Pavio

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Upon completion of this activity, participants will be able to:

- Describe epidemiologic features of autochthonous hepatitis E virus (HEV) infections based on a French study
- Compare the genetic identity of HEV strains found in humans and swine during an 18-month period in France
- Describe the public health implications of these findings.

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Frequent zoonotic transmission of hepatitis E virus (HEV) has been suspected, but data supporting the animal origin of autochthonous cases are still sparse. We assessed the genetic identity of HEV strains found in humans and swine during an 18-month period in France. HEV sequences identified in patients with autochthonous hepatitis E infection (n = 106) were compared with sequences amplified from swine livers collected in slaughterhouses (n = 43). Phylogenetic analysis showed the same proportions of subtypes 3f (73.8%), 3c (13.4%), and 3e (4.7%) in human and swine populations. Furthermore, similarity of >99% was found between HEV sequences of human and swine origins. These results indicate that consumption of some pork products, such as raw liver, is a major source of exposure for autochthonous HEV infection.

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Hepatitis E virus (HEV) is a causative agent of enterically transmitted acute hepatitis in humans (*1*). It is a major

public health issue in developing countries, where it causes large waterborne epidemics (*2*). In industrialized countries, it is an emerging problem, as an increasing number of sporadic cases for which the origins are still unclear (*3*) have been reported for patients who have not traveled to HEV-endemic areas.

HEV is a nonenveloped virus with a single-stranded positive RNA genome of 7.2 kb composed of 3 open reading frames (ORFs). HEV is the sole member of the family *Hepeviridae* (*4*) and has been classified into 4 major genotypes and 24 subtypes. Genotype 1 is divided into 5 subtypes (1a to 1e), genotype 2 into 2 subtypes (2a and 2b), genotype 3 into 10 subtypes (3a to 3j), and genotype 4 into 7 subtypes (4a to 4g) (*5*). Although genotypes 1 and 2 are endemic to developing countries, genotypes 3 and 4 are the cause of sporadic cases. HEV is the only hepatitis virus that is also found in a wide variety of animals (*6*). Genotype 3 can infect humans as well as swine, wild boar, deer, and mongoose (*7–10*). It is generally agreed that swine are widely infected all over the world (*6*). HEV seroprevalence varies greatly depending on countries; 22.7% to 88.4% of pigs are seropositive at 6 months of age (*11*,*12*). Among pigs slaughtered at ≈25 weeks of age, the prevalence of HEV fecal excretion ranges from 4% to 41% (*13*,*14*). Viral RNA sequences from pigs and humans can be closely related (*15*,*16*), and cross-species infection of genotypes 3 and 4 from human to pig and pig to nonhuman primate has been demonstrated experimentally (*17*). To date, only 2 cases of zoonotic transmission from consumption of raw or undercooked sika deer and wild boar meat have been clearly identified in Japan with near or 100% homology between the sequences from the patient and the consumed meat (*7*,*8*).

A few reports have shown close phylogenetic relationships between sequences identified in swine and in humans. However, these studies were based on limited numbers of sequences with little geographic or temporal data (*18–21*).

In France, HEV seroprevalence in the human population ranges from 3.2% to 16.6%, depending on the geographic regions studied (*22*,*23*). The number of reported viral hepatitis E cases is increasing. Although only 38 cases were reported in 2006, a total of 340 cases were diagnosed in 2010, of which 70% were declared autochthonous with no recent history of patients traveling abroad (French National Reference Laboratory, unpub. data). In the swine reservoir, a recent nationwide survey performed at slaughterhouses showed high prevalence of HEV. HEV seroprevalence in swine ranges from 31% at the individual level to 65% at the farm level. In that study, HEV prevalence in pig liver was estimated at 4%, meaning that HEV-infected pig livers can enter the food chain (*24*). Moreover, it has been shown that regional products made from raw pig liver may contain HEV (*25*). In France, pork is the most widely eaten type of meat (*26*) and could represent an HEV reservoir with a high risk for zoonotic transmission.

To assess the zoonotic risk for transmission from swine to humans in France, we studied HEV sequences in both hosts. HEV sequences collected from every human autochthonous case of hepatitis E infection and HEV-positive pig livers collected at slaughterhouses, both within 18 months, were analyzed. Epidemiologic and spatial–temporal data corresponding to phylogenetic analyses of partial ORF2 sequences were used to investigate whether swine are a major source of HEV contamination in France.

Materials and Methods

HEV Patients

Persons who had autochthonous hepatitis E virus infection during May 2008–November 2009 and had no travel history outside France were included in the study. RNA was extracted from patient serum or fecal samples by using a MagNA Pure LC RNA Isolation Kit (MagNA Pure LC Instrument; Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions. HEV RNA was amplified by using a nested reverse transcription PCR for the ORF2 gene as described (*27*). Sequencing was performed on amplified strands with an automated DNA sequencer (CEQ8000; Beckman-Coulter Inc., Fullerton, CA, USA). Patients' demographic and epidemiologic features were collected anonymously from a questionnaire on age, sex, recent travel (within the past 4 months), and medical history.

Swine Sample Collection

As part of a national survey on the prevalence of swine infected with HEV, 3,715 liver samples were collected at slaughterhouses from May 2008 through November 2009. Pig farms were selected through random sampling from 35 slaughterhouses accounting for 95% of the national pig production. Herds were selected randomly from a database table indicating dates and times of slaughter regardless of the herd size, leading to a random distribution of small and large types of farms (*24*). Thirty milligrams of liver was excised with sterile surgical blades. Tissues were disrupted in bead-milling tubes (FastPrep 24; MP Biomedicals, Illkrish, France). RNA was extracted by using the RNeasy Viral RNA extraction kit (QIAGEN, Courtaboeuf, France) according to the manufacturer's instructions.

HEV RNA was detected by nested reverse transcription PCR with the same primers used for human HEV amplification (*27*). Positive samples were sequenced by the Sanger method (Cogenics, Grenoble, France or Eurofins MWG Operon, Ebersberg, Germany).

Phylogenetic Analysis

We deposited 106 HEV sequences from human patients (1 sequence/patient) in GenBank under accession nos. JF730329–JF730434 and 43 HEV sequences from swine livers (1 sequence/farm) under accession nos. JF718787–JF718829. Human and swine HEV RNA sequences of 204 to 306 nt were analyzed by using MEGA4 (*28*), with a set of sequences available from GenBank (online Appendix Table, wwwnc.cdc.gov/EID/article/17/11/11-0616-TA1.htm), to determine genotypes and subtypes as described by Lu et al. (5). Alignment was performed by using ClustalW (MEGA4, www.megasoftware.net). Phylogenetic trees were built by using the neighbor-joining method with a bootstrap of 1,000 replicates.

Statistical Analyses

Statistical analyses were performed by using a χ^2 distribution with 1 df and the Fisher exact probability test to compare proportions between the 2 groups. Differences were considered to be statistically significant when $p<0.05$.

Results

Epidemiologic Data

During May 2008–November 2009, hepatitis E was diagnosed for 305 patients in France. Only the 106 patients who had answered and returned the questionnaire and who had no recent history of traveling abroad were included in the study.

Of the 106 patients with HEV viremia, information on sex and age was available for 103 patients, of whom 72% were men; the mean age was 55 years (Figure 1). The 40–69-year age group had a significantly predominant number of male patients (81%). All patients had acute resolving hepatitis E, except for 1 in whom chronic hepatitis E developed after a liver transplant.

Geographic Distribution of Human Cases and HEV-positive Swine Herds

Geographic data on place of residence were available for 100 patients. Most human HEV cases were diagnosed in southern France (67%), especially in the southeastern region, Provence-Alpes-Côte-d'Azur, which accounted for 30% of the cases (Figure 2) and contains 7.6% of the national population. In northern France, where 33% of the cases were observed, a high density of HEV cases (11%) were clustered in the Paris region (Figure 2). The Paris area, Ile-de-France, is the most populated region and contains 18% of the total population (*29*). In contrast, most of the HEV-positive swine herds were found in northern France (77%), particularly in the western region, Brittany, which is the largest swine-producing region, accounting for 52% of national production (Figure 2). Fewer positive swine samples were found in southern France (23%), where there is a lower density of pig herds than in Brittany (*24*).

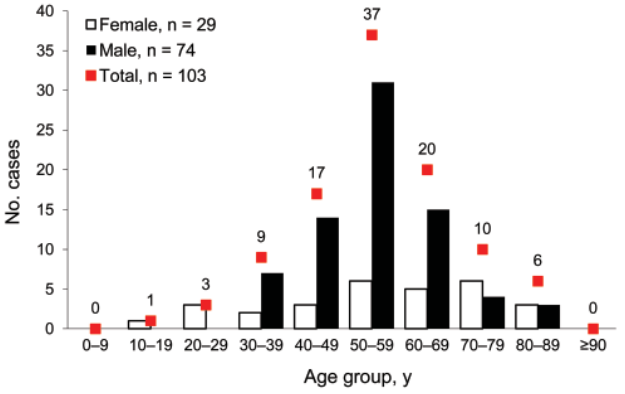

Figure 1. Distribution of age and gender for 103 hepatitis E virus (HEV) viremic patients, France, May 2008–November 2009.

Human and Swine HEV Sequences

To characterize HEV circulating in humans and swine from May 2008 through November 2009, we subjected partial ORF2 HEV sequences, amplified for both populations, to phylogenetic analysis. This ORF2 genomic region seems to match the classification of full-

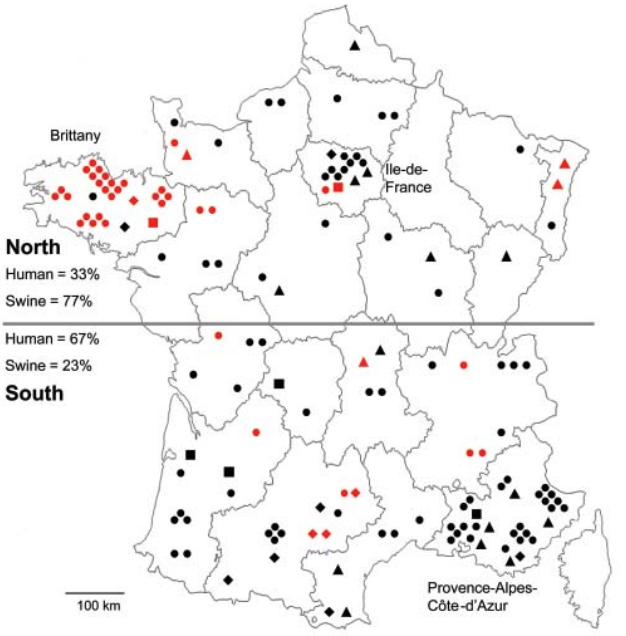

Figure 2. Geographic distribution of hepatitis E virus (HEV) subtypes recovered from humans (n = 100) and swine (n = 43), France, May 2008–November 2009. Black, human HEVs; red, swine HEVs; triangles, subtype 3c; squares, subtype 3e; dots, subtype 3f; diamonds, strains of undefined subtype. Regions with a high density of HEV are named.

length HEV sequences according to Lu et al. (*5*) and gives similar phylogenetic topologies to the ORF1 region RdRp (*30*). For each human case, a single HEV sequence was retrieved (n = 106). One HEV RNA sequence from each positive farm was included when the same sequence was recovered from several pig livers from the same farm (n = 43). To define genotypes and subtypes, we added 22 reference sequences of human and swine origins to the analysis (online Appendix Table). Genotype 4 HEV was used as the outgroup.

Human and swine strains were scattered homogeneously on the phylogenetic tree (Figure 3); no specific cluster in relation to the host was considered. All sequences belonged to genotype 3 and more specifically to subtypes 3f, 3c, and 3e. There was some difficulty in identifying a specific subtype to a cluster of 12 sequences, 8 from humans and 4 from swine. These sequences were close to 7 subtypes (3a, 3b, 3c, 3d, 3h, 3i, and 3j) but shared <90% homology with any of them (*5*). The term undefined subtype was given to this cluster (Figure 3).

Subtype Proportions and Distribution

A comparison of subtype proportions in swine and human populations did not reveal any significant differences (p>0.05) (Table 1). Subtype 3f was the largest cluster, accounting for 73.8% of the strains sequenced (72.6% in humans and 76.7% in swine). Subtype 3c was the second largest group, accounting for 13.4% of HEV strains (15.1% in humans and 9.3% in swine). The set of sequences of undefined subtype accounted for 8.1% of the total strains and was also homogeneously represented (no statistical difference in proportion) between human (7.6%) and swine strains (9.3%). Finally, the proportion of subtype 3e was smallest, 4.7% in the swine and the human groups.

Geographic distribution of subtypes showed that 3f was found all over the territory; 3c seemed to be missing in Brittany, where the largest number of samples was collected (1,760 livers). Most sequences of the undefined subtype originated from southern France (Figure 2).

Nucleotide Variations among Human and Swine HEV Sequences

To investigate whether some nucleotide positions would be host strain specific, a p-value was calculated for each nucleotide position. No significant difference (p<0.05) between human and swine HEV was obtained for the short nucleotide sequence studied (data not shown). The same observation was made at the amino acid level, where there was no significant difference at any position between human and swine HEV (data not shown).

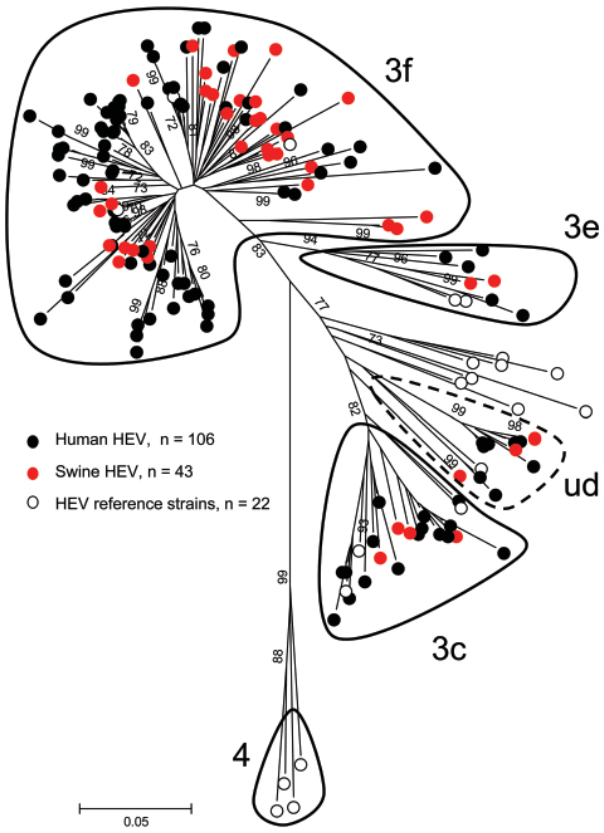

Figure 3. Phylogenetic tree of hepatitis E virus (HEV) detected in human and swine constructed by the neighbor-joining method with a bootstrap of 1,000 replicates based on the ClustalW alignment (MEGA4, www.megasoftware.net) of 204- to 306-nt sequences within open reading frame 2. The 106 HEV sequences recovered from patients from France are displayed as black dots (GenBank accession nos. JF730329–JF730434), the 43 HEV sequences recovered from swine from France are displayed as red dots (accession nos. JF718787–JF718829), and the 22 reference strains from GenBank are displayed as white dots (GenBank accession nos. in online Appendix Table, wwwnc.cdc.gov/EID/article/17/11/11-0616-TA1.). Genotype 4, subtypes 3f, 3c, and 3e, as defined by Lu et al. (*8*), are encircled by a solid black line; undefined subtype is encircled by a dashed black line. Bootstrap values >70% are indicated on respective branches. Scale bar represents nucleotide substitutions per site.

HEV Sequence Similarities

Human Sequence Similarities

The 106 sequences recovered from human patients were compared with each other at the nucleotide level. The percentage of nucleotide sequence identities ranged from 67.8% to 100% (Table 2). Four groups of 2–3 patients had 100% nt similarity. These sequences were detected in patients living in different regions, at intervals ranging from 6 days to 6 months (Figure 4).

Table 1. Proportions of subtypes of HEV strains identified in 106 humans and 43 swine, France, May 2008–November 2009*

Sequences	No. (%) isolates				Total no.
	Subtype 3c	Subtype 3e	Subtype 3f	Undefined subtype	
Human HEV	16 (15.1)	5 (4.7)	77 (72.6)	8 (7.6)	106
Swine HEV	4 (9.3)	2 (4.7)	33 (76.7)	4 (9.3)	43
Total HEV	20 (13.4)	7 (4.7)	110 (73.8)	12 (8.1)	149

*HEV, hepatitis E virus.

Swine Sequence Similarities

First, to evaluate HEV within-farm homology, we compared 10 sequences recovered on the same day from 10 animals from the same farm. Similarities of 99% to 100% were found (Table 2). The 43 sequences recovered from independent farms all over France were then compared with each other. Similarities ranged from 71.7% to 99.3% (Table 2). Three pairs of sequences were found with similarities of >99% (Figure 4). These pairs of sequences originated from neighboring farms (Figure 4). Two pairs of sequences were sampled on the same day (August 30, 2008 or November 18, 2009), and the third pair was sampled at a 6-month interval (June 10, 2008, and November 24, 2008).

Similarities between Human and Swine Sequences

Similarities ranged from 68.4% to 99.3% (Table 2). These minimum and maximum similarities do not significantly differ from those found in each separate population ($p<0.05$).

Two pairs of sequences were found to have >99% similarity. In both cases, human and animal HEV sequences were identified in different geographic regions at intervals of 5 months (human, August 3, 2008; and swine, April 9, 2008) to 1 year (human, May 22, 2009; and swine, May 27, 2008). In both cases, swine sequences were sampled first, before the onset of the disease in the patient.

Discussion

Although zoonotic transmission of hepatitis E virus from swine to human has been well accepted, little data are available on HEV sequences circulating in human and swine populations within a country during a restricted period. We investigated a large number of HEV sequences, collected from 106 patients and 43 swine over an 18-month period. The patients were mostly male (72%) and >55 years of age. This finding is in agreement with a previous report on acute HEV infection in France, which found that men accounted for 68% (36/53) of the cases (*31*). The situation in industrialized countries contrasts with that in regions where attack rates for waterborne outbreaks of HEV genotype 1 are higher among young adults (15–40 years of age) (*1*). This observation suggests that the 2 epidemiologic profiles may involve different contamination routes. There are differences in hygiene and meat consumption habits in these regions. Moreover, no animal reservoirs have been yet described for the genotypes involved in waterborne outbreaks (genotypes 1 or 2) (*6*), suggesting that zoonotic transmission might be limited in any HEV-endemic areas.

Table 2. Percentage nucleotide similarities of 204 to 306 HEV nucleotide sequences from 106 humans and 43 swine, France, May 2008–November 2009*

Sequences	Minimum	Average	Median	Maximum
Human HEV	67.8	85.2	87.7	100
Swine HEV				
Same farm	99.0	99.7	99.7	100
Different farms	71.7	86.1	88.2	99.6
Human and swine HEV	68.4	85.4	87.6	99.3

*HEV, hepatitis E virus.

All 149 HEV sequences belonged to genotype 3 and were divided into at least 3 subtypes according to the classification elaborated by Lu et al. (*5*). Sequences sharing a minimum of 90% similarity were considered as belonging to the same subtype. Among these sequences, 137 belonged to subtypes 3f, 3c, and 3e. For 12 sequences, 8 human HEV and 4 swine HEV, there was some classification uncertainty because they were close to 7 different subtypes but shared <90% homology. The difficulty in classifying this undefined subtype might be because of partial sequencing of the strains identified, although Lu et al. showed that the 5′ end of the ORF2 region matches the complete genomic sequence for HEV classification better than other regions of the HEV genome (*5*). Using the nucleotide BLAST database (http://blast.ncbi.nlm.nih.gov/Blast), sequences from this undefined subtype are close but share <90% homology to 3a and 3c sequences detected in the Netherlands or 3h and 3i sequences detected in Germany. This undefined subtype also clusters on its own (>90% homology) and could be a new subtype that is specific to France. Comparison of autochthonous HEV from France with HEV from neighboring countries shows that the same main subtypes are found: 3f is found all over Europe; 3c in the Netherlands, Italy, and Hungary; and 3e in the UK, the Netherlands, Germany, and Hungary (*15*,*18*,*32*–*35*). This finding suggests that some subtypes may have emerged and evolved locally through animal trading.

The proportion of each subtype in both species was then estimated, and the proportions of subtypes 3f, 3c, and 3e were found to be almost the same. Such a similar distribution of subtypes suggests an active circulation

of the virus between the 2 host species in France. In the Netherlands, proportions of subtypes in human compared with animals or environmental strains were found to differ markedly, 6% versus 43% for 3f and 75% versus 35% for 3c (*18*), suggesting a limited number of contamination events through these 2 possible contamination pathways in this country.

Although HEV is widely distributed across France, some geographic regions showed higher rates of infection in humans. Most (67%) cases of autochthonous hepatitis E were found in southern France and particularly in the Provence-Alpes-Côte-d'Azur region (30%). These results are consistent with HEV seroprevalence in blood donors being higher in southern (16.6%) than in northern France (3.2%) (*22*,*23*). Furthermore, this observation correlates with results of a previous national survey in France showing an increasing north-to-south gradient of acute hepatitis E (*31*). In contrast, in the animal reservoir, most HEV sequences were detected in the main pig-producing area located in northwestern France. Nevertheless, the low number (only 2) of human cases observed in this region with a high density of pig farms suggests that the number of contamination events through the environmental pathway is limited. In the Ile de France region (Paris area), a high number (11%) of cases of hepatitis E was also reported. This finding could be partially explained by the high population density (18%) in this area; a few cases were reported after traveling and eating uncooked pork products in southern France.

To further analyze sequence similarities between human and swine HEV strains, we determined the similarities in nucleotides between human and swine sequences. HEV has a high mutation rate because of its error-prone RNA-dependent RNA polymerase and is probably present as a quasispecies in an infected host (*36*). Thus, low (<1%) variability in nucleotides may correspond to a unique strain. Analyzing human HEV sequences, 100% nt similarity was found in 4 groups of 2–3 patients. These patients were not related, but they may have been exposed to an unknown common source of contamination.

Swine sequences amplified from livers of animals within the same herd were found to be homogeneous, with a maximum difference of 3 nt along the 306 nt sequenced. Except for 3 groups of 2 herds, all the sequences were different (<99%). These 3 groups included herds that were sampled at the same time or 5 months apart and that were geographically close (a few kilometers). This high similarity of partial sequences might be explained by a possible exchange of animals between nearby herds, which is a common practice. However, it cannot be excluded that movements of farm workers and veterinarians or spreading of infected slurry might contribute to HEV transmission between herds. Swine HEV infection spreads easily within a herd through the fecal–oral route (*37*). This geographic clustering of HEV strains detected in animals was also observed in Sweden (*19*).

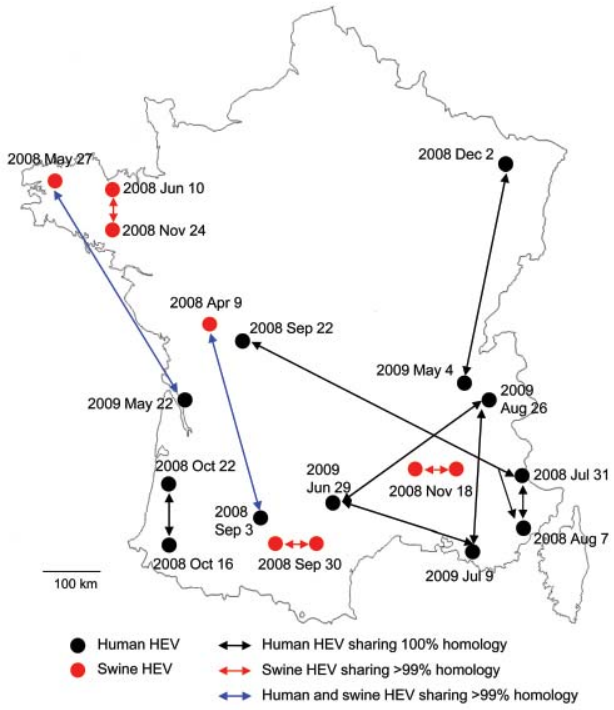

Figure 4. Geographic distribution and sampling date of human and swine hepatitis E virus (HEV) sequences sharing >99% identities, France, May 2008–November 2009.

Because animals from the same herd can have a difference of 3 nt over the same amplified sequence, human and porcine HEV sequences with >99% similarities may be considered as coming from related strains. A comparison of human and swine sequences showed that 2 pairs of sequences were similar (99.3%). In both cases, swine and human sequences were detected in different geographic areas. The swine sequences were identified first and later in humans. Pork meat is the most widely eaten meat in France (34.7 kg/inhabitant/year), and it is distributed and consumed throughout France (*26*). In our study, HEV sequences were amplified from liver, but other meat might be a vector for HEV infection because it has been shown that other organs such as muscles can be HEV positive (*38*). Considering the geographic distances and the detection of these HEV sequences in animals first, it seems reasonable to assume that foodborne infection may play a major role in autochthonous cases of hepatitis E. The high similarity observed suggests that these 2 cases could be the result of zoonotic transmission. Furthermore, because since these sequences are not geographically linked, contamination through environmental exposure can be ruled out.

In addition to the high degree of similarity observed between human and swine sequences and the identical proportion of each subtype in both hosts, no specific nucleotide substitutions have been identified when sequences from different host species were compared. These results are in line with the possible absence of a species barrier for HEV strains of genotype 3. However, before concluding that there are no host restriction determinants, further analysis of longer sequences is required.

This unique large-scale study on human and swine sequences with spatial–temporal data suggests that zoonotic transmission of HEV is involved in autochthonous cases. The swine reservoir is widely infected with HEV, and infected livers enter the food chain. Living in southern France seems to be associated with more frequent exposure to HEV (67% of cases). This observation might be linked to cultural food habits specific to southern France and frequent consumption of products made from raw swine liver (*25*).

Slurry from swine is often spread onto local fields, but there are few (only 2) cases reported in Brittany compared with other regions. The spread of HEV into the environment may not have major consequences but cannot be ignored. Contact with animals; consumption of contaminated water, vegetables, or shellfish; or unknown routes of transmission need to be investigated. In conclusion, taken together, these results confirm the major role played by the swine reservoir of HEV in autochthonous cases of hepatitis E. This study underlines the need for a surveillance and control plan, either at the level of pig production or at the level of food processing, to limit human exposure to HEV through consumption of pork products.

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Mr Bouquet is a PhD student working at the French Agency for Food, Environmental and Occupational Health and Safety. His research focuses on the molecular biology, genetics, and epidemiology of hepatitis E virus in human and animals and the risk for zoonotic transmission.

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Dynamics of Cholera Outbreaks in Great Lakes Region of Africa, 1978–2008

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Cholera outbreaks have occurred in Burundi, Rwanda, Democratic Republic of Congo, Tanzania, Uganda, and Kenya almost every year since 1977–1978, when the disease emerged in these countries. We used a multiscale, geographic information system–based approach to assess the link between cholera outbreaks, climate, and environmental variables. We performed time-series analyses and field investigations in the main affected areas. Results showed that cholera greatly increased during El Niño warm events (abnormally warm El Niños) but decreased or remained stable between these events. Most epidemics occurred in a few hotspots in lakeside areas, where the weekly incidence of cholera varied by season, rainfall, fluctuations of plankton, and fishing activities. During lull periods, persistence of cholera was explained by outbreak dynamics, which suggested a metapopulation pattern, and by endemic foci around the lakes. These links between cholera outbreaks, climate, and lake environments need additional, multidisciplinary study.

In Asia, the endemic and seasonal character of cholera largely depends on human exposure to the aquatic reservoirs of *Vibrio cholerae* (*1*). Culturable *V. cholerae* as well as viable but nonculturable *V. cholerae* (i.e., those that have entered into a dormant stage because of conditions unfavorable for growth or reproduction) attach to zooplankton and phytoplankton, especially in estuarine areas (*2*). In these areas, the incidence of cholera is influenced by local factors, such as rainfall and plankton blooms, and by global climatic conditions, such as increased sea surface temperatures linked to El Niño Southern Oscillation events (*3*,*4*). This link between cholera, the aquatic environment, and climate, named the "cholera paradigm" by Colwell (*5*), was highlighted by numerous studies in coastal areas.

Except for Haiti, where an epidemic of cholera began in mid-October 2010 (*6*), the area experiencing the worst cholera epidemics is sub-Saharan Africa. During 1995–2005, a total of 632 cholera outbreaks were reported worldwide; 66.0% of the total cases and 87.6% of fatal cases were reported from sub-Saharan Africa (*7*). Specifically, according to the World Health Organization (WHO), only 5 countries (Burundi, Cameroon, the Democratic Republic of the Congo [DRC], Ghana, and Tanzania) have reported cases of cholera every year since 1990 (*8*). Three of these countries—Burundi, DRC, and Tanzania—are partially or totally located in the African Great Lakes region (AGLR), an area including Lakes Tanganyika, Victoria, Kivu, Edward, and Albert. This region also includes Rwanda and part of Kenya and Uganda, which have also reported cases of cholera nearly every year since 1991 (except for 3 years for Kenya and 2 years for Rwanda and Uganda). Except for some limited epidemics, AGLR was long free from cholera, which emerged in 1977–1978 when the 6 countries were simultaneously affected (*9*). Since then, AGLR has become one of the most active foci of cholera, declaring 322,532 cases during 1999–2008 (20% of all cholera cases officially reported worldwide to WHO for these 10 years). Nevertheless, these numbers are widely underestimated because many patients cannot access health care facilities

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(*10*). This worrying evolution of cholera outbreaks in AGLR went unnoticed in the scientific community, and no serious attempts have been made to describe these new endemic foci of a waterborne disease originating from coastal marine environments.

Considering the established link between rainfall, El Niño events, sea surface temperature, plankton, and cholera in the coastal areas, the emergence of cholera and its spread in AGLR can be hypothesized to have been facilitated by global climatic and local environmental factors. However, the AGLR environment differs widely from estuarine environments, which are known to harbor favorable ecosystems for *V. cholerae* survival during interepidemic periods (*1*–*5*). Our study aimed to describe 1) cholera outbreak dynamics in the AGLR, 2) the modes of persistence of *V. cholerae* during lull periods, and 3) the role of specific climatic conditions that might trigger widespread epidemics.

Methods

Data Collection and Case Definitions

Annual reports of cholera cases during 1978–2008 in Burundi, Rwanda, DRC, Tanzania, Uganda, and Kenya were retrieved from a WHO website (*8*), but it provided information only to the country level. To obtain more detailed information, we also referred to the ProMED website (*11*), which compiles information about cholera outbreaks reported by official government and international agencies, print and online media, and local observers. However, ProMED can miss some outbreaks and possibly bias the spatial distribution toward areas with major outbreaks. In addition, outbreak data on the website are not always accurate. Therefore, with the help of local and national staff of the DRC Ministry of Health, information about cholera cases was collected weekly in each DRC health district during 2002–2008. Attack rates were calculated by using population data provided by the Congolese Ministry of Health. Cholera cases were diagnosed on the basis of the WHO standard case definition (i.e., acute watery diarrhea, with or without vomiting, in a patient ≥5 years of age). In DRC, the national surveillance system lowers the age limit to 2 years for case-patients associated with confirmed cholera outbreaks. Samples for only a small percentage of suspected cholera case-patients were submitted for laboratory confirmation. Nevertheless, as recommended by WHO, outbreaks are usually confirmed by culture results and by identification of *V. cholera* O1 in fecal samples. This confirmatory testing is performed by national health staff, sometimes with the support of staff from WHO or international nongovernmental organizations. For instance, in 2009 WHO helped to confirm 38 cholera outbreaks in Africa (*12*).

Statistical Analysis and Geographic Information System

Pearson correlations were computed between the time series of annual data of cholera cases in the 6 AGLR countries (*13*). Significance was estimated by computing H_0 (the null hypothesis) probability using the Monte Carlo method (999 replicates).

A geographic information system was established on the basis of data collected in the 515 DRC health districts during 2002–2008. Six health districts were not included in the statistical analysis because >10% of their weekly reports were missing. We examined the relationship between the number of cholera cases in each health district and the following variables: population, presence or absence of railways, presence or absence of roads, and lakeside location. Populations of each health district were log-transformed, and log(population) was included as an offset term in the model. Because of the overdispersion of cholera incidence, several generalized linear models belonging to the negative binomial family were compared and checked for spatial structure. Stepwise selection of variables was performed in each case, and the best models were selected by using the Akaike index criterion, following Venables and Ripley (*14*) and Rigby and Stasinopoulos (*15*). We checked model residuals for spatial structure by plotting an empirical variogram. A variogram envelope was then computed by performing 1,000 permutations of the residual values on the spatial locations (the health district centroids). All semivariances that were observed were within the limits of the envelope, indicating that no spatial correlation could be detected in the residuals.

To investigate for case clustering, we used SaTScan software (Kulldorf, Cambridge, UK) to analyze the case numbers in each Congolese health district during 2002–2008. To detect clusters, this software systematically moves a circular scanning window of increasing diameter over the studied region and compares observed case numbers inside the window to the numbers that would be expected under the null hypothesis (i.e., a random distribution of cases) (*16*). The radius of the maximum allowed cluster size was 200 km. The significance for each cluster was obtained through Monte Carlo hypothesis testing (i.e., results of the likelihood function were compared with 999 random replications of the dataset generated under the null hypothesis) (*17*,*18*).

Time series of cholera cases that occurred in the health district belonging to the main clusters identified by the Kulldorf method were decomposed into a trend, a seasonal component, and a residual by using a seasonal-trend decomposition procedure based on Loess regression following the method of Cleveland et al. (*19*). Cross-correlations between time series were computed, and

health zones with synchronous patterns were grouped into 5 hotspots.

To investigate the possible link between cholera and rainfall, we analyzed the rainfall time series obtained for the 5 hotspots from January 1, 2002, through December 31, 2008, and decomposed the time series into trend, seasonal, and residual components as explained by Venables and Ripley (*14*). We then checked for a correlation between the residual components of rainfall and cholera data. Rainfall data were obtained from the International Research Institute for Climate and Society IRI/LDEO Climate Data Library, providing the estimated daily precipitation in Africa from the National Oceanic and Atmospheric Administration Climate Prediction Center (*20*). The daily estimated precipitation from January 1, 2002, through December 31, 2008, was extracted for 5 areas, including the 5 hotspots, and aggregated on a weekly basis. The areas were North Kivu (28.7°–29.7°E, 1.2°–1.7°S), South Kivu (28.7°–29.2°E, 1.7°–2.2°S), Uvira (28.6°–29.3°E, 2.6°–3.9°S), Kalemie (28.1°–29.5°E, 5.6°–7.2°S), and Upper Congo Basin (25.5°–26.6°E, 8.0°–9.9°S).

The link between dynamics of cholera and fluctuation of phytoplankton in Lake Tanganyika was studied by using remote sensing data of chlorophyll-*a* (in μg/L) and field measurements from 2002 through 2006 (*21*–*23*). This dataset, which was computed by using daily MODIS/Aqua Level 1B images (http://oceancolor.gsfc.nasa.gov), was specifically optimized for the monitoring of plankton blooms in Lake Tanganyika (*21*). Chlorophyll-*a* is a usual proxy for phytoplankton concentration (*24*). Whole-lake chlorophyll-*a* data were specifically investigated near Uvira (3°23′18″ S, 29°12′27″ E) and Kalemie (5°55′91″ S, 29°15′00″ E) for this study. Computations were done and graphical displays were made by using R 2.12.2 (*25*), with MASS 7.3–11 and GAMLSS 4.0–8 (both from The R Foundation for Statistical Computing, Vienna, Austria), and by using ArcGIS 9.3 (Environmental Systems Research Institute, Inc., Redlands, CA, USA). Finally, because human activities may also influence the seasonal pattern of cholera, we conducted field observations and systematic interviews in each hotspot to understand the lifestyles of fishermen, tradesmen, artisans, and other inhabitants of the region.

Results

Temporal Dynamics of Cholera and El Niño Warm Events

The annual cholera cases for 1978–2008 for Burundi, DRC, Tanzania, Uganda, and Kenya (but not for Rwanda) were synchronized without a time lag (Table). We found a large increase in cholera for 8 years (the numbers in parentheses after the years show the increase over the preceding year): 1982 (1.9×), 1991 (3.8×), 1992 (2.8×), 1994 (25.8×), 1997 (6.1×), 1998 (1.9×), 2002 (5.0×), and 2006 (1.8×) (Figure 1). By extracting El Niño southern oscillation events indices from the National Oceanic and Atmospheric Administration website (www.cpc.ncep.noaa.gov/data/indices/wksst.for), we found 7 warm events (abnormally warm El Niños). These events lasted ≥5 months and corresponded to periods during which the monthly sea surface temperature exceeded the expected sea surface temperature by at least 0.5°C at the same time in the Niño 3 and Niño 4 regions. The 7 warm events peaked during the last trimester of 1982; the third trimester of 1987; the first trimester of 1992; and the last trimesters of 1994, 1997, 2002, and 2006, which exceeded the expected sea surface temperature by 1.81°C, 1.28°C, 1.14°C, 1.01°C, 2.27°C, 1.26°C, and 1.16°C, respectively. All but 1 of these warm events corresponded to the years cited above that had large increases in cholera; thus, we tested the hypothesis that this was a random occurrence but found that to be an unlikely hypothesis ($p = 0.0003$, Fisher exact test).

Cholera Epidemics and Lakeside Area

Using the ProMED website, we identified and localized 252 cholera epidemics for 1999–2008 (Figure 2) (*11*). Of the outbreaks, 63.5% occurred in districts in lake areas, mainly around Lakes Victoria, Kivu, Albert, and Edward and the northern half of Lake Tanganyika. By contrast, only 12% of outbreaks occurred in seaside areas of Kenya and Tanzania. We then analyzed data provided by DRC, which reported 159,086 cholera cases and 4,912 cholera-related deaths during 2002–2008, corresponding to 66% of the cases and 71% of the deaths reported to WHO from the 6 AGLR countries. Using the type II negative binomial model (lowest Akaike information criterion and

Table. Annual correlations for cholera cases between 6 countries in the African Great Lakes region, 1978–2008*

	Correlation coefficient (p value)					
Country	Burundi	DRC	Kenya	Rwanda	Tanzania	Uganda
Burundi						
DRC	**0.4937 (0.0048)**					
Kenya	**0.4789 (0.0064)**	0.3133 (0.0861)				
Rwanda	0.1307 (0.4833)	0.2665 (0.1473)	0.168 (0.3665)			
Tanzania	0.327 (0.0725)	0.1721 (0.3545)	**0.4338 (0.0148)**	0.2792 (0.1282)		
Uganda	**0.5631 (0.001)**	**0.7284 (0.00001)**	**0.4304 (0.0157)**	0.2884 (0.1157)	**0.5076 (0.0036)**	

*Values in **boldface** are significant. DRC, Democratic Republic of Congo.

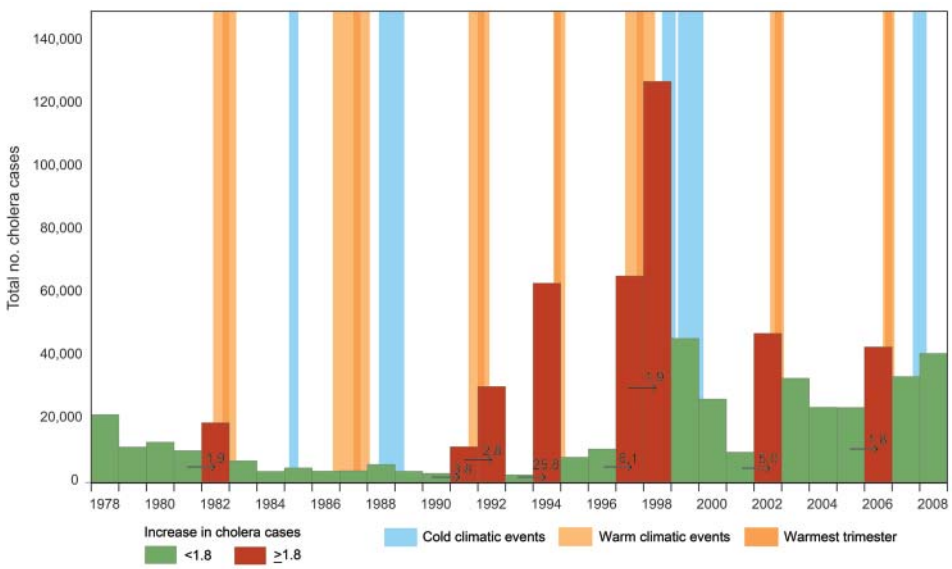

Figure 1. Yearly number of cholera cases in the African Great Lakes region (Burundi, Democratic Republic of Congo, Kenya, Rwanda, Tanzania, and Uganda), 1978–2008. Red bars indicate years with large increases in cholera cases. Numbers on arrows represent the increase factor in cholera cases. Warm climatic events (indicated by light orange background) had a duration of ≥5 months and a sea surface temperature increase of ≥0.5°C simultaneously in Niño 3 (eastern Pacific, from 90°W–150°W and 5°S–5°N) and Niño 4 (western Pacific, from 160°E–150°W and 5°S–5°N) regions.

sigma coefficient = 8) and including the presence of roads and lakeside location, we found that the number of cholera cases in each health district in DRC was significantly higher in areas with roads (risk ratio [RR] 1.4, 95% confidence interval 1.1–1.9) and lakes (RR 7.0, 95% confidence interval 4.9–10.0). Results of the SaTScan analysis showed that the spatial clusters that were associated with significant RRs were all located in eastern DRC (Figure 3). The 3 clusters with maximal RR ($p<0.001$) were 1) Kalemie, on the shore of the Lake Tanganyika (RR 17.1); 2) the area bordering lakes on the Upper Congo basin (RR 12.7); and 3) an area including the northern shore of Lake Tanganyika, Lake Kivu, and the southern shore of Lake Edward (RR 6.0). These 3 clusters represented 28 health districts and 107,826 cases of cholera (68% of the total cases and <10% of the total population of DRC). When considering these 3 clusters altogether, cholera cases were reported every week during the 7-year period studied. The lowest incidence was 8 cases per million inhabitants (week 24, 2004). By contrast, outside of these 3 clusters, we identified numerous periods with no or almost no cholera cases (<1 case/1 million inhabitants/week), many of them lasting >1 month (weeks 24–30, 2002; weeks 19–28 and 36–40, 2004; weeks 9–13, 20–34, and 44–49, 2005; weeks 16–25, 2006; and weeks 23–27, 2007).

By using time-series analysis to search for synchronous patterns, we identified 5 hotspots within these spatial clusters, accounting for 84,465 cholera cases. The

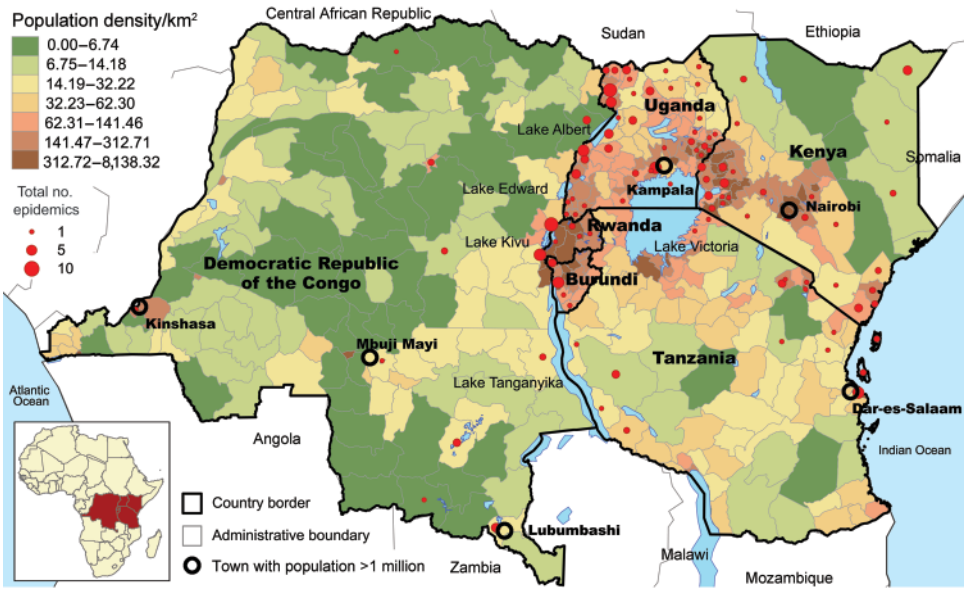

Figure 2. Number of reports and localization of cholera outbreaks in the African Great Lakes region, 1999–2008, as reported by ProMED (*11*).

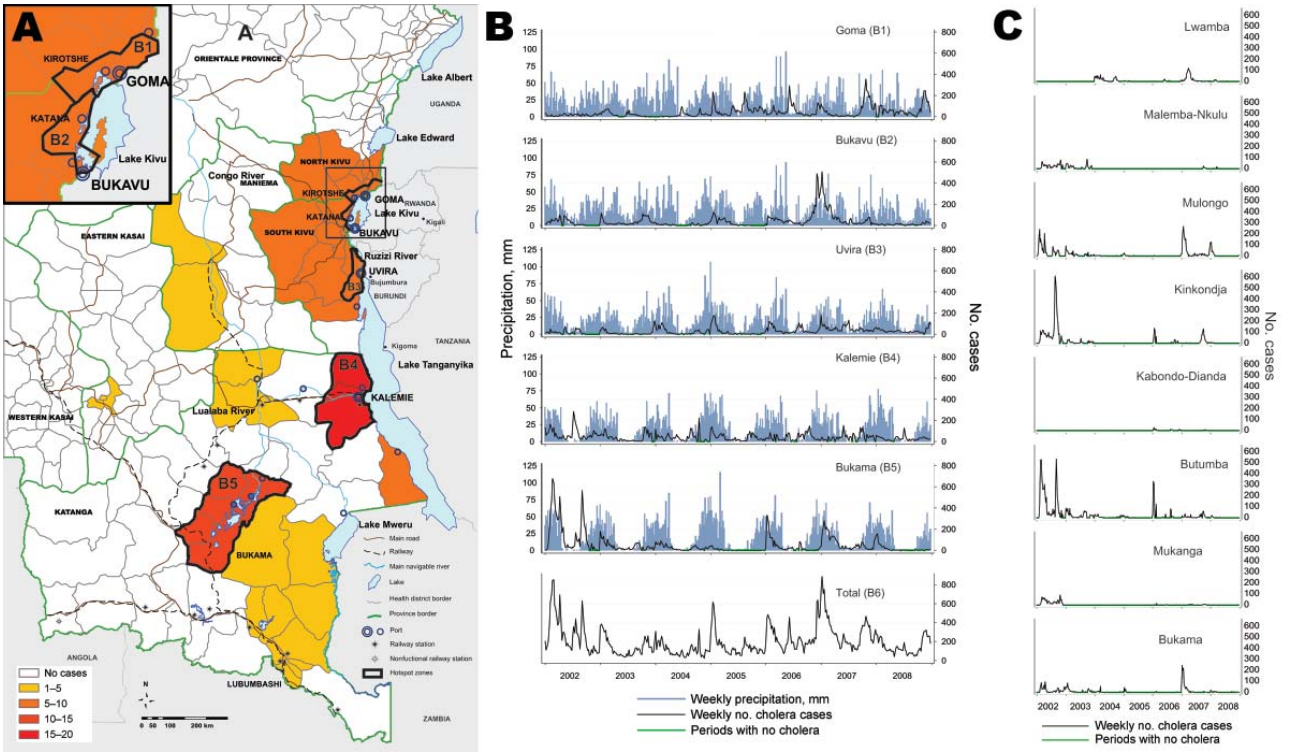

Figure 3. Temporal-spatial evolution of cholera cases in 5 hotspots in the African Great Lakes region, 2002–2008. A) Spatial distribution of cholera in the provinces of Katanga, North Kivu, and South Kivu (Democratic Republic of Congo). Health districts are colored according to the risk ratio of the cluster, as calculated by using SatSCan software (Kulldorf, Cambridge, UK). B) Evolution of the weekly number of cholera cases in the 5 hotspots (B1–B5). B1) Goma and Kirotshe health districts; B2) Bukavu and Katana health districts; B3) Uvira health district; B4) Kalemie and Nyemba health districts; B5) 8 health districts in the Upper Congo River Basin (see district names in panel C); B6) total cases for the 5 hotspots. Green indicates periods without cholera; blue indicates estimated weekly rainfalls. The global curve did not show any remission periods. C) Evolution of the weekly number of cholera cases in the 8 health districts composing the Upper Congo Basin hotspot. The epidemic curve in B5 was composed of partially synchronous epidemics separated by periods of lull.

first hotspot was around Goma in North Kivu (including Goma and Kirotshe health districts; Figure 3); the second hotspot was around Bukavu in the north of South Kivu (Bukavu and Katana health districts); the third hotspot was in Uvira, in the south of South Kivu (Uvira health district); the fourth hotspot was around Kalemie, near Lake Tanganyika in Katanga (Kalemie and Nyemba health districts); and the fifth hotspot was in the Upper Congo River Basin in Katanga (Bukama, Butumba, Kinkondja, Kabondo-Dianda, Malemba-Nkulu, Lwamba, Mukanga and Mulongo health districts). In each of these hotspots, cholera cases were reported almost every week except for a few short interruptions (Figure 3).

Cholera Weekly Incidence by Season, Rainfall, Plankton Abundance, and Fishing Activities

Seasonal patterns of cholera varied according to the location of the hotspots (Figure 4). Around Goma, where no dry season could be determined, time-series analyses did not identify any seasonal component in the occurrence of cholera. Around Bukavu and Uvira—2 hotspots characterized by a short dry season—a clear trend toward a lull in cholera cases during the dry season was observed. Further south, in Kalemie and the Upper Congo River Basin, cholera outbreaks started before the end of the dry season and worsened during the rainy season.

Cross-correlations between residual components of cholera and rainfall time series showed a significant positive relationship in Uvira after a latency of 2–5 weeks and in the Upper Congo River Basin after no latency. In Kalemie and Bukavu, the link between rainfall and cholera was supported only by the seasonal trend. Therefore, the deleterious effect highlighted during the El Niño years might, at least partly, have resulted from excess rainfall in the Great Lakes region.

We further studied the links between the dynamics of cholera and the fluctuations of phytoplankton in Lake Tanganyika by using chlorophyll-*a* concentration estimates (in µg/L) derived from remote sensing. Cholera epidemics and blooms of phytoplankton occurred almost simultaneously in Uvira and Kalemie (Figure 5). However, after removal of the seasonal components of the time series,

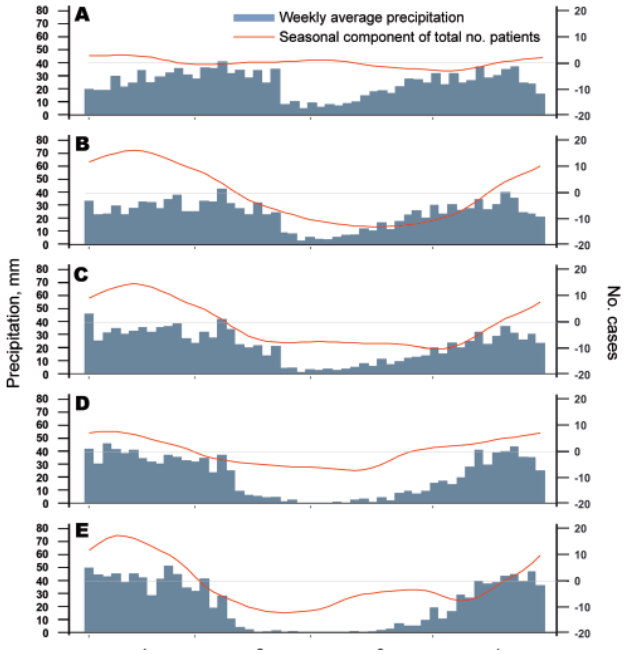

Figure 4. Seasonal patterns/components of cholera outbreaks for 5 hotspots in the African Great Lakes region, 2002–2008. Hotspots are Goma (A), Bukavu (B), Uvira (C), Kalemie (D), and Upper Congo Basin (E). Blue indicates the weekly average precipitation (in mm); red indicates the seasonal component of the total number of patients after the time series was decomposed into a trend and seasonal and residual components by using a seasonal-trend decomposition procedure based on loess regression. Horizontal gray lines indicate seasonal component = 0.

no additional significant relationship was found between these 2 phenomena.

In Kalemie and the Upper Congo River Basin, which are among the main fishing areas in DRC, field investigations and interviews focused on descriptions of the behaviors of fishermen and of the seasonal variations in trading and fishing activities. In Kalemie, fishing activities peaked from mid-July to September (the dry season), when fishermen move into settlements located on the shore of Lake Tanganyika. In the Upper Congo River Basin, the fishing season is slightly earlier (mid-June to September), and fishermen crowd into camps on islands that emerge during the dry season but are below water during the rainy season. These fishing settlements are characterized by poor sanitary conditions, which lack clean water and a system for disposing of excreta (Figure 6). In both areas, the mild increase in cholera cases during the dry season is associated with the traveling of fishermen and traders between the main towns and fishing camps.

Persistence of Cholera during Lull Periods

Because of the combined effects of seasonal patterns and interannual trends of cholera, short lulls in cholera outbreaks occurred in the 5 hotspots. However, these lull periods were not completely synchronous (Figure 3). Although the number of cholera cases fell to zero in a given hotspot, neighboring hotspots were still undergoing outbreaks and served as starting points for cholera to recolonize other lakeside areas. The high and still increasing density of population has resulted in less frequent and shortened periods with complete interruption of cholera transmission in Kalemie, Uvira, Bukavu, and Goma, adding to the stability of this pattern of epidemics (Figure 3). Therefore, spontaneous and simultaneous extinction in every hotspot was never observed during this 7-year survey.

Discussion

Our findings show that cholera in AGLR greatly increases during years of El Niño warm events, and it decreases or remains stable between these warm events. In this region of Africa, which is located near the equator, rainfall affects the epidemiologic patterns of cholera. Therefore, the deleterious effect during El Niño warm event years might at least partly result from the excess of rainfall during the corresponding years. Seasonal patterns

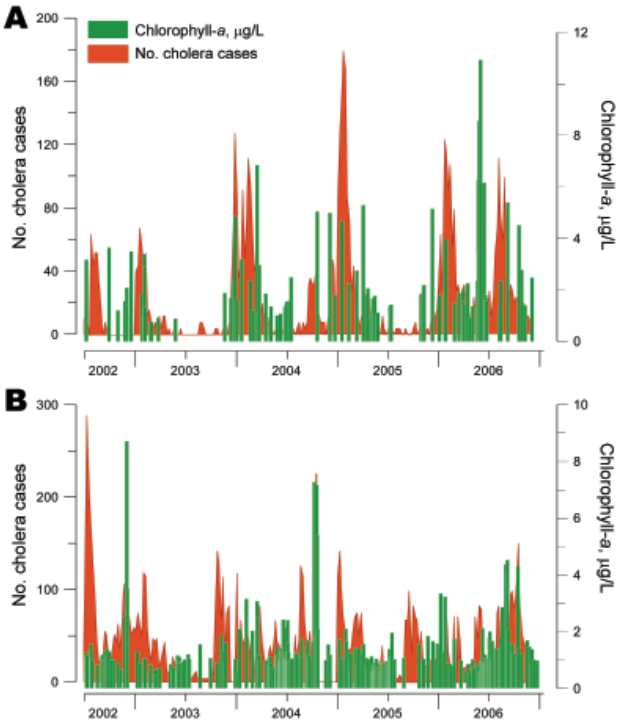

Figure 5. Link between the number of cholera cases and fluctuations in phytoplankton abundance (chlorophyll-*a* concentrations) in Lake Tanganyika, Africa Great Lakes region, January 2002–December 2006. Two of 5 cholera hotspots in the region were tested, both of which face Lake Tanganyika: Uvira (A) and Kalemie (B). Green indicates median concentrations of chlorophyll-*a* in surface water; red indicates cholera cases.

Figure 6. Fishing camp on an island in Lake Upemba, upper Congo River Basin. Fishermen and their families usually spend several weeks every year in camps like this, in which the lake is the only source of water. Because there is no firewood in such areas, campaigns promoting the boiling of water are useless. (Photograph by Didier Bompangue.)

of cholera and the effects of the rainfall shown by our results corroborate the findings from a study performed in Zambia, a country bordering the southern Katanga Province, DRC (*26*). There, the risk for cholera epidemics increases when the rainy season begins earlier and is preceded 6 weeks earlier by a period of warm temperature.

Our results also show that a few lakeside areas play a crucial role in maintaining endemic cholera in AGLR. Two case-control studies, including 1 on Lake Tanganyika (*27*), showed a statistical correlation between contracting cholera and living on the shores of a lake or a river in Africa (*27*,*28*). The link between high incidence of cholera and presence of lakes has also been noted in DRC at the provincial level (*29*,*30*). We addressed this issue through a multiscale approach and obtained data suggesting that lakeside areas were the source of the disease in the entire AGLR. Indeed, lakeside areas were the only areas where the disease persisted continuously during the study period. Therefore, we believe that in the absence of lakeside areas, the disease would have disappeared from AGLR.

Two hypotheses emerged to explain how cholera took root in AGLR, an area far from the coastal marine environments known to be the original biotope of *V. cholerae*. The first hypothesis involves the possible persistence of some cholera strains in the lakes of AGLR. Weather conditions (i.e., seasonal rainfall and the multiannual recurrence of El Niño warm events) might promote plankton growth and *V. cholerae* multiplication, similar to the epidemiology of cholera in South Asia (*3*). Climatic changes have resulted in biological modifications of the lakes. The temperature of the African Great Lakes has increased during the past 3 decades (*31*–*33*). Changes in algal community structure have also occurred; for example, the reported Lake Tanganyika cyanobacteria-chrysophytes-chlorophytes community of 1975 was replaced by a cyanobacteria-chlorophytes-diatom community (*34*). These environmental changes, which were observed in Lakes Victoria, Malawi (another African Great Lake, also known as Lake Nyasa), and Tanganyika, could have affected the dynamics of cholera. Although our results showed a relationship between the abundance of phytoplankton and the number of cholera cases, we acknowledge that we did not demonstrate a causal relationship. Others causes, such as seasonal rainfall, may explain increased plankton bloom (because of an increase in nutrients) and increased cases of cholera (due to fecal contamination of lake water). Seasonal patterns of cholera around the lakes may also be partly explained by the seasonal variation of human exposure to aquatic reservoirs of *V. cholerae*, especially in fishing settlements.

The second hypothesis explains the persistence of cholera during the lull periods by outbreak dynamics evoking a metapopulation pattern (cholera stability on a regional scale originates from interactions between asynchronous local foci prone to extinction) and by densely populated endemic foci around the lakes. Most of these foci are towns in which humans live in close proximity to each other with poor hygiene conditions and little access to clean water. In such situations, cholera could persist during the dry season through a mix of human-to-human and waterborne transmission. Even if the African Great Lakes lack cholera strains that persist for extended periods, sewage seeping into the lakes from the towns and camps may result in transient but repetitive contamination of the water, which many AGLR residents use for cooking and drinking. In addition, cholera epidemics among fishing communities help maintain a human reservoir of the disease. At the end of the lull periods, the spread of cholera is then favored by several factors, including rainfall, which enhances water contamination, and commercial activities, which facilitate the spread of the disease.

To further understand the mechanisms and conditions that enabled cholera to take root in AGLR, an interdisciplinary study will investigate the role of freshwater environments and climatic factors in cholera dynamics in this region of Africa. This study, named CHOLTIC, is just beginning around Lake Tanganyika and involves specialists in various topics, including epidemiology, microbiology, limnology, hydrodynamics, phytoplankton, zooplankton, fisheries, remote sensing, and modeling. Our initial results support a link between cholera outbreaks, climate, and lake environment, and provide an encouraging basis for further investigation.

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International Spread of MDR TB from Tugela Ferry, South Africa

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We describe a death associated with multidrug-resistant tuberculosis and HIV infection outside Africa that can be linked to Tugela Ferry (KwaZulu-Natal, South Africa), the town most closely associated with the regional epidemic of drug-resistant tuberculosis. This case underscores the international relevance of this regional epidemic, particularly among health care workers.

Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) pose an increasing challenge to international health (*1*), particularly in the context of HIV infection. An outbreak of XDR TB around a rural hospital in Tugela Ferry (KwaZulu-Natal Province, South Africa) in 2006 received widespread international attention, in part because of the high case-fatality rate. More recent work has highlighted the risk for MDR TB among health care workers (*2*) in South Africa. We report a death outside of Africa associated with MDR TB that can be directly related to the epidemic of drug resistance in Tugela Ferry, the center most closely associated with the epidemic.

The Study

A 42-year-old South Africa–born health care worker was admitted to a regional hospital in the United Kingdom with a 1-month history of fever, cough, and weight loss associated with cervical lymphadenopathy, choroidal tuberculoma (Figure), and pleural effusion. The patient had no history of TB treatment and no known family contact with TB. He had moved to the United Kingdom 6 years earlier. Extrapulmonary TB diagnosis was based on microscopic examination of a cervical lymph node specimen, and co-infection with HIV was identified (CD4 count 5 cells/μL).

After arrival in the United Kingdom, the patient worked as a temporary nurse in several health care facilities. Before that, the patient worked at the Church of Scotland Hospital in Tugela Ferry during 1996–2002 in general medical wards. This facility was the focus of the 2006 report of XDR TB, and most MDR isolates identified there during 2005–2007 were XDR TB (*3*).

A presumptive diagnosis of MDR TB was made 7 days after the patient sought care at the hospital. A line probe assay (INNO-LiPA Rif.TB; Innogenetics, Ghent, Belgium) performed on cervical lymph node aspirates identified *Mycobacterium tuberculosis* and a hybridization pattern consistent with *rpoB* gene mutation (associated with rifampin resistance and a high risk for MDR TB). Treatment was altered from weight-appropriate doses of rifampin, isoniazid, pyrazinamide, and ethambutol to include levofloxacin, amikacin, cycloserine, and protionamide. Because of known sensitivity patterns from isolates at Tugela Ferry and the possibility of XDR TB, para-aminosalicylic acid and linezolid were added to treatment, and intravenous amikacin was changed to capreomycin. Treatment was subsequently changed on the basis of culture results. The patient required 35 days of mechanical ventilation for likely pulmonary immune reconstitution syndrome after initiation of antiretroviral treatment. Although discharged from intensive care after successful treatment of TB, the patient remained hospitalized and died 90 days after first seeking care. Samples cultured from >1 site showed evidence of widespread MDR TB. More than 500 potentially infectious contacts were identified, but no secondary cases of TB have been diagnosed.

Culture confirmed the *M. tuberculosis* isolate from the patient to be resistant to rifampin, isoniazid, pyrazinamide, and ethambutol but sensitive to amikacin, capreomycin, moxifloxacin, para-aminosalicylic acid, and linezolid. These resistance and sensitivity characteristics identified the isolate as MDR TB rather than an XDR TB strain. Automated sequencing of *rpoB* revealed the L533P mutation previously associated with rifampin resistance (*4*–*6*) but not the D516G mutation found in XDR TB strains previously isolated from patients at Church of Scotland Hospital and from other hospitals within KwaZulu-Natal (*7*).

To explore whether the patient's isolate was related to the F15/LAM4/KZN strain genotypes associated with the Tugela Ferry outbreak (*8*) and the broader population of drug-resistant strains in the region, we performed

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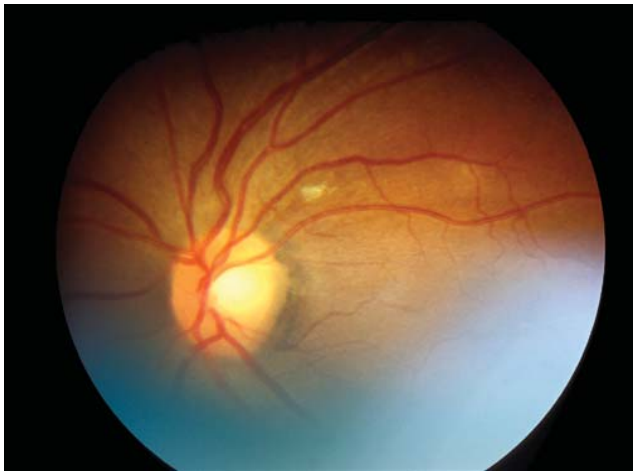

Figure. Retinal image from patient with evidence of choroidal tuberculosis.

mycobacterial interspersed repetitive units (MIRU)–variable number tandem repeats (VNTR) typing and spoligotyping (*9*). The isolate displayed a typical LAM4 spoligotype (1111111111111111111100001111111100001110111), consistent with a F15/LAM4/KZN genotype. In the absence of full MIRU-VNTR typing for Tugela Ferry strains, the alleles of 15 loci were compared with those inferred by in silico analysis of publicly available genome sequences for five F15/LAM4/KZN strains from Tugela Ferry or KwaZulu-Natal (*7*,*10*). The patient's isolate differed by only a single locus from the F15/LAM4/KZN 605 reference strain, whereas the 4 other strains of this genotypic family varied by 1 to 3 loci (Table). Single-locus variation is prognostic of a close relationship within a clonal complex with confidence exceeding 99% (*9*). Furthermore, the patient's isolate genotype also specifically best matched that of an F15 strain (also differing by 1 locus), in a database of 209 South African isolates containing a large variety of genotypic families (*11*).

Conclusions

The risk for TB among health care workers in developing countries is well recognized (*12*) but has become more of a public health concern with evidence of the nosocomial transmission of MDR and XDR TB in South Africa. Nosocomial transmission of drug-resistant TB has been reported from well-resourced settings (*13*), but this case highlights how migration of health care workers can link the 2 settings.

When taken together, the clinical and molecular epidemiologic data in this case support the hypothesis that infection was acquired in South Africa and most likely while the patient was based in Tugela Ferry. Health care workers in South Africa are significantly more likely to be admitted to a hospital with MDR or XDR TB than are population controls (*2*), and this patient had prolonged occupational exposure at a hospital strongly associated with drug-resistant TB. Although F15/LAM4/KZN strains are frequent in South Africa, MDR versions of this genotype emerged in the KwaZulu-Natal Province in the mid-1990s, at the same time that the prevalence of HIV was increasing to hyperendemic levels and before antiretroviral therapy was widely available (*8*). The fact that this patient's isolate and the XDR strains from Tugela Ferry have different *rpoB* mutations suggests that these strains arose independently from the locally circulating F15/LAM4/KZN strain pool, as already suggested for other MDR F15/LAM4/KZN strains (*7*).

Since this patient left South Africa, multidrug resistance has continued to grow as a challenge to public health, fueled in part by the HIV epidemic (*14*) and despite greater availability of HIV treatment. Given the long latent period between infection and the time when patients seek care for symptoms, it is reasonable to expect that such cases may become increasingly common outside Africa. If they do become more common, this change has implications for diagnostics, clinical management, and public health policy. In settings where prevalence is low but availability of resources is high, such as Europe and the United States,

Table. Locus typing for tuberculosis patient isolate according to standard nomenclature based on chromosomal locations*

Genotypic family and strain or isolate	Allele at MIRU-VNTR locus														
	154	577	580	802	960	1644	2059	2165	2461	2531	2687	2996	3007	3192	4348
F15/LAM4/KZN															
Patient isolate	1	4	3	4	4	3	2	2	2	6	1	5	3	3	2
605 (reference)	1	4	2	4	4	3	2	2	2	6	1	5	3	3	2
1435	1	4	2	5	4	3	2	2	2	6	1	5	3	3	2
R506	1	4	2	3	3	3	2	2	2	6	1	4	3	3	2
V2475	1	4	2	3	3	3	2	2	2	6	1	4	3	3	2
4207	1	4	2	3+4	3+4	3	2	2	2	6	1	4+5	3	3	2
F11/LAM															
F11	2	4	2	3	3	3	2	2	2	6	1	4	3	3	2

*Patient's isolate most closely matches F15/LAM4/KZN605 reference strain by a single locus variation, a genetic distance equal to or lower than that separating other known F15/LAM4/KZN strains (i.e., 1–3 locus variations). MIRU-VNTR, mycobacterial interspersed repetitive units–variable number tandem repeats.

access to molecular diagnostic testing remains variable despite recent advances (*15*). The INNO-LiPA Rif.TB assay used here provided useful information with an indication of potential multidrug resistance. However, despite early molecular testing, identification of MDR or XDR TB still depended on conventional methods of susceptibility testing. Potentially toxic medications could have been avoided if more extensive molecular testing for resistance to second-line drugs (particularly quinolones and aminoglycosides) were achievable in close physical proximity to the patient.

The poor outcome for MDR and XDR TB in HIV-positive patients is well recognized in disease-endemic settings (*3*), and this case confirms the high risk for death even in well-resourced settings. This case also highlights deficiencies in screening policies at national borders and within occupational health, particularly for health care workers not in permanent employment. Barriers to HIV and TB testing are multifactorial, and TB screening methods have limitations; nonetheless, in this situation, there were several missed opportunities for diagnosis of TB and HIV that could have prevented this patient's death.

This report serves as a reminder that XDR or MDR TB and HIV in sub-Saharan Africa represent not just a regional epidemic but also a challenge to international health. Although this challenge has yet to fully emerge, this case highlights the potential risk to health care workers in areas of low and high transmission.

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Dr Cooke is a clinician scientist based at Imperial College London and an honorary associate professor of infectious diseases at the University of KwaZulu-Natal, South Africa. He has an interest in HIV co-infections, particularly MDR TB.

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Seasonal Influenza A Virus in Feces of Hospitalized Adults

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In a cohort of hospitalized adults with seasonal influenza A in Hong Kong, viral RNA was frequently (47%) detected in stool specimens. Viable virus was rarely isolated. Viral RNA positivity had little correlation with gastrointestinal symptoms and outcomes. In vitro studies suggested low potential for seasonal influenza viruses to cause direct intestinal infections.

Although influenza predominantly causes respiratory diseases, gastrointestinal signs such as diarrhea and vomiting are not uncommonly reported, particularly among young, hospitalized children (8%–38%) and immunocompromised persons (*1*–*3*). Influenza virus RNA has been detected in feces, but its role is unknown (*4*–*7*). We investigated fecal viral RNA shedding in a large cohort of hospitalized adults with seasonal influenza A in Hong Kong Special Administrative Region, People's Republic of China. The potential of seasonal influenza viruses to cause direct intestinal infections was examined.

The Study

We conducted a prospective, observational study among adults hospitalized with laboratory-confirmed seasonal influenza A infections during 2006–2009. Hospital admission, diagnosis, and management procedures have been described (*8*). Briefly, patients were admitted if severe symptoms, respiratory or cardiovascular complications, or exacerbations of underlying conditions developed. When the patients sought care, nasopharyngeal aspirates (NPAs) were collected for diagnosis by using immunofluorescence assay or reverse transcription PCR. Patients with confirmed influenza A were recruited if they were >18 years of age and sought care within 1 week of illness onset. Patients with pandemic (H1N1) 2009 virus infections were excluded and reported separately (*9*).

After providing written, informed consent, patients were asked to submit 1 stool specimen for viral RNA detection during hospitalization, regardless of gastrointestinal symptoms. Clinical information was prospectively recorded (*8*). For comparison, fecal shedding of respiratory syncytial virus (RSV) and parainfluenza virus (PIV) were studied during a 10-month period by using a similar approach. Ethical approval for the study was obtained from the institutional review boards of The Chinese University of Hong Kong.

All stool specimens were subjected to influenza viral RNA detection by using quantitative real-time reverse transcription PCR targeting the matrix gene (*6*). If positive, virus subtyping was performed by using H1- and H3-specific conventional PCRs. Freshly collected stool specimens during 1 seasonal peak were simultaneously subjected to viral RNA detection and virus isolation by using MDCK cells. Detailed methods for fecal detection of influenza viruses and RSV and PIV are provided in the online Technical Appendix (wwwnc.cdc.gov/EID/pdfs/11-0205-Techapp.pdf).

Lectin histochemical analysis and double immunofluorescence staining were used to study the distribution of influenza virus receptors on human small and large intestinal tissues. An in vitro virus binding study on intestinal tissues was also performed by using inactivated human virus isolates of subtypes H1N1 (A/HongKong/CUHK-13003/2002) and H3N2 (A/HongKong/CUHK-22910/2004) (online Technical Appendix).

A total of 119 hospitalized adults with seasonal influenza A infections were studied (Table 1). Their median age was 71 years (interquartile range [IQR] 57–79 years), and most (66%) had concurrent conditions; ≈5% were profoundly immunosuppressed. Vomiting and diarrhea were reported by 15 (13%) and 7 (6%) patients, respectively. Influenza A viral RNA was detected in 56 of 119 of stool samples, collected at a median interval of 3 days (IQR 3–5 days) from onset (detection rates by study year and virus subtype are shown in Table 2). Detection rate by day from onset ranged from 31% to 63% and showed a trend to decrease toward the end of the week (Figure 1, panel A). Overall, the mean ± SD fecal viral RNA concentration was 4.4 ± 0.8 $\log_{10}$ copies/g of feces and the median (IQR) was 4.2 (3.8–5.0) $\log_{10}$ copies/g of feces; concentrations tended to decrease with longer time elapsed from onset (Figure 1, panel B).

In most (77%) viral RNA–positive samples, further H1- or H3-specific PCRs identified 7 cases as H1 and 36 cases as H3; unsuccessful amplification was associated with lower viral (matrix gene) concentrations (median [IQR] 3.9 [3.8–4.1] vs. 4.4 [3.8–5.1] $\log_{10}$ copies/g of stool; $p = 0.04$). No discrepancy was found between these and the subtyping results of the virus isolates from NPAs. Fecal

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Table 1. Comparisons of baseline clinical and laboratory variables between influenza patients with positive and negative fecal viral RNA detection test results, Hong Kong, 2006–2009*

Patient characteristics	Fecal viral RNA–positive, n = 56	Fecal viral RNA–negative, n = 63	p value
Mean age, y (SD)	65.3 (18.6)	69.9 (13.4)	0.12
Age group, y, no. (%)			0.35†
18–49	7 (13)	6 (10)	
50–65	18 (32)	14 (22)	
>65	31 (55)	43 (68)	
Female sex, no. (%)	33 (59)	30 (48)	0.27
Interval from illness onset to sample collection ≤5 d, no. (%)	52 (93)	53 (84)	0.14
Concurrent condition, no. (%)			
Any	36 (64)	43 (68)	0.65
Major‡	31 (55)	37 (59)	0.72
Virus isolation, nasopharyngeal aspirates	50 (89)	49 (77)	0.06
Signs and symptoms when care was sought, no. (%)			
Fever	48 (86)	53 (84)	1.00
Cough and sputum	39 (74)	45 (76)	0.83
Sore throat	15 (28)	18 (31)	0.84
Rhinorrhea	21 (40)	21 (36)	0.70
Shortness of breath	18 (34)	27 (46)	0.25
Vomiting or diarrhea	10 (18)	9 (14)	0.63
Vomiting	7 (13)	8 (13)	1.00
Diarrhea	5 (9)	2 (3)	0.25
Laboratory parameters when care was sought			
Total leukocyte count, × 10^9 cells/L, median (IQR)	7.3 (5.9–8.9)	7.9 (6.1–10.3)	0.18
Neutrophil count, × 10^9 cells/L, median (IQR)	5.1 (4.2–7.0)	6.0 (4.1–7.7)	0.22
Lymphocyte count, × 10^9 cells/L, median (IQR)	0.8 (0.6–1.1)	0.9 (0.6–1.2)	0.20
Lymphocyte count <1.0 × 10^9 cells/L, no. (%)	37 (66)	29 (46)	0.03
Monocyte count, × 10^9 cells/L, median (IQR)	0.7 (0.5–0.9)	0.7 (0.5–0.9)	0.51
Platelet count, × 10^9 cells/L, median (IQR)	180 (142–228)	196 (156–248)	0.20
Alanine aminotransferase level, IU/L, median (IQR)	19 (13–32)	22 (15–36)	0.54
Antiviral treatment, no. (%)			
Oseltamivir§	35 (63)	42 (67)	0.70
Zanamivir	6 (11)	5 (8)	0.75
Specimen collected after starting antiviral drugs, no. (%)	39 (70)	42 (67)	0.84
Complication, no. (%)			
Any	39 (70)	44 (70)	0.98
Cardiorespiratory¶	29 (52)	38 (60)	0.36
Clinical outcome, no. (%)			
ICU admission	2 (4)	0 (0)	0.22
Death	0	0	NA
Median duration of hospitalization, d (IQR)	6 (4–12)	5 (4–13)	0.50

*Univariate comparisons of categorical and continuous variables were performed by using Fisher exact test and Mann-Whitney U test or Student *t* test, whenever appropriate. IQR, interquartile range; ICU, intensive care unit; NA, not applicable.
†Fisher 2×3 exact test.
‡Defined as congestive heart failure; cerebrovascular, neoplastic, chronic liver, and renal diseases; diabetes; ischemic heart disease; or use of immunosuppressant drugs (*8*). Approximately 5% of patients were profoundly immunocompromised.
§Oseltamivir (standard oral regimen, 75 mg 2×/d for 5 d). Amantadine was coadministered to 7 patients during the 2008–09 influenza season.
¶Defined as pneumonia, acute bronchitis, acute exacerbation of chronic respiratory conditions (e.g., chronic obstructive pulmonary disease, asthma); acute coronary syndrome, decompensated heart failure, arrhythmia, or acute cerebrovascular events (*8*).

viral RNA detection rate and concentrations were similar between H1 and H3 subtypes (Table 2).

Thirty-eight stool samples from 1 seasonal peak were subjected to virus isolation and viral RNA detection. In 10 cases, cytotoxicity occurred (procedure discontinued); in the remaining 28 cases, 12 were viral RNA positive; only 1 showed virus growth. This sample was from an 82-year-old man with dilated cardiomyopathy hospitalized for seasonal influenza A (H1N1) pneumonia and heart failure; diarrhea was absent.

Among 25 confirmed RSV or PIV infections (median [IQR] age 71 [55–79] years), viral RNA was detected in 5 fecal samples (collected at median [IQR] 4 [3–6] days after onset); none was culture positive. Fecal viral RNA positivity was lower compared with that of seasonal influenza viruses (p = 0.01).

Table 2. Fecal detection of seasonal influenza A viral RNA in stool samples, by year of study and virus subtype, Hong Kong, 2006–2009*

Year of study	No. fecal viral RNA–positive/ no. tested (%)	Fecal viral RNA–positive by virus subtype, no. (%)		Fecal viral RNA concentration by virus subtype, log_{10} RNA copies/g stool, median (IQR)	
		H1	H3	H1	H3
2006	11/20 (55)	7/7 (100)	2/4 (50)	5.0 (4.6–6.0)	NA
2007	19/35 (54)	0/0	19/31 (61)	NA	4.2 (4.0–5.1)
2008	11/26 (42)	1/5 (20)	10/21 (48)	NA	3.9 (3.7–4.4)
2009	15/38 (39)	5/10 (50)	10/27 (37)	3.9 (3.8–4.1)	3.9 (3.7–4.7)
All	56/119 (47)	13/22 (59)	41/83 (49)	4.6 (3.9–5.2)	4.2 (3.8–5.0)

*Virus subtyping results were unavailable for 9 cases in 2006, 4 cases in 2007, and 1 case in 2009. IQR, interquartile range; NA, not applicable.

Patients with positive and negative fecal viral RNA detection results were compared (Table 1). Positive fecal viral RNA detection was associated with younger age, shorter interval from illness onset to sample collection, lymphopenia, and positive virus isolation. Multivariate logistic regression showed that lymphopenia (adjusted odds ratio 2.36, 95% confidence interval 1.02–5.47; p = 0.045) and positive virus isolation in NPAs (adjusted odds ratio 3.76, 95% confidence interval 1.07–13.20; p = 0.039) were significant explanatory variables. No significant association was found between fecal viral RNA detection and clinical outcomes. Fecal viral RNA concentrations were also analyzed, and no association with clinical outcomes was found (data not shown), except for a negative correlation with lymphocyte count (Spearman ρ –0.37, p = 0.047).

Lectin histochemical analysis showed that sialic acid α 2,6-Gal (human-like influenza virus receptor) was absent from epithelial surface of small and large intestines and was found only in lamina propria cells. In contrast, sialic acid α 2,3-Gal (avian-like influenza virus receptor) was found on large intestinal epithelial cells and in lamina propria cells. Virus-binding study showed that neither seasonal influenza A (H1N1) nor A (H3N2) virus bind to small and large intestinal epithelial surface, but they bind to a subset of CD45+ leukocytes (Figure 2).

Conclusions

Direct intestinal infection by seasonal influenza viruses seems an unlikely explanation for the frequent fecal detection of viral RNA in the patients reported here. No clinical correlation was shown for RNA positivity (but was shown with lymphopenia and positive virus isolation in NPA, indicating higher virus load), and culture positivity is rare (*4,5,10,11*). Human-like influenza virus receptor is

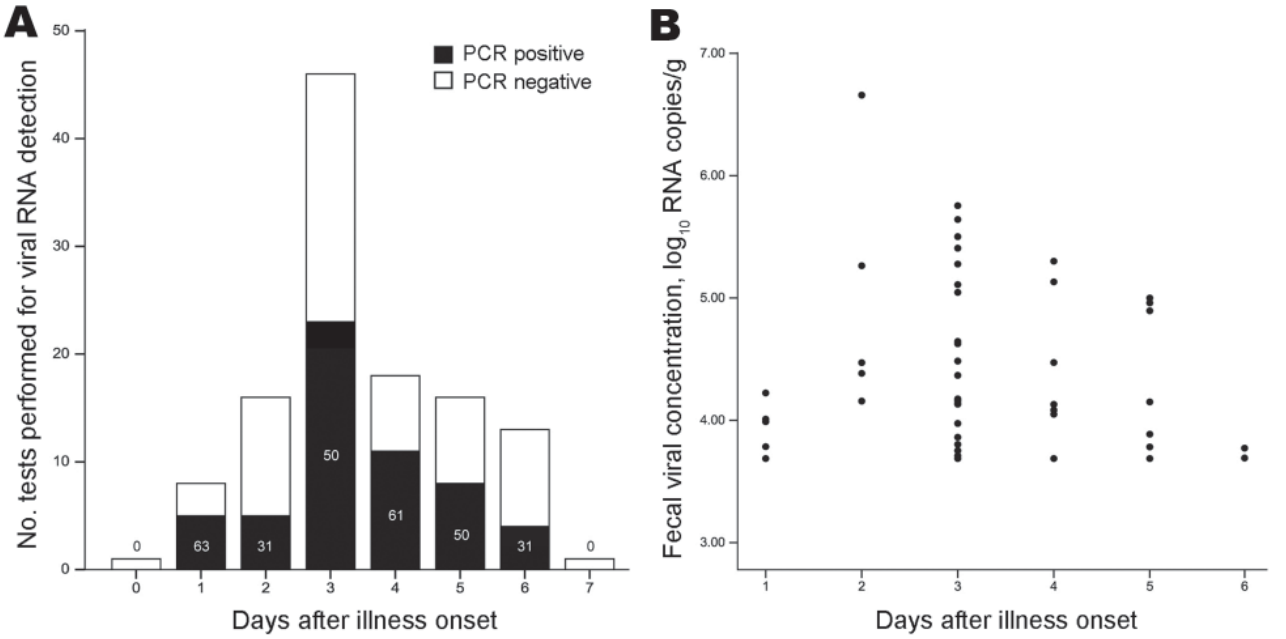

Figure 1. Fecal seasonal influenza A viral RNA detection rate and its concentration, by number of days after illness onset, Hong Kong, 2006–2009. A) Fecal viral RNA detection rate. Numbers in bars represent percentage of cases with positive viral RNA detection. B) Fecal viral RNA concentration. Three outliers were omitted from the figure for better illustration. Fecal viral RNA concentration was determined by using quantitative real-time reverse transcription PCR specific for the viral matrix gene and was expressed as log_{10} RNA copies/g of stool. The lower detection limit of the assay was 3.7 log_{10} RNA copies/g of stool.

not found to express on normal intestinal epithelial cells (*12*). These findings agree with reports which showed that intestinal cells and tissues do not support efficient replication of seasonal viruses (*12*,*13*), thus their low potential to cause direct intestinal infection. Alternatively, swallowing of virus-containing nasopharyngeal secretions (although it seems inadequate to explain the higher rate of detecting fecal viral RNA than RSV or PIV) and hematogenous dissemination to organs through infected lymphocytes or macrophages in severe influenza cases with high virus load (spillover) are possible explanations for fecal viral RNA detection (*2*,*14*). Our findings on virus receptor distribution and in vitro virus binding to intestinal lamina propria leukocytes lends support to the latter hypothesis. Notably, viral RNA positivity in nonpulmonary tissues infiltrating mononuclear cells without detectable viral particles or antigens or tissue damage has been reported (*15*). Our study does not reject the possibility of seasonal influenza viruses causing occasional, disseminated infection in profoundly immunosuppressed persons because receptor affinity is not absolute (*2*). Conversely, highly pathogenic avian influenza (H5N1) and pandemic (H1N1) 2009 viruses have the ability to bind to avian-like influenza virus receptors on colonic epithelium and to replicate efficiently in intestinal cells and tissues (*12*). Their enhanced potential to cause direct intestinal infections and fecal–oral transmission deserve further investigation.

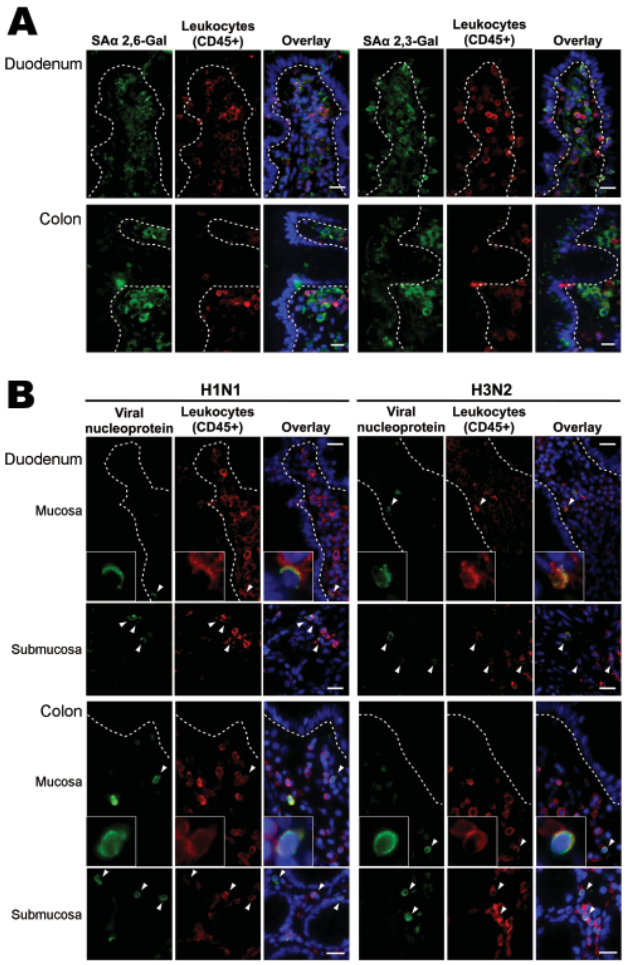

Figure 2. Intestinal distribution of influenza virus receptors and in vitro binding of inactivated seasonal influenza A (H1N1) and A (H3N2) viruses to human duodenal and colonic tissues. Images in the panels labeled Overlay show the green, red, and blue (nuclei counterstain) color channels in the same view. Dotted lines outline basal lining of intestinal epithelium. Arrowheads denote virus-bound cells. Scale bars = 20 μm. A) Double immunofluorescence staining showing that human-like influenza virus receptor sialic acid (SA) α 2,6-Gal; green) was not found on epithelial surface of small and large intestines but in lamina propria cells. Avian-like influenza virus receptor (SAα 2,3-Gal; green) was found on colonic epithelial surface and in lamina propria cells. Part of receptor-positive cells coexpressed CD45 (leukocyte common antigen; red), representing leukocytes. B) In vitro virus binding showing that neither seasonal influenza A (H1N1) nor A (H3N2) viruses bind to epithelial surface of small and large intestines but only to a subset of intestinal CD45+ leukocytes interspersed in the lamina propria and submucosa.

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Influenza B Viruses with Mutation in the Neuraminidase Active Site, North Carolina, USA, 2010–11

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Oseltamivir is 1 of 2 antiviral medications available for the treatment of influenza B virus infections. We describe and characterize a cluster of influenza B viruses circulating in North Carolina with a mutation in the neuraminidase active site that may reduce susceptibility to oseltamivir and the investigational drug peramivir but not to zanamivir.

Influenza B viruses are responsible for sporadic seasonal influenza illness and can be associated with severe illness and death. In the United States, there are 2 classes of antiviral drugs licensed by the Food and Drug Administration for treatment of influenza infections. The adamantanes are ineffective against influenza B viruses, which limits the available antiviral options to 2 neuraminidase inhibitors (NAIs), inhaled zanamivir and oral oseltamivir. Influenza B viruses seem to have reduced susceptibility to NAIs compared with influenza A viruses on the basis of neuraminidase inhibition (NAI) assays (*1*,*2*). Furthermore, in clinical studies, changes conferring either resistance or reduced susceptibility to NAIs have been identified in the neuraminidase (NA) of influenza B viruses isolated from patients after treatment (*3*–*6*). Although the use of an antiviral agent can lead to the development of drug resistance, influenza B viruses with a reduced NAI susceptibility have also been recovered from patients with no history of exposure (*5*,*7*–*10*). It is therefore plausible that such mutations may be naturally occurring within the NA of influenza B viruses.

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The Study

During routine influenza antiviral susceptibility surveillance, an influenza B virus, B/North Carolina/11/2010, with reduced susceptibility to oseltamivir and the investigational NAI peramivir was detected by using the fluorescent NAI assay based on IC_{50} values (amount of NAI required to inhibit 50% of viral NA activity). According to the current algorithm, viruses with elevated IC_{50} values, when compared with a drug-susceptible control reference virus, are further investigated by using either conventional sequencing or pyrosequencing. Sequence analysis for the NA gene of B/North Carolina/11/2010 showed a novel substitution, present as a mixed population, of isoleucine (I) to valine (V) at position 221 (B NA numbering corresponds to 222 in N2 NA amino acid numbering). A substitution of I to threonine (T) at 221 has previously been associated with reduced susceptibility to NAIs in influenza B viruses (*1*,*5*,*9*). Moreover, reduced susceptibility to oseltamivir has been reported in viruses with variation at the corresponding residue (223, N1 NA numbering) in the pandemic (H1N1) 2009 virus (*11*,*12*) and in influenza A/H5N1 (*13*) and A/H3N2 viruses (*14*).

Subsequent fluorescent NAI testing of isolates recovered during surveillance showed a cluster of 14 influenza B viruses from North Carolina with elevated oseltamivir IC_{50} values compared with reference wild-type influenza B, wild-type pandemic (H1N1) 2009, and wild-type A(H3N2) viruses; a similar trend was observed for peramivir IC_{50} values (Table 1). When comparing the pandemic (H1N1) 2009 virus with the oseltamivir-resistance conferring H275Y substitution and an influenza A (H3N2) virus with the oseltamivir-resistance conferring E119V substitution, the North Carolina B viruses showed intermediate susceptibility (Table 1). The influenza B virus carrying the R152K substitution was resistant to all NAIs compared with the influenza B viruses with I221V (Table 1). In the chemiluminescent NAI assay, the oseltamivir IC_{50} values for the I221V variants were greater than that for the E119V influenza A (H3N2) virus variant, which has been associated with oseltamivir resistance (Table 1) (*15*). Pyrosequencing analysis showed I221V as well as wild-type (I221) in the propagated viruses used in the NAI assays. The presence of wild-type variants is likely to reduce IC_{50} values.

A total of 258 influenza B virus isolates from domestic and foreign laboratories submitted to the Centers for Disease Control and Prevention for routine surveillance were screened for the I221V substitution by using single-nucleotide polymorphism (SNP) pyrosequencing analysis (*10*). All viruses were wild type at this position, with the exception of the 14 viruses from North Carolina with reduced susceptibility in the NAI assay (Table 2). All 14 viruses were collected from patients in North Carolina during November 2010 through February 2011.

Table 1. Comparison of influenza virus susceptibility, North Carolina, USA, 2010–11 influenza season*

Type/subtype and strain designation	Virus subset	NA change	Fluorescent NAI assay, IC_{50} ± SD, nmol/L (-fold)			Chemiluminescent NAI assay, IC_{50} ± SD nmol/L (-fold)		
			Zanamivir	Oseltamivir	Peramivir	Zanamivir	Oseltamivir	Peramivir
Influenza B								
B/North Carolina/11/2010	Test	I221V/I	8.58 (3)	20.39 (6)	2.77 (8)	5 (2)	8.97 (5)	1.26 (5)
B/North Carolina/03/2011	Test	I221V/I	8.13 (3)	18.98 (6)	2.43 (7)	6.37 (2)	4.98 (3)	1.19 (4)
B/North Carolina/13/2010	Test	I221V/I; K360E	6.42 (2)	17.76 (5)	2.6 (7)	3.67 (1)	4.84 (3)	1.29 (5)
B/North Carolina/07/2011	Test	I221V/I; S283N	7.93 (3)	22.31 (7)	2.95 (8)	4.82 (2)	6.01 (3)	1.26 (5)
B/North Carolina/10/2011	Control	WT	4.34 (2)	10.67 (2)	0.64 (2)	2.82 (1)	2.72 (1)	0.41 (1)
2010–2011 influenza B, n = 39	Surveillance	WT	3.31 ± 0.91 (1)	8.33 ± 0.28 (3)	0.61 ± 0.18 (2)	2.48 ± 0.89 (1)	2.77 ± 0.79 (1)	0.38 ± 0.20 (1)
B/Memphis/20/1996	Reference	WT	2.59 ± 0.50 (1)	3.25 ± 0.9 (1)	0.35 ± 0.03 (1)	2.96 ± 0.64 (1)	1.88 ± 0.33 (1)	0.28 ± 0.04 (1)
B/Memphis/20/1996	Reference	R152K	66.14 ± 28.45 (26)	700.25 ± 61.06 (215)	269.78 ± 38.21 (771)	53.46 ± 11.59 (18)	177.20 ± 36.72 (94)	62.83 ± 38.02 (224)
Pandemic (H1N1) 2009								
A/California/07/2009	Reference	WT	0.26 ± 0.02 (1)	0.22 ± 0.08 (1)	0.08 ± 0.01 (1)	0.24 ± 0.04 (1)	0.21 ± 0.03 (1)	0.07 ± 0.01 (1)
A/Texas/48/2009	Reference	H275Y	0.37 ± 0.04 (1)	165.42 ± 24.15 (752)	16.32 ± 2.12 (204)	0.36 ± 0.06 (1)	58.77 ± 10.99 (280)	7.50 ± 1.30 (107)
Influenza A (H3N2)								
A/Washington/01/2007	Reference	WT	0.40 ± 0.07 (1)	0.12 ± 0.01 (1)	0.13 ± 0.01 (1)	0.79 ± 0.08 (1)	0.11 ± 0.02 (1)	0.13 ± 0.01 (1)
A/Texas/12/2007	Reference	E119V	0.40 ± 0.05 (1)	43.81 ± 1.90 (365)	0.16 ± 0.02 (1)	0.57 ± 0.09 (1)	3.37 ± 0.63 (31)	0.16 ± 0.03 (1)

*NA, neuraminidase; NAI, neuraminidase inhibition; IC_{50}, 50% inhibitory concentration; WT, wild type. Mean IC_{50} values for wild-type reference control viruses for each type/subtype are in **boldface** and were used to calculate the -fold differences for their respective type/subtypes as indicated in parentheses. Test, influenza B viruses from North Carolina study carrying the I221V substitution. Control, influenza B virus with identical NA sequence as B/North Carolina/11/2010 with the exception of being wild-type (I) at position 221. Surveillance, influenza B viruses collected in the US during October 2010–December 2010 tested in routine influenza surveillance activities in both the fluorescent and chemiluminescent NAI assays. Reference, susceptible and resistant reference viruses used as controls in NAI assays. IC_{50} values for reference viruses represent the average taken from 5 replicates.

Because some susceptibility-altering NA mutations have been shown to arise from virus propagation in tissue culture (15), pyrosequencing analysis at position 221 in available matching clinical specimens was performed to rule out cell culture selection. The I221V substitution was identified in the 9 available matching clinical specimens (Table 2). Notably, most of the clinical specimens contained higher percentages of the V221 variant compared with the

Table 2. Percentage composition of isoleucine and valine at position 221 in the neuraminidase of virus isolates and clinical specimens with reduced susceptibility to oseltamivir and peramivir, North Carolina, USA, 2010–11 influenza season*

Strain designation	Date of collection	Clinical specimen		Virus isolate	
		% Isoleucine	% Valine	% Isoleucine	% Valine
B/North Carolina/02/2010	2010 Nov 10	NA	NA	20	80
B/North Carolina/11/2010	2010 No 29	5	95	38	62
B/North Carolina/06/2010	2010 Dec 6	NA	NA	15	85
B/North Carolina/07/2010	2010 Dec 6	NA	NA	19	81
B/North Carolina/08/2010	2010 Dec 7	NA	NA	24	76
B/North Carolina/10/2010	2010 Dec 17	8	92	13	87
B/North Carolina/12/2010	2010 Dec 20	9	91	15	85
B/North Carolina/13/2010	2010 Dec 21	10	90	17	83
B/North Carolina/03/2011	2011 Jan 5	38	62	36	64
B/North Carolina/06/2011	2011 Jan 24	7	93	15	85
B/North Carolina/01/2011	2011 Feb 1	11	89	13	87
B/North Carolina/02/2011	2011 Feb 1	8	92	14	86
B/North Carolina/07/2011	2011 Jan 31	8	92	15	85
B/North Carolina/08/2011	2011 Feb 10	F	F	12	88
B/Memphis/20/1996	NA	NA	NA	100	0

*NA, not available; F, failed in pyrosequencing analysis. 10% is the standard cutoff value for the presence of a single-nucleotide polymorphism because of limitations of the pyrosequencing assay.

matching virus isolates, which may indicate a potential selective pressure for the wild-type variant (I221) in cell culture.

An epidemiologic investigation and enhanced surveillance was initiated in cooperation with the North Carolina Department of Health and Human Services. Of 220 patients with influenza B virus infections in North Carolina during November 2010 through March 2011, specimens from 209 patients underwent pyrosequencing analysis. Specimens from 45 (22%) patients from 13 counties contained the I221V mutation based on SNP pyrosequencing analysis; patient median age was 12 years (range 6 months–60 years). Among 199 patients with available antiviral treatment information, specifically for oseltamivir use, none had documented exposure to the virus before specimen collection. This finding may indicate that influenza B viruses carrying the I221V mutation are co-circulating with wild-type influenza B viruses in North Carolina.

Conclusions

Although the NA change I221V has been seen among the N1 NA subtype of influenza A viruses (*1*,*13*), such a change has not been reported in influenza B viruses. Amino acid 221 is known to be a highly conserved residue of the NA enzyme active site. To date, all influenza B viruses with the I221V substitution appear to be limited geographically; however, monitoring is ongoing. Although oseltamivir IC_{50} values obtained with the influenza B viruses carrying the I221V substitution are similar to those seen with influenza A(H3N2) viruses carrying the oseltamivir-resistance conferring substitution E119V (Table 1), the clinical significance of the altered susceptibility associated with I221V in influenza B viruses is unknown at this time and warrants further investigation. Furthermore, such variant-dependent elevated IC_{50} values highlight the need for establishing a correlation between laboratory-determined IC_{50} values and clinical resistance.

Phylogenetic analysis of the hemagglutinin gene of the North Carolina B viruses carrying the I221V change in the NA is consistent with the B Victoria lineage (Figure,

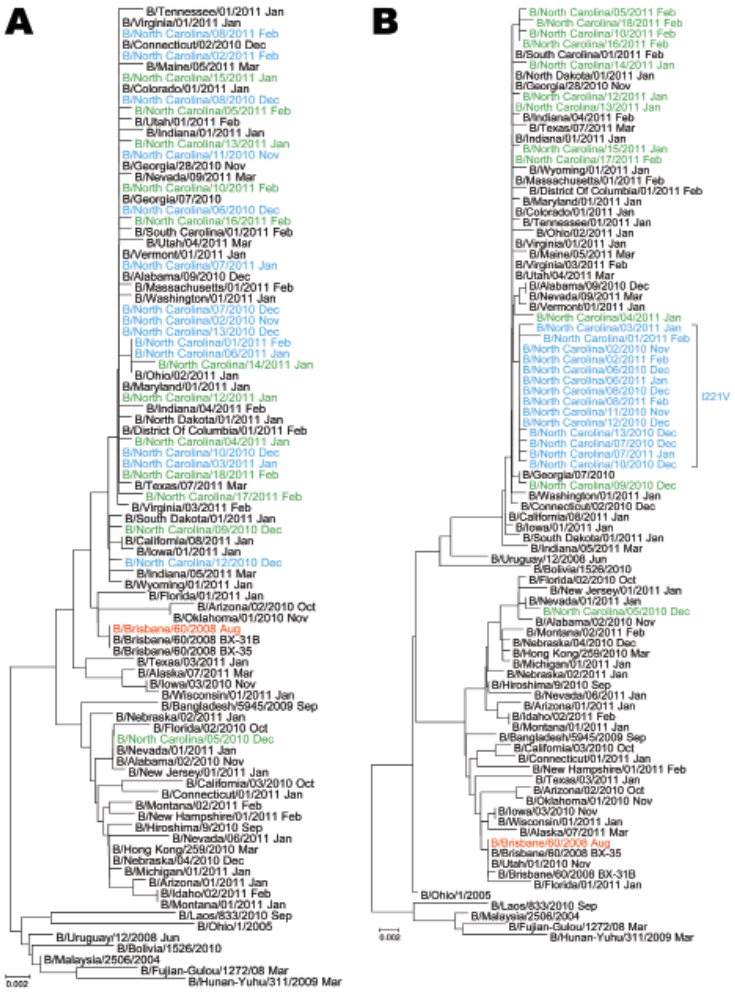

Figure. Phylogenetic analysis of A) hemagglutinin and B) neuraminidase genes of Victoria lineage type B influenza viruses (n = 89). Red indicates the 2010-2011 Northern Hemisphere vaccine strain; blue indicates the cluster of influenza B viruses identified in North Carolina carrying the I221V substitution in the neuraminidase; green indicates viruses collected from North Carolina with wild-type sequence at position 221 in the neuraminidase; black indicates representatives of globally circulating influenza B viruses. Month of collection is shown after virus strain designation. Evolutionary distances were computed by using the Tamura-Nei method (www.megasoftware.net/WebHelp/part_iv___evolutionary_analysis/computing_evolutionary_distances/distance_models/nucleotide_substitution_models/hc_tamura_nei_distance.htm). Scale bars indicate number of base substitutions per site.

panel A). Similarly, phylogenetic analysis of the NA gene demonstrated that the North Carolina B viruses with the I221V change also belong to the B Victoria lineage and form a cluster because of the I221V substitution (Figure, panel B). As of March 2011, of the 438 influenza B viruses isolated in the United States, 94% were antigenically characterized as B/Brisbane/60/2008-like (B-Victoria lineage) (www.cdc.gov/flu/weekly). The cluster of North Carolina influenza B viruses carrying the I221V mutation antigenically matched the current influenza B component of the seasonal influenza vaccine. Data collected from an ongoing epidemiologic and clinical correlation study will be the subject of a more detailed future report.

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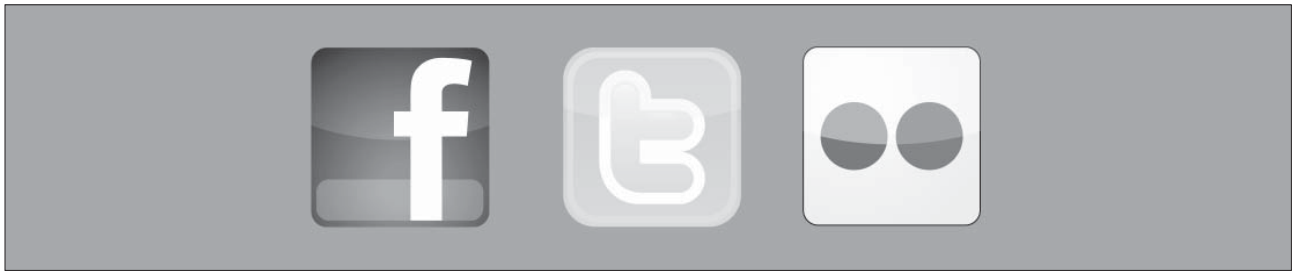

Hepatitis E Virus in Rabbits, Virginia, USA

Caitlin M. Cossaboom, Laura Córdoba, Barbara A. Dryman, and Xiang-Jin Meng

We identified hepatitis E virus (HEV) in rabbits in Virginia, USA. HEV RNA was detected in 14 (16%) of 85 serum samples and 13 (15%) of 85 fecal samples. Antibodies against HEV were detected in 31 (36%) of 85 serum samples. Sequence analyses showed that HEV from rabbits is closely related to genotype 3.

Hepatitis E virus (HEV), the causative agent of hepatitis E, is a major human pathogen and a public health concern in many developing countries. Sporadic cases of acute hepatitis E have also been reported in many industrialized countries, including the United States (*1*). In addition to humans, strains of HEV have also been genetically identified from other animal species, including pigs, chickens, rats, mongooses, and deer (*2*). A unique strain of HEV was recently identified from farmed rabbits in the People's Republic of China (*3*,*4*), although its prevalence in other regions is unknown.

At least 4 major genotypes of HEV that infect mammals have been identified (*5*). Genotypes 1 and 2 are restricted to humans, and genotypes 3 and 4 have an expanded host range and are capable of causing zoonotic disease (*6*–*10*). Avian HEV from chickens likely represents a new genus within the family *Hepeviridae* (*1*,*2*). More recently, additional putative new genotypes of HEV were identified in rats in Germany (*11*) and wild boars in Japan (*12*). The objectives of this study were to determine if farmed rabbits in the United States are infected by HEV, and if so, to genetically identify these viruses from rabbits in the United States.

The Study

Fecal swab specimens and serum samples were obtained from 85 rabbits from 2 rabbit farms in Virginia, USA (25 rabbits from Farm A and 60 rabbits from Farm B). The 2 rabbit farms, 1 in eastern Virginia and 1 in southwestern Virginia, raised rabbits for meat consumption, fur, and pets. Ages of rabbits ranged from 3.9 to 36.8 months (mean 7.0 months) on Farm A and from 3.0 to 56.9 months (mean 10.8 months) on Farm B. Rabbits were of various breeds, including Californian, Flemish X, Lop, MiniRex, New Zealand, New Zealand X, Rex X, Salitan, and TN Redback. All rabbits appeared to be healthy.

Serum samples were tested for antibodies against HEV by using an ELISA essentially as described (*13*). A truncated recombinant genotype 1 HEV capsid protein containing the immunodominant 452–617 aa region (GenWay Biotech, Inc., San Diego, CA, USA) was used as antigen. Horseradish peroxidase–conjugated goat anti-rabbit IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA) was used as the secondary antibody. Preinoculation serum and convalescent-phase serum obtained from 2 rabbits experimentally infected with rabbit HEV were included as negative and positive controls, respectively. The ELISA cutoff was calculated as the mean negative control optical density value plus 3 SD. The prevalence of IgG against HEV was 36.5% (31/85): 52% (13/25) for Farm A rabbits and 30% (18/60) for Farm B rabbits (Table 1).

All rabbit serum and fecal swab samples were tested for HEV RNA by using a nested reverse transcription PCR and a set of degenerate primers that amplify a conserved region of the HEV capsid gene. These primers were designed on the basis of multiple sequence alignment of the 2 known rabbit strains of HEV from China (*3*,*4*) and 75 other genotype 3 HEV strains. Primers used for the first-round PCR were forward primer RabdegF1 (5′-GCMACACGKTTYATGAARGA-3′) and reverse primer RabdegR1 (5′-ACYTTRGACCAATC VAGRGARC-3′). Primers used for the second-round PCR were forward primer RabdegF2 (5′-GCTGAYACRC TTCTYGGYG-3′) and reverse primer RabdegR2 (5′-TGAMGGRGTRGGCYGRTCYTG-3′).

HEV RNA was detected in 19 (22%) of 85 rabbits, including 14 (16%) of 85 serum samples and 13 (15%) of 85 fecal samples (Table 1). Authenticity of amplified PCR products was confirmed by sequencing. More rabbits were positive for HEV RNA on Farm A (48% and 40% in serum and fecal samples, respectively) than on Farm B (3% and 5% in serum and fecal samples, respectively) (Table 1). A total of 42 (49%) of 85 rabbits on the 2 farms were infected (fecal shedding, viremia, or seropositive): 20 (80%) of 25 on Farm A and 22 (37%) of 60 on Farm B (Table 1).

A 181-bp sequence within the capsid gene was identified in all 27 PCR-positive samples amplified. Sequence analyses identified 4 HEV isolates: USRab-14, USRab-16, USRab-31, and USRab-52. The 4 rabbit HEV isolates shared ≈81.2%–97.8% nt sequence identity with each other and 80.1%–95.6% nt sequence identity with the 2 rabbit HEV isolates from China (GDC9 and GDC46) (*3*). Small amounts of available clinical samples limited our ability to perform extensive genetic characterization of rabbit HEV isolates.

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Table 1. HEV in rabbits from 2 farms in Virginia, USA*

Farm	No. rabbits tested	Mean age, mo	No. (%) positive for antibodies against HEV	No. (%) positive for HEV RNA		No. (%) exposed to HEV
				Serum	Feces	
A	25	7.0	13 (52.0)	12 (48.0)	10 (40.0)	20 (80.0)
B	60	10.8	18 (30.0)	2 (3.3)	3 (5.0)	22 (36.7)
Total	85	9.7	31 (36.5)	14 (16.5)	13 (15.3)	42 (49.4)

*HEV, hepatitis E virus.

However, we amplified a larger 765-bp sequence within the capsid gene of isolate USRab-14 (Figure) by using a set of heminested primers: first-round PCR with primers RabdegF2 and RabOrf2R1 (reverse 5′-TTAAAACTCCCGGGTTTTACC-3′) and second-round PCR with primers RabOrf2F2 (forward 5′-CAG GTATTCTACTCCCGC-3′) and RabOrf2R1. Analysis of the 765-bp sequence (GenBank accession no. JN383986) showed that the USRab-14 isolate shared ≈87%–89% nt sequence identity with the 2 rabbit HEV strains from China (Table 2). Phylogenetic analysis showed that the USRab-14 isolate grouped with the 2 rabbit HEV strains from China (GDC9 and GDC46) (Figure), which are more closely related to genotype 3 HEV than to any other known HEV genotypes (Figure; Table 2). These results suggest that the rabbit HEV is likely a distant member of genotype 3 (*14*).

Conclusions

We report that farmed rabbits in the United States are naturally infected with antibodies against HEV and that HEV RNA was detected in various breeds of rabbits from 2 farms in Virginia, USA. The prevalence of antibodies against HEV and HEV RNA was higher on Farm A than on Farm B. This variation may reflect differences in rabbit housing practices on the 2 farms: rabbits on Farm A were caged in groups of 2–9 and rabbits on Farm B were each caged individually. Because HEV is transmitted by the fecal–oral route, virus likely spreads between cage mates on Farm A, thus increasing the numbers of HEV-positive rabbits.

The overall prevalence of antibodies against HEV (36%) among rabbits from the United States was lower than that among rabbits from Gansu and Beijing, China (57% and 55%, respectively) (*3*,*4*). The prevalence of HEV

Figure. Phylogenetic tree for the 765-bp sequence of open reading frame 2 of the capsid gene of rabbit hepatitis E virus (HEV) isolate USRab-14 from the United States, 2 rabbit HEV isolates (GDC9 and GDC46) from China, representative genotype 1–4 HEV strains, avian HEV, rat HEV, and novel wild boar HEV. GenBank accession numbers are shown for each HEV strain used in the phylogenetic analysis.

Table 2. Nucleotide sequence identities of a 765-bp capsid gene sequence among HEV strains from rabbits in China and the United States and other HEV strains*

Virus isolate	HEV strains, % identity					
	China	Genotype 1	Genotype 2	Genotype 3	Genotype 4	Avian
USRab-14	87.2–89.0	78.3–79.3	74.8	80.1–82.3	78.9–81.0	34.9–35.9
China GDC9/GDC46	NA	77.2–78.6	74.5–75.5	78.7–83.1	78.0–82.0	35.3–36.0

*HEV, hepatitis E virus; NA, not applicable.

RNA in serum and fecal samples on rabbit farms in the United States (16.5% and 15.3%, respectively) was higher than that on farms in Gansu and Beijing, China (7.5% and 6.96%, respectively). Ages of rabbits, animal housing practices, and hygienic conditions on rabbit farms may explain the observed difference in HEV prevalence.

Sequence analysis of the 765-bp capsid gene showed that HEV isolate USRab-14 from the United States is genetically different from the 2 rabbit strains of HEV from China (Table 2; Figure). Genetic variations were also observed among the 4 rabbit HEV isolates from the 2 rabbit farms in the United States. Rabbit HEV strains from the United States and China clustered into a distinct branch closely related to genotype 3 HEV.

Thus, similar to swine HEV in pigs (*13*) and avian HEV in chickens (*15*), rabbit HEV is also widespread in the rabbit population in the United States. The fact that rabbit HEV appears to be closely related to genotype 3 HEV raises a potential concern for zoonotic infection because genotype 3 HEV from other animal species is known to infect humans (*1*,*2*). Therefore, cross-species infection and zoonotic risk for infection with rabbit HEV should be evaluated.

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Astrovirus MLB2 Viremia in Febrile Child

Lori R. Holtz, Kristine M. Wylie, Erica Sodergren, Yanfang Jiang, Carl J. Franz, George M. Weinstock, Gregory A. Storch, and David Wang

Astroviruses cause diarrhea, but it is not known whether they circulate in human plasma. Astrovirus MLB2 was recently discovered in diarrhea samples from children. We detected MLB2 in the plasma of a febrile child, which suggests that MLB2 has broader tropism than expected and disease potential beyond the gastrointestinal tract.

Approximately 10% of nonbacterial, sporadic diarrhea is caused by infection with astroviruses (*1*). Until 2008, astroviruses that infect humans were thought to be limited to 8 closely related serotypes. However, 5 highly divergent astroviruses (MLB1, MLB2, VA1, VA2, and VA3) were discovered recently in stool samples from patients with (*2*–*4*) and without (*5*) diarrhea. No definitive disease association has been established for these 5 astroviruses.

A few reports have described enteric viruses in blood and other parts of the body, but none have described astroviruses in human plasma. For example, rotavirus RNA has been detected in serum (*6*), cerebrospinal fluid (*7*), and throat swab specimens (*7*); and rotavirus viral protein 6 and nonstructural protein 4 also have been detected in serum (*6*,*8*). Norovirus RNA also has been reported in human serum (*9*) and in cerebrospinal fluid (*10*), and enterovirus RNA has been reported in human serum (*11*). We report a case of astrovirus MLB2 viremia.

The Study

As part of a broad effort to define the human virome, we performed high-throughput sequencing (Genome Analyzer IIX; Illumina Inc., San Diego, CA, USA) on several plasma samples from children with febrile illness (K.M. Wylie et al., unpub. data). The Human Research Protection Office, Washington University (St. Louis, MO, USA) approved this study. The case report in this article describes results generated from study of a 20-month-old boy with a history of transient and resolved neutropenia. The child was evaluated in the emergency department of St. Louis Children's Hospital for petechial rash (3-day history), fever ≤40°C (1-day history), cough, and nasal congestion. He did not have vomiting or diarrhea.

The evaluation included a leukocyte count, with results (7.8×10^3 cells/mm^3) within the reference values and with a differential count of 26% bands, 59% neutrophils, 8% lymphocytes, 6% monocytes, and 1% atypical lymphocyte; blood culture results were negative. Nasopharyngeal swab specimen was negative for respiratory syncytial virus, influenza types A and B, parainfluenza, and adenovirus by fluorescent antibody testing, and culture results were negative for respiratory viruses. Chest radiograph was interpreted as showing mild peribronchial thickening, which may represent a viral process. In addition, plasma or blood samples from the patient were subjected to a battery of PCR screenings for the following viruses, the results of which were all negative: adenovirus; enteroviruses (Enterovirus ASR; Cepheid Inc., Sunnyvale, CA, USA); human herpesvirus 6 and 7; parvovirus B19 (RealStar Parvovirus B19 PCR Kit 1.0; Altona Diagnostics, Hamburg, Germany); human bocavirus; cytomegalovirus (whole blood); Epstein-Barr virus (whole blood); and JC, BK, WU, and KI polyomaviruses.

Total nucleic acid was extracted from 100 μL of the patient's plasma by using the Roche (Indianapolis, IN, USA) MagNa Pure System and randomly amplified by using a sequence-independent PCR strategy as described (*12*). Amplicons were sheared and, following standard library construction, were sequenced by using the Genome Analyzer IIX (Illumina Inc.) according to the manufacturer's protocol. Sequencing resulted in 6,394,424 sequence reads of 100 nt. When present in the sheared amplicons, the primer used for random amplification was removed, resulting in 83-nt sequences. From all reads, 238 had >80% nt identity to the partial MLB2 sequence in GenBank (GQ502192.1) when aligned by using Cross_match software (www.phrap.org/phredphrapconsed.html#block_phrap). In addition, 374 sequence reads with similarity to anelloviruses were detected in this sample by alignment of the reads to the GenBank NT and NR databases, using Cross_match and Blastx (http://blast.ncbi.nlm.nih.gov/Blast.cgi), respectively. Anelloviruses are commonly detected in human blood (*13*) and have no known disease association. No reads aligned with any other viruses, except endogenous human retrovirus sequences.

Given the number of sequence reads from the plasma sample that could be aligned with the 3,280-nt sequence of MLB2 (accession no. GQ502192.1) in GenBank, we reasoned that additional reads were likely to be present from parts of the MLB2 genome that had not yet been sequenced. To provide a complete reference genome for such an analysis, we sequenced the complete MLB2 genome from a previously described isolate (GenBank accession

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no. GQ502192.1) (*3*) from a stool sample by using a combination of reverse transcription PCR (RT-PCR), 3′ and 5′ rapid amplification of cDNA ends, and pyrosequencing on a genome sequencer (Roche) as described (*4*). The complete MLB2 genome of 6,119 nt, excluding the polyA tail, was confirmed by Sanger sequencing of overlapping RT-PCR amplicons and has been deposited in GenBank (accession no. JF742759).

Comparison of the high-throughput sequencing reads from the plasma to the complete genome yielded an additional 199 reads with >80% nt identity. Assembly of all reads yielded 10 contigs, with an average length of 305 bp, which aligned throughout the MLB2 genome (Figure). Conventional RT-PCR and quantitative TaqMan RT-PCR independently confirmed the presence of MLB2 in the plasma sample. The complete sequence of the capsid (open reading frame 2) of this plasma-derived MLB2 strain was obtained by RT-PCR (GenBank JF742760) by using primers designed from the stool-derived MLB2 strain. The capsid of the plasma-derived MLB2 strain has 99% nt identity with the stool-derived MLB2 strain. Of the 27 nt substitutions, 25 were synonymous. Because of limited quantities of the plasma sample, we were unable to sequence the complete genome of the plasma-derived MLB2.

To quantify the MLB2 virus load in the plasma specimen, we developed a quantitative RT-PCR TaqMan assay targeting the capsid (forward primer LG0169 5′-ACAACTGGCCCTACATTGAATTC-3′, reverse primer LG0170 5′-CCGACACGCACATCTCGAT-3′, and probe FAM-TCGGGTCTTGGCGCGCGAT-tam). We used the MAXIscipt Kit (Ambion, Austin, TX, USA) to generate in vitro–transcribed RNA from a plasmid containing the region of interest to establish a standard curve for the assay. On the basis of the results of this assay, this sample has 4.5×10^5 copies of MLB2 per mL of plasma.

To evaluate how frequently astroviruses may be present in human plasma, we screened archived plasma samples from 90 children with fever and 98 afebrile controls by using an astrovirus consensus RT-PCR (*3*). Total nucleic acid was extracted as described above. All 188 plasma samples were negative, which suggests that astrovirus MLB2 viremia is relatively rare, at least in the cohort analyzed.

Conclusions

The role of novel astrovirus MLB2 in human health and disease and the clinical consequence of MLB2 viremia are not yet known. This case report raises the possibility that astrovirus MLB2 may be a cause of febrile illness. In addition, the finding of MLB2 viremia suggests that astrovirus MLB2 may have effects outside the enteric system. These data, combined with the recent detection of an astrovirus in brain tissue of an immunocompromised patient (*14*) and the brain tissue of mink with shaking mink syndrome (*15*), demonstrate a broader distribution of astroviruses in the body than previously recognized. The possibility that additional disease states may be linked to astrovirus infection is intriguing. For example, no other known pathogen was detected in the patient in this case report, and he had mostly upper respiratory signs, raising the possibility that MLB2 may play a role in respiratory illness. These new hypotheses warrant further investigation.

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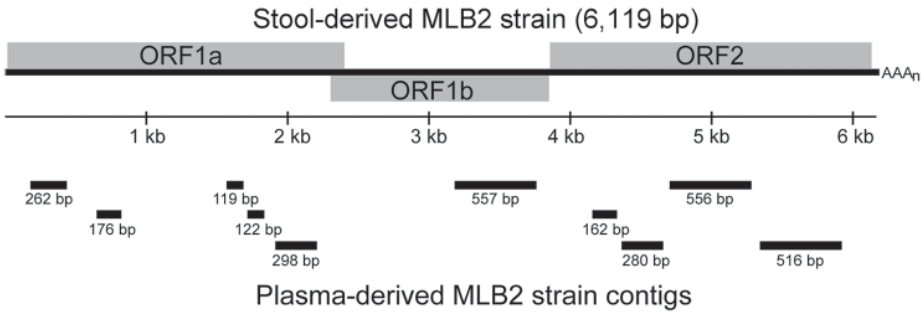

Figure. Map of 10 plasma-derived astrovirus MLB2 strain contigs generated by high-throughput sequencing (Genome Analyzer IIX; Illumina Inc., San Diego, CA, USA). ORF, open reading frame.

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New Dengue Virus Type 1 Genotype in Colombo, Sri Lanka

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The number of cases and severity of disease associated with dengue infection in Sri Lanka has been increasing since 1989, when the first epidemic of dengue hemorrhagic fever was recorded. We identified a new dengue virus 1 strain circulating in Sri Lanka that coincided with the 2009 dengue epidemic.

Dengue virus (DENV) is a flavivirus transmitted by *Aedes* spp. mosquitoes. There are 4 distinct DENV serotypes (DENV-1–4). Infection with a single serotype leads to long-term protective immunity against the homologous serotype but not against other serotypes (*1*). Globally, dengue is an emerging disease that causes an estimated 50–100 million infections, 500,000 dengue hemorrhagic fever (DHF) cases, and 22,000 deaths annually (*2*,*3*).

Epidemiologic and other studies indicate that risk factors for severe dengue include secondary infection with a heterologous serotype, the strain of infecting virus, and age and genetic background of the host. Studies are under way to further explore the role of these factors in severe disease (*1*,*4*).

In Sri Lanka, serologically confirmed dengue was first reported in 1962 (*5*), but although all 4 virus serotypes were present and there were cases of DHF, only since 1989 has DHF been considered endemic to Sri Lanka (*5*). Dengue was made a reportable disease in Sri Lanka in 1996, and the largest epidemic (35,008 reported cases, 170 cases/100,000 population, and 346 deaths) occurred in 2009 (*6*). DHF epidemics in 1989 and 2002–2004 were associated with emergence of new clades of DENV-3 (*7*,*8*). We report a new DENV-1 genotype introduced to Sri Lanka before the 2009 epidemic.

The Study

The study was approved by the Ethical Review Committee of the Faculty of Medicine, University of Colombo, Sri Lanka, and the Institutional Research Board of the International Vaccine Institute, Seoul, South Korea. Serum samples were obtained in 2009 and early 2010 from patients as part of a Pediatric Dengue Vaccine Initiative (PDVI) fever surveillance study in Colombo, Sri Lanka. Samples were originally tested for dengue by reverse transcription PCR at Genetech Research Institute (Colombo, Sri Lanka). A random subset of dengue-positive samples of all 4 serotypes was sent to the Program in Emerging Infectious Diseases Laboratory at Duke–National University of Singapore Graduate Medical School, Singapore, for virus isolation and sequencing.

RNA was extracted from virus isolates, subjected to standard reverse transcription PCR to confirm the presence

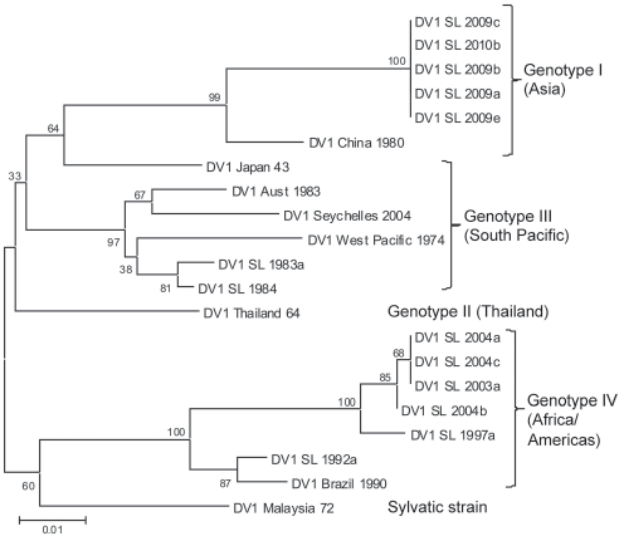

Figure. Phylogenetic tree of dengue virus 1 (DENV-1) serotype viruses from Sri Lanka (SL), 2009–2010, and other DENV-1 viruses. The tree is based on a 498-bp (nt 2056–2554) fragment that encodes portions of the envelope protein and nonstructural protein 1. Phylogenetic analysis was conducted by using MEGA5 (*10*). Percentages of replicate trees in which the associated taxa clustered in the bootstrap test (1,000 replicates) are shown next to the branches. Genotype I (Asia) includes SL isolates from 2009–2010; genotype III (South Pacific) includes SL isolates from the early 1980s; genotype IV (Africa/Americas) includes SL isolates from the 1990s and early 2000s. Classification and naming of DENV-1 genotypes are based on the report by Rico-Hesse (*11*). DV, dengue virus. Scale bar indicates nucleotide substitutions per site.

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of dengue virus, and serotyped as described (*7*). Samples processed at Duke–National University of Singapore underwent whole-genome sequencing as described (*9*). Using DENV-1 isolates from Sri Lanka obtained from dengue cases in 1983, 1984, 1997, 2003, and 2004 (*7*) and representative DENV-1 sequences for the 4 genotypes, we constructed a phylogenetic tree by using MEGA5 software (*10*) (Figure; Table).

The 4 DENV serotypes found in Sri Lanka have been classified into genotypes according to the nomenclature described by Rico-Hesse (*11*). The earliest isolates found in 1983 and 1984 belong to South Pacific genotype III. More recent isolates obtained during surveillance efforts during 1997–2004 belong to Africa/America genotype IV, indicating that at some point between the early 1980s and the mid 1990s, there was a DENV-1 genotype shift. Analysis of viruses isolated in 2009 indicated that another Asia genotype I of DENV-1 has been introduced into Sri Lanka (Figure) (*7*). This Asia genotype I virus appears to be responsible for the 2009 epidemic of dengue fever and DHF.

Conclusions

A feature of the epidemiology of dengue in Sri Lanka was the lack of DHF in the early 1980s and the increase in the number of severe dengue cases since 1989, more so after 2000. This finding was observed despite seroprevalence rates remaining largely the same over time as reported in a previous study (*12*) and in the current PDVI study (*13*).

Previous epidemics (1989 and 2002–2004) showed a correlation with evolution of DENV-3 genotype III in Sri Lanka, where emergence of new clades of DENV-3 genotype 3 showed a correlation with large increases in the number of reported cases and the geographic range of the virus (*7*,*8*). A similar observation was reported for Puerto Rico by Bennett et al., who compared data for DEN2 and DEN4 over 20 years and found that dominant clades were replaced by viral subpopulations existing within the population (*14*) and in the South Pacific region for DENV-2, where a similar clade replacement occurred (*15*). These clade changes were accompanied by positive selection in the nonstructural protein 2A (NS-2A) gene for DENV-4 and the envelope, premembrane, NS-2A, and NS-4A genes for DENV-2.

Our results indicate that introduction of a new DENV-1 genotype coincided with the 2009 dengue epidemic in Sri Lanka. Studies are underway to determine if the proportion of DENV-1 cases in 2009 was greater than in previous years and to assess the role of this new DENV-1 genotype in the severe epidemic of 2009. Further studies are needed to determine if this new genotype has spread to other countries in the region.

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Table. Dengue virus type 1 strains used in analysis of new dengue virus genotype, Colombo, Sri Lanka

Virus strain*	Location	Subtype	Year isolated	GenBank accession no.
DV1_Aust_1983	Australia	III	1983	AB074761
DV1_West_Pacific_1974	Western Pacific	III	1974	U88535
DV1_Brazil_1990	Brazil	IV	1990	AF226685
DV1_China_1980	People's Republic of China	I	1980	AF350498
DV1_Japan_43	Japan	I	1943	AB074760
DV1_Malaysia_72	Malaysia	Sylvatic	1972	EF457905
DV1_Seychelles_2004	Seychelles	III	2004	DQ285561
DV1_SL_1983a	Sri Lanka	III	1983	FJ225443
DV1_SL_1984	Sri Lanka	III	1984	FJ225444
DV1_SL_1992a	Sri Lanka	IV	1992	FJ225445
DV1_SL_1997a	Sri Lanka	IV	1997	FJ225446
DV1_SL_2003a	Sri Lanka	IV	2003	FJ225447
DV1_SL_2004a	Sri Lanka	IV	2004	FJ225448
DV1_SL_2004b	Sri Lanka	IV	2004	FJ225449
DV1_SL_2004c	Sri Lanka	IV	2004	FJ225450
DV1_Thailand_64	Thailand	II	1964	AF180818
DV1_SL_2009a	Sri Lanka	I	2009	HQ891313
DV1_SL_2009b	Sri Lanka	I	2009	HQ891314
DV1_SL_2009c	Sri Lanka	I	2009	HQ891315
DV1_SL_2009e	Sri Lanka	I	2009	JN054256
DV1_SL_2010b	Sri Lanka	I	2010	JN054255

*Strains are indicated as genotype_location_year.

interests are the epidemiology, virology, surveillance, and control of dengue.

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Ultrastructural Characterization of Pandemic (H1N1) 2009 Virus

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We evaluated pandemic influenza A (H1N1) 2009 virus isolates and respiratory tissues collected at autopsy by electron microscopy. Many morphologic characteristics were similar to those previously described for influenza virus. One of the distinctive features was dense tubular structures in the nuclei of infected cells.

In April 2009, a novel influenza A (H1N1) virus was first detected in 2 children in California; the same virus was found to be circulating in Mexico and then spread rapidly worldwide (*1*). The virus became known as pandemic (H1N1) 2009 and generally caused a mild-to-moderate illness, although severe and fatal cases were reported.

Influenza A virus is a member of the family *Orthomyxoviridae* and contains a genome that is composed of single-stranded negative-sense RNA that, with the viral nucleoprotein, is formed into 8 separate ribonucleoprotein segments (*2*). The pandemic (H1N1) 2009 virus contains a unique combination of RNA segments from North American and Eurasian swine lineages and is capable of human-to-human transmission (*3*).

The pathologic features of fatal cases of pandemic (H1N1) 2009 have been described (*4*,*5*). Notably, in addition to infection of the tracheobronchial epithelium, as is seen with seasonal influenza, pandemic (H1N1) 2009 virus also extensively infects the lower respiratory system. The most common histopathologic finding was diffuse alveolar damage comprising intraalveolar edema, hyaline membranes, fibrin, and hemorrhage. Immunohistochemical examination detected influenza virus antigens in type II pneumocytes, in epithelial cells in the upper airways, and in submucosal glands.

In this study, we examined the morphologic properties of pandemic (H1N1) 2009 virus in cultured cells and in human tissues obtained at autopsy. Immunogold labeling was used to further analyze various aspects of the morphogenesis of this novel influenza virus.

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The Study

Infected and uninfected MDCK cells were embedded for standard electron microscopy or immunogold electron microscopy in a mixture of Epon-substitute and Araldite or in LR White resin (Ted Pella, Inc., Redding, CA, USA), respectively, as described (*6*). The immunogold electron microscopy protocol used a goat antibody raised against the matrix protein of influenza A virus as a primary antibody and a donkey anti-goat antibody conjugated to 12-nm colloidal gold particles as a secondary antibody. In addition, lung tissues were obtained from the upper and lower respiratory tracts of 2 patients who died of pandemic (H1N1) 2009. Samples from each patient were negative for parainfluenza viruses and respiratory syncytial virus by PCR. Areas for examination were selected on the basis of strong immunohistochemical labeling for influenza virus. Sections for light microscopy sections were cut from the electron microscopy blocks, and areas were selected for either bronchus with submucosal glands or lung with alveoli.

Pandemic (H1N1) 2009 virus isolates grown in MDCK cells were morphologically similar to those of other influenza A viruses. In negative stain preparations, virions appeared mostly spherical (average diameter 104 nm) with some filamentous particles (up to 3.3 μm in length) and contained surface projections of the hemagglutinin and neuraminidase glycoproteins (Figure 1, panel A). By thin section electron microscopy, infected cells showed virus particles being assembled mostly at the plasma membrane. Extracellular virions, averaging 86 nm in diameter, were mostly ovoid or filamentous (Figure 1, panel B). The virions were surrounded by the glycoprotein spikes, and the individual nucleocapsids inside the virions were seen as thin threads (when cut longitudinally) or dark dot-like figures (when cut in cross-section) and measured ≈8 nm in diameter (Figure 1, panels B and C).

The nuclei of some infected cells contained dense tubular structures, which had a rough outer edge and averaged 37 nm in width (Figure 1, panel D). Immunogold electron microscopy labeling that used an anti–matrix protein goat antibody detected matrix (M) proteins on the dense tubular structures (Figure 1, panel E) as well as on virions (C. Goldsmith et al., unpub. data).

Lung tissues from patients with fatal cases of pandemic (H1N1) 2009 were examined by electron microscopy. Infected cells and virions were observed in the alveolar spaces and in the submucosal glands (Figure 2, panels A, B, and C). Spherical or ovoid extracellular viral particles, which may represent cross-sections through filamentous particles, were seen in respiratory tissues. Dense, amorphous material was associated with the virions. In

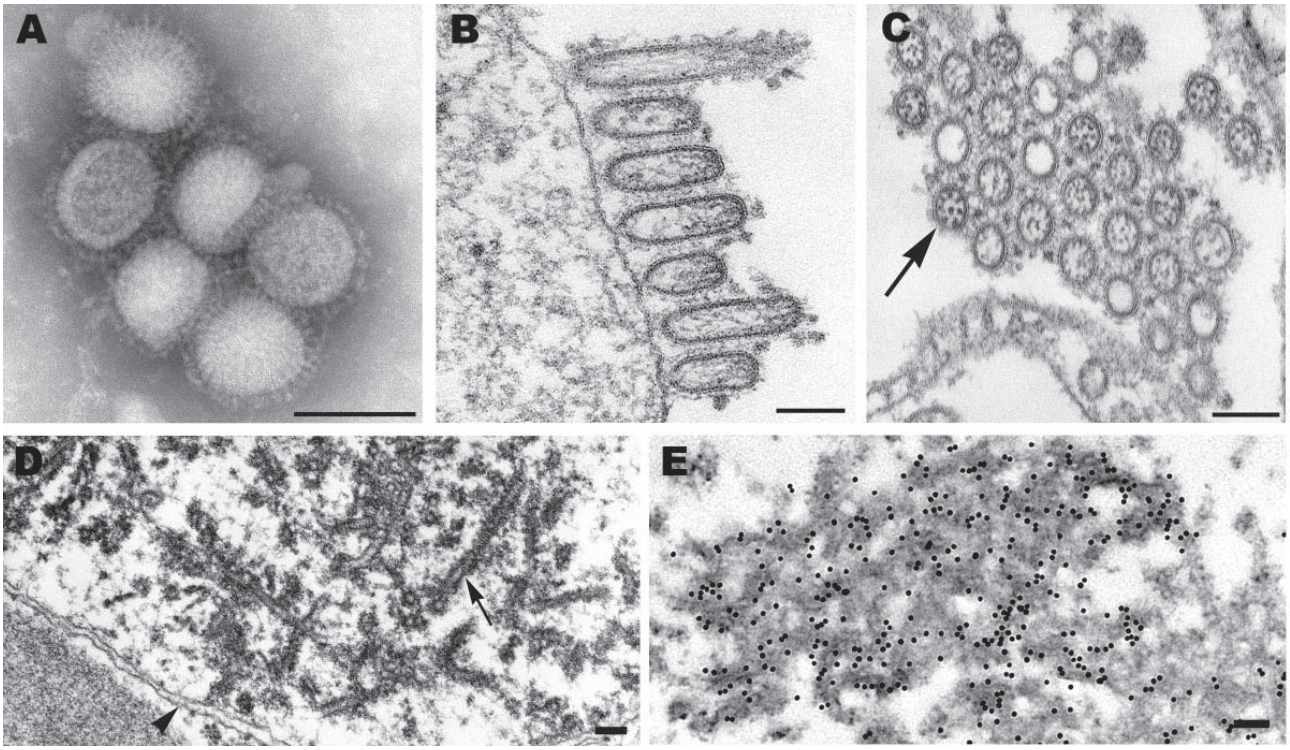

Figure 1. Electron microscopy of pandemic (H1N1) 2009 virus. A) Negatively stained virions grown in MDCK cells showing spherical particles with distinct surface projections. Scale bar = 100 nm. B) Filamentous and ovoid particles assembling at the plasma membrane. Scale bar = 100 nm. C) Extracellular particles showing internal nucleocapsids, seen in cross-section, surrounded by an envelope with prominent spikes. Note all 8 nucleocapsids present in 1 virion (arrow). Scale bar = 100 nm. D) Dense tubules (arrow), which were found in the nuclei of some MDCK-infected cells. Arrowhead, nuclear envelope. Scale bar = 100 nm. E) Immunogold labeling of the nuclear tubules by using an antibody against the matrix protein. Scale bar = 100 nm.

addition, intranuclear dense tubules, similar to those seen in tissue culture–infected cells, were recognized in infected cells (Figure 2, panel D).

Conclusions

This study shows that the morphologic features of pandemic (H1N1) 2009 virus in infected cells were similar to other members of the family *Orthomyxoviridae* (*7*). Enveloped particles, either spherical or filamentous, were surrounded by a fringe of surface projections and enclosed viral nucleocapsids. As is typical for influenza viruses, virions assembled and budded at the plasma membrane. In addition, inclusions consisting of dense tubules were seen in the nuclei of some infected cells in cell culture and tissues collected during autopsy. Although intranuclear dense tubules have been previously reported for other influenza A-infected cells, these were either smooth or helical (*8*,*9*). Of note, the dense tubules in the nuclei of pandemic (H1N1) 2009–infected cells were much larger (37 nm vs. 8 nm) and did not resemble the more thread-like nucleocapsids of influenza virus.

Of particular interest is the labeling of the nuclear dense structures in pandemic (H1N1) 2009–infected cells by an antibody directed against the matrix protein. M1 serves as the matrix protein during the formation of viral particles. The M1 protein is also transported from the cytoplasm into the nucleus during viral replication and, in conjunction with nonstructural protein 2, is involved in the export of the ribonucleoproteins out of the nucleus (*10*,*11*). The immunolabeling of the dense tubular structures seen in the pandemic (H1N1) 2009–infected cells reported here confirms the presence of matrix protein in the nuclei of infected cells.

Generally, freshly isolated viruses from human tissues are predominantly filamentous, whereas laboratory-adapted strains are predominantly spherical (*12*). This study and other recent reports of early isolates of pandemic (H1N1) 2009 revealed both spherical and filamentous forms (*4*,*13*). Similarly, in this study, mostly spherical particles were found in lung autopsy tissues, whereas filamentous particles were as described by Nakajima et al. (*4*). The determinants of influenza virus morphology, i.e., spherical versus filamentous, have been evaluated by many researchers and found to be influenced by the M1 and M2 proteins and by polarization of the host cell (*14*,*15*). The biological relevance of finding both

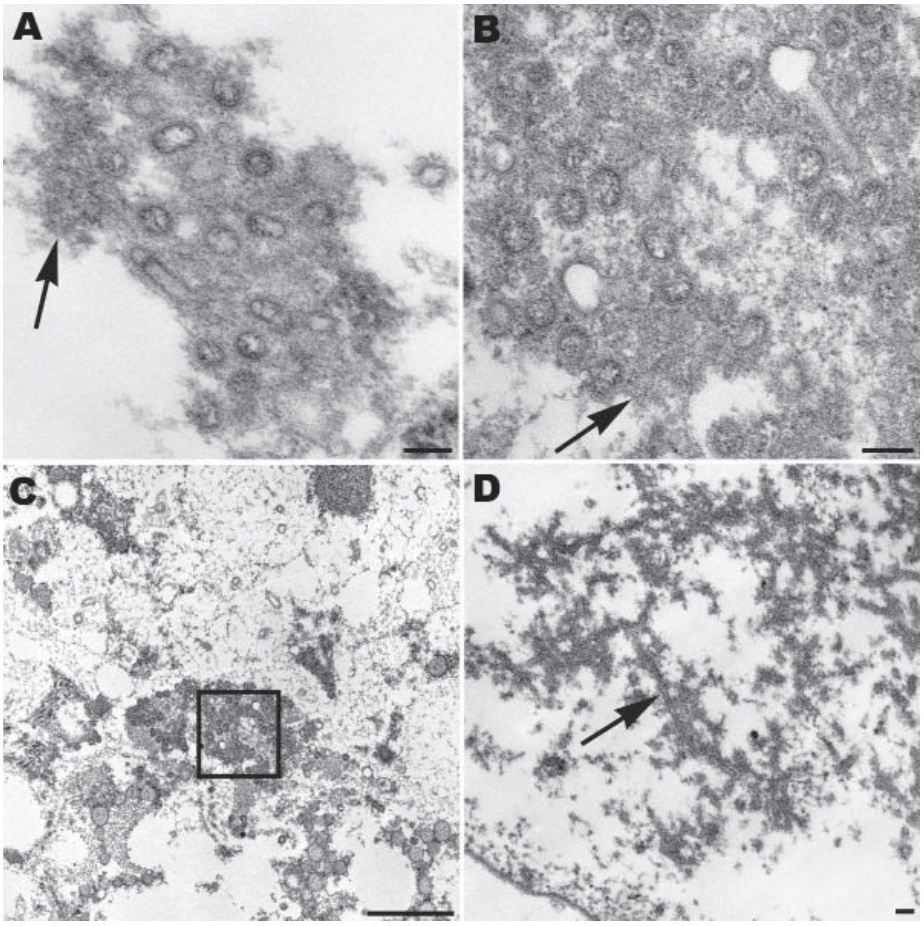

Figure 2. Spherical and ovoid extracellular pandemic (H1N1) 2009 virus particles in human lung tissue found in the alveolar space (A) and in a submucosal gland (B). Nucleocapsids and surface projections are visible on some virions. Note the dense material (arrows) associated with the particles. Scale bars = 100 nm. C) Low-power magnification of the aggregation of virus particles seen in panel B, showing virions (box) in the mucus of the submucosal gland. Scale bar = 1 μm. D) Dense tubules (arrow) found in the nucleus of an infected cell in alveolar space. Scale bar = 100 nm.

spherical and filamentous particles in autopsy tissues and in early isolates of pandemic (H1N1) 2009 virus requires further studies.

Another distinctive feature of pandemic (H1N1) 2009 virus was infection of the lower respiratory tract, as evidenced by the presence of viral particles in the alveoli. This finding helps explain the diffuse alveolar damage associated with hyaline membranes seen in severe cases of infection. Pandemic (H1N1) 2009 virus particles were also found in the mucus of the submucosal glands and may play a major role in human-to-human transmission through aerosolization of respiratory secretions.

Pandemic (H1N1) 2009 virus was the causative agent of the first influenza pandemic since 1968, and much has been learned since the pandemic began. However, there is still much to be elucidated about this emerging virus, and electron microscopic studies have revealed distinctive features of the pandemic (H1N1) 2009 virus that may help in understanding its morphogenesis and pathogenesis.

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Molecular Subtyping in Cholera Outbreak, Laos, 2010

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A cholera outbreak in Laos in July 2010 involved 237 cases, including 4 deaths. Molecular subtyping indicated relatedness between the *Vibrio cholerae* isolates in this and in a 2007 outbreak, uncovering a clonal group of *V. cholerae* circulating in the Mekong basin. Our finding suggests the subtyping methods will affect this relatedness.

Cholera is a major public health concern in countries where access to safe water and adequate sanitation cannot be guaranteed for all residents. *Vibrio cholerae* serogroups O1 and O139 are the causative agents of cholera (*1*). A major virulence factor is cholera toxin (Ctx) encoded by the *ctxAB* gene and located on the Ctx prophage. *V. cholerae* O1 is classified into 2 biotypes, classical and El Tor. The El Tor biotype is responsible for the ongoing seventh pandemic of cholera (*2*). Since the early 1990s, the El Tor variant strains, which are biotypes of El Tor but carry the classical type of *ctxB*, have emerged and prevail in multiple regions where cholera is endemic (*1*,*3*–*6*).

The Study

In July 2010, a cholera outbreak began in Attapeu Province in southern Laos along the Cambodian border. Onset dates were July 5–September 16. The outbreak spread to 17 villages of the province and involved 237 persons, including 4 who died. To isolate the suspected *V. cholera* colonies, we screened specimens on thiosulfate citrate bile salt sucrose agar with or without enrichment in alkaline peptone water. Suspected colonies were examined by conventional biochemical tests and PCR amplification of *ctx* (*7*,*8*). Of the 42 fecal specimens tested, 9 were culture positive. The isolates were toxigenic *V. cholerae* O1 serotype Ogawa with features of the El Tor variant, according to the *ctxB*-typing method of Morita et al. (*9*).

We analyzed the 9 *V. cholerae* isolates from the Attapeu outbreak. We performed pulsed-field gel electrophoresis (PFGE) according to the PulseNet protocol (*10*) and multilocus variable number tandem repeat analysis (MLVA) using the 7 loci, as described (*5*,*11*).

The isolates of the Attapeu outbreak had almost indistinguishable PFGE profiles and MLVA repeat copy numbers. In PFGE analysis, 8 of the 9 isolates showed indistinguishable profiles (PFGE-A). The profile of the remaining isolate differed from the dominant isolates by 2 bands (PFGE-B) (Figure). In MLVA, 8 isolates showed the same MLVA type (MLVA-I), and 1 isolate showed another MLVA type that differed from the major MLVA type by being a single-locus variant of MLVA-I with only 1 locus and 1 repeat copy number (MLVA-II) (Table). Seven of the MLVA-I and 1 of the MLVA-II isolates showed the PFGE-A profile, and 1 of the MLVA-I isolates showed the PFGE-B profile. Although the source of contamination remains unknown, these results indicate that all isolates were indistinguishable from or similar to each other and that the outbreak could have been caused by a single source of contamination.

For comparison, we also examined 19 isolates from an outbreak that occurred in Xekong Province in 2007. These isolates also were toxigenic *V. cholerae* O1 serotype Ogawa of the El Tor variant (*12*). MLVA results clearly indicate that the isolates of the Attapeu outbreak in 2010 differed from those of the Xekong outbreak in 2007. The isolates from the Xekong outbreak comprised 3 MLVA types; 17 isolates were MLVA-III, 1 was MLVA-IV, and 1 was MLVA-V. MLVA-IV and MLVA-V were single-locus variants of MLVA-III (Table). Of the 7 loci tested, 3 or 4 displayed different repeat copy numbers than did those of the Attapeu and Xekong outbreaks. In PFGE analysis, however, the profiles were similar to each other; the isolates from the Xekong outbreak showed a PFGE-B profile (Figure).

These results suggest that strains with a specific PFGE type and the related strains have been circulating in the area for at least 3 years. Nguyen et al. suggested that another cholera outbreak in Vietnam that occurred from the end

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Table. MLVA types identified in study of cholera, Laos, 2010*

MLVA type	No. isolates	Outbreak location	*Vibrio cholerae* repeat copy no.							PFGE profile (no. isolates)
			1	2	3	5	6	7	8	
I	8	Attapeu	8	6	NA	NA	7	17	17	A (7), B (1)
II	1	Attapeu	8	6	NA	NA	7	18	17	A (1)
III	17	Xekong	10	6	NA	4	7	16	16	B (17)
IV	1	Xekong	10	6	NA	4	7	16	17	B (1)
V	1	Xekong	9	6	NA	4	7	16	16	B (1)

*MLVA, multilocus variable number tandem repeat analysis; PFGE, pulsed-field gel electrophoresis; NA, no PCR products amplified.

of 2007 to the beginning of 2008 was associated with the Xekong outbreak (*13*). Choi et al. also studied isolates from Vietnam in 2007 and 2008 by using MLVA, wherein they used 5 loci that are in common with those in this study (VC-1, -2, -6, -7, and -8) (*14*). The MLVA results obtained in our study indicated that the repeat copy numbers of the compatible loci of the Xekong outbreak isolates were the same as those of some of the isolates described in the study by Choi et al. This finding strongly suggests that the causative agents of the Xekong outbreak of Laos and the Vietnam outbreak in 2007–2008 were the same. Moreover, the strains were speculated to circulate widely in the Mekong basin, although the similarity between the PFGE profiles of the isolates from Laos and Vietnam remain to be studied.

Recently, another *ctxB* type of *V. cholerae* O1 biotype El Tor serotype Ogawa was reported in Orissa in eastern India (*15*). Representatives of the Xekong and Attapeu isolates also were subjected to sequence analysis of *ctxB*. The results showed that their *ctxB* sequences were identical with that of the original classical type, which suggests that the clonal group in the Mekong basin differs from the new Orissa type of *V. cholerae* in India.

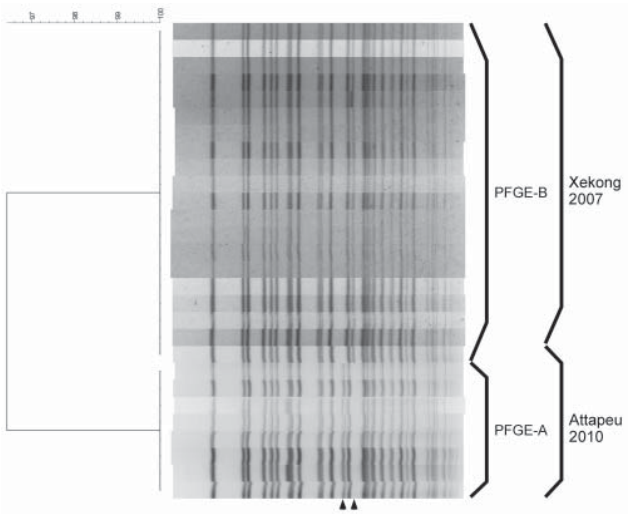

Figure. *Not*I-digested pulsed-field gel electrophoresis (PFGE) profiles of *Vibrio cholerae* isolates, Laos, 2010. The names of the profiles and the sources of the isolates are shown on the right. A dendrogram was created with BioNumerics software (Applied Maths, Kortrijk, Belgium) by using the Dice coefficient, unweighted pair-group method with arithmetic means, and a band-position tolerance of 1.2%. Arrowheads at bottom indicate location of bands differing in PFGE-A and PFGE-B.

Conclusions

Our study clearly indicates that the 2010 cholera outbreak at Attapeu was caused by 1 source of contamination. Furthermore, isolates from the Attapeu outbreak and the 2007 Xekong outbreak showed similar PFGE profiles, but they were differentiated by MLVA, consistent with their origin. This study suggests that PFGE analysis is useful for identifying the kinds of *V. cholerae* clones circulating in a specific geographic region and might be useful for determining a long-term framework of the region-specific *V. cholerae* because PFGE profiles are probably more stable than the MLVA types. By contrast, MLVA is useful for investigating and discriminating short-term individual outbreaks in a region. Another cholera outbreak in Cambodia in 2010 also might be related to the Attapeu outbreak. Combined use of both molecular subtyping methods would indicate the relatedness of cholera in the 2010 Cambodian outbreak and the others in the Mekong basin.

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Clonal Origins of *Vibrio cholerae* O1 El Tor Strains, Papua New Guinea, 2009–2011

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We used multilocus sequence typing and variable number tandem repeat analysis to determine the clonal origins of *Vibrio cholerae* O1 El Tor strains from an outbreak of cholera that began in 2009 in Papua New Guinea. The epidemic is ongoing, and transmission risk is elevated within the Pacific region.

In July 2009, an outbreak of cholera began in the Morobe Province of Papua New Guinea (PNG) (*1*), and in the following months the disease spread throughout the coastal regions of the country. Although environmental and social conditions are conducive to the transmission and sustained presence of cholera, to our knowledge, this was the first outbreak of cholera in PNG. Sporadic outbreaks have occurred in the nearby Indonesian province of West Papua (formerly Irian Jaya) in the 1960s, 1990s, and, most recently, in 2008 (*2*,*3*). As such, conjecture has existed about whether this outbreak was the result of a new incursion of *Vibrio cholerae* or a reemergence of previously undetected strains endemic to PNG. We used multilocus sequence typing (MLST) and variable number tandem repeat (VNTR) analysis to investigate the diversity of the PNG *V. cholerae* strains and to elucidate the origin of this outbreak.

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The Study

The PNG cholera outbreak was first reported in Lambutina and Nambariwa villages in Morobe Province on the northeast coast of mainland PNG in July 2009 (*1*). The outbreak spread within the province and then to Madang and East Sepik Provinces along the northwest coast. In January 2010, the epidemic reached the national capital, Port Moresby, resulting in a large sustained outbreak in National Capital District and surrounding Central Province. In the following months, the outbreak spread along the south coast to Gulf and Western Provinces. Recently, the cholera outbreak has spread to the Autonomous Region of Bougainville. Since July 2009, >15,500 cases of cholera have been reported in PNG, with 493 recorded deaths (Figure).

Clinical isolates from Morobe Province (n = 2), Madang Province (n = 2), Eastern Highlands Province (n = 2), East Sepik Province (n = 2), and National Capital District (n = 4) were isolated and identified as *V. cholerae* O1 El Tor Ogawa by using standard bacterial culture methods. The isolates were confirmed as *V. cholerae* serogroup O1 and carriers of the *ctxA* gene by multiplex PCR (*4*). All isolates were identified as altered El Tor through PCR detection of the classical *rstR* gene (*5*).

Nine loci were targeted for MLST analysis: *dnaE*, *lap*, *recA*, *pgm*, *gyrB*, *cat*, *chi*, *rstA*, and *gmd*, as described (*6*). The PCR products were visualized on an agarose gel, and direct sequencing was performed in both directions by using the MLST primers (Macrogen, Seoul, Korea). Contiguous nucleotide sequences were assembled by using Sequencher software (www.genecodes.com), and all nucleotide positions were confirmed by ≥2 independent sequencing

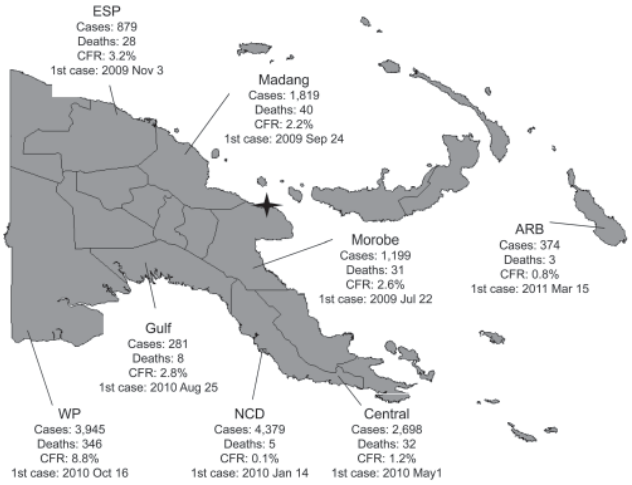

Figure. Cholera outbreak, Papua New Guinea, 2009–2011. Total cases: 15,582. Total deaths: 493. Overall case-fatality rate (CFR): 3.2%. Star denotes original outbreak sites of Lambutina and Nambariwa villages. ESP, East Sepik Province; ARB, Autonomous Region of Bougainville; WP, Western Province; NCD, National Capital District.

reactions in each direction. The PNG MLST sequences were compared with previously reported sequences by using the same 9 loci outlined in previous studies (*5*–*7*). All PNG isolates displayed 100% nt identity across the 9 MLST loci and were identical to the Bangladesh strain MJ-1236 (*8*).

Five loci were analyzed for tandem repeats by using VNTR-specific primers, as described (*9*). The targeted regions were VC0147, VC0436–7, VC1650, VCA0171, and VCA0283. Contiguous nucleotide sequences were prepared as described above. Sequence types were designated by the actual number of repeats at the target loci as described in recent studies (*10*,*11*) and compared with sequence-derived VNTR data from the international literature and databases. Three sequence types were detected that were all within clonal complex 10,6,8,X,X (Table). The isolates from PNG were most closely related to strains from Vietnam (1995–2004) in the MLVA group III reported by Choi et al. (*12*). As reported, loci on the small chromosome (VCA0171 and VCA0283) were more variable than the loci on the large chromosome (*9*,*11*,*12*).

Conclusions

The homogeneity of outbreak strains in PNG and the relatedness to strains from Vietnam by VNTR analysis is indicative of a recent incursion from the Southeast Asia region. It is unlikely that this outbreak is because of a previously undetected autochthonous endemic strain, given the close relationship with the isolates from Vietnam.

MLST and VNTR have been used by numerous research groups to analyze the diversity of outbreak strains and to investigate the origin of epidemics (*6*,*9*–*11*). MLST analysis of the *V. cholerae* strains and comparison with previously published MLST data suggested that the most closely related isolate was from Bangladesh (*8*). All isolates from PNG were identical to the strain MJ-1236 across the 9 housekeeping genes examined by sequence analysis. In contrast, the VNTR sequence analysis suggested that the PNG outbreak strains were most closely related to strains isolated from Vietnam in 1995, 2002, 2003, and 2004 (*12*). MLST data were not available for the Vietnam strains in the international literature or databases; therefore, a direct comparison cannot be made between the PNG MLST and VNTR results.

This outbreak highlights the continued challenge that cholera presents to authorities worldwide: the disease can spread rapidly and the causative organism persists in the environment (*13*), which makes prevention and control of the disease complex. In PNG the large estuarine waterways (e.g., Sepik and Fly Rivers) and the settlement areas (which are often in estuarine areas with limited water and sanitary infrastructure and are more densely populated than rural and urban areas) present potential reservoirs for *V. cholerae*. The prevalence of enteric diseases remains high in PNG where access to safe drinking water is limited, particularly in rural areas where an estimated 87% of the population lives (*14*). These factors may aid the persistence of *V. cholerae* and result in a transition to endemicity of cholera in PNG.

During this outbreak, a relatively high national case-fatality ratio (CFR) of 3.2% was recorded. The provincial CFRs varied widely from 0.1% in the National Capital District, where oral rehydration salts points and treatment centers provided timely accessible treatment, to 8.8% in Western Province, where health system access and preparedness were weak. Strong leadership and coordination contributed to effective response but were limited where CFRs were high.

Table. Variable number tandem repeat profiles of Papua New Guinea *Vibrio cholerae* isolates and comparison with related international strains*

Isolate	Source and year	VC0147	VC0436–7	VC1650	VCA0171	VCA0283	Reference
M1	Madang, PNG, 2010	10	6	8	8	12	This study
M2	Madang, PNG, 2010	10	6	8	9	12	This study
G1	EHP, PNG, 2010	10	6	8	8	12	This study
G2	EHP, PNG, 2010	10	6	8	8	12	This study
L1	Morobe, PNG, 2010	10	6	8	8	11	This study
L2	Morobe, PNG, 2010	10	6	8	8	11	This study
W1	ESP, PNG, 2010	10	6	8	8	12	This study
W2	ESP, PNG, 2010	10	6	8	8	12	This study
P1	NCD, PNG, 2011	10	6	8	8	11	This study
P3	NCD, PNG, 2011	10	6	8	8	12	This study
P4	NCD, PNG, 2011	10	6	8	8	12	This study
P5	NCD, PNG, 2011	10	6	8	8	11	This study
07.95/Vc.P	Vietnam, 1995	10	6	8	16	26	(*12*)
272.03/Vc.P	Vietnam, 2003	10	6	8	17	28	(*12*)
43.04/Vc.P	Vietnam, 2004	10	6	8	16	29	(*12*)
MJ-1236	Bangladesh, 1994	8	7	8	12	19	(*12*)

*Variable number tandem repeat profiles indicate the actual number of repeats. PNG, Papua New Guinea; EHP, Eastern Highlands Province; ESP, East Sepik Province; NCD, National Capital District.

Despite road networks linking affected coastal areas to the mountainous interior, where most of the country's population resides, imported cases have not resulted in ongoing transmission. This lack of transmission may be related to a less favorable habitat for environmental persistence of the organism and ongoing transmission. A similar situation was reported from the current outbreak of cholera in Haiti, where location on a coastal plain was a notable risk factor for cholera cases (*15*). Nonetheless, cholera remains a high risk for both affected and unaffected provinces in PNG. Moreover, the frequent international migration between PNG and neighboring communities with no prior cholera exposure and with vulnerable sanitary conditions heightens the risk for international spread.

Although the MLST and VNTR results concur that the PNG strains are closely related, our data suggest that VNTR has greater discriminatory power when used for investigations into the clonality and relatedness of *V. cholerae* strains. Other studies have highlighted the value of VNTR for strain typing of *V. cholerae* (*9–12*), but reports that directly compare VNTR and MLST are lacking. However, analysis by either VNTR or MLST is hampered by the paucity of data available to compare outbreak strains from around the world. An online *V. cholerae* VNTR database would enable more accurate tracking of the evolution of outbreaks and provide evidence for the mode of spread of *V. cholerae* strains between countries and geographic areas. A more comprehensive analysis of *V. cholerae* strains from around the world is also required to gain a better understanding of the global and regional spread of these strains.

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In Memoriam: David Judson Sencer, A Public Health Giant

Jeffrey P. Koplan

Many of the contributors to Emerging Infectious Diseases journal and its readers recently lost a dear friend, personally and in the field of public health. In medical school, professors often regaled us with tales of the "giants," master diagnosticians and clinicians, physicians who could proficiently teach, investigate, care, and inspire. There is no question that David J. Sencer was a "giant" who left his footprint (indeed, one with a worn oval in the forefoot) on many careers, institutions, and programs, and on the many people around the world who were spared illness and premature death by his efforts.

This reflection on David Sencer is not an obituary or a biography but rather a series of observations that hope to capture his intelligence, wisdom, dedication, and humor. As he was the longest serving director of the Centers for Disease Control and Prevention (CDC) from 1966 to 1977 and one who made it his business to know virtually the whole workforce and what they did, there are many current and former CDC employees with personal accounts and recollections. Whenever possible I've tried to incorporate their insights and memories.

David Sencer by any measure was a great director of CDC. His accomplishments and legacy still provide a strong backbone for the agency in its structure, performance, and values. He understood that CDC had a global health mission before global health became popular and was a strong advocate for states and their primary role in our national public health system. Dr Sencer was uncompromising in the need for strong science to drive public policy. He expanded CDC's responsibilities in keeping with the expanded mission of public health, adding nutrition, health education, and cancer epidemiology among others to a largely infectious disease portfolio. He was willing to err on the side of protecting the public's health even if it meant a risk of critical perceptions of him and the CDC. Dr Sencer established a management system at CDC with a deep bench that was as strong as the scientific workforce. He extended CDC's influence, strengthened our partners, and developed new skills and experiences for his staff by attaching promising and accomplished employees to state health departments, the World Health Organization (WHO), and other countries.

David J. Sencer

David Sencer was a great judge of people. He recognized talent and integrity, could see through phonies, valued colleagues who communicated with clarity and brevity, didn't confuse science with opinion, and put the best interests of public health first above the parochial interests of their agency. He surrounded himself with talented leaders. There are far too many to name here, but David Sencer's appointees shared his values and operating style. They gave CDC a unique personality and esprit de corps. Under Dave's leadership, CDC grew into an agency with purpose, talent, integrity, compassion, courage, and responsiveness, and, when appropriate, an ability to laugh at itself and the ironies of public service and life's curve balls.

David Sencer was impressive at every stage of his career, from being a young TB investigator to distinction as a CDC director and commissioner of health of New York City. But he was just as impressive in retirement. He was great fun to be with. Whatever the topic, he would be well informed, have an opinion, challenge yours, add in personal

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recollections and current gossip, and generate a laugh. At a dinner or public function where side commentary improved the main event, he was the person who you wanted sitting next to you. His memory was truly extraordinary. He could effortlessly embroider discussions of public health events long past with details of conversations and biographical scoop on all the players while illuminating the scientific and political elements that made the events important. He read widely, mastered the Internet, blog sites, and search engines and easily accessed archival government documents that supported his recollection of events. His emails were always informative and often offered a lesson for the recipient.

While Dave remained fully engaged in contemporary public health, he was a lifelong supporter of succession planning, long before the term was invented. He was a master mentor who never stopped mentoring. Public health workers, junior and senior, governmental and academic, who never knew him in his top leadership roles, came under his wing during his retirement. He was a frequent visitor to CDC and Emory's Rollins School of Public Health (an institution in whose creation he played a key role) and loved attending epidemiology seminars and guest lectures. Dave remained an active public health professional throughout his life, serving as a reviewer for the Emerging Infectious Diseases journal and as active volunteer consultant on public health matters, most recently pandemic (H1N1) 2009 influenza.

Dave enjoyed visiting people in their labs and offices and learning firsthand what they did. He did this as CDC Director and at every stage of his career, including during his "retirement," when he would seek out students at Emory and young staff at CDC. Many young people at both institutions relished his visits and conversation and note that such an introduction often led to a long-term friendship and indeed an opportunity for Dave to mentor another promising newcomer to public health. Some topics were surefire icebreakers for Dave to initiate such conversations. They included an interest in TB or Chinese food, a connection to Michigan or New York City, and any item that would permit him to segue into a favorite anecdote.

A classic tale is his description, from early in his tenure as New York City Commissioner of Health, of his newfound responsibility for horses and their droppings in New York City. It is a tale worthy of the New Yorker and a most artful mix of public health and humor. It also illustrates his lifelong practice of getting advice from all quarters and addressing politics with a wise mix of pragmatism and principle.

On one of his first days occupying the office of Commissioner of Health, he was visited by the Assistant Commissioner for Public Affairs, who told his boss that he had to be at City Hall that afternoon for a bill signing. When asked what the bill was, he was told, it was "Public Health Law No. 1," the bill that requires the Commissioner to license carriage and riding horses in New York City.

Dave firmly responded, "I'm not going to do that. It's not a health problem."

His Assistant Commissioner replied, "Yes, you are."

A test of power, political reality, working relationships was unfolding as Dave tried to raise the stakes with, "I'll go discuss it with the Mayor."

"No, you won't," said the Assistant Commissioner.

And Dave recollected, "I didn't."

He had a canny ability to recognize the battles that needed fighting and those that didn't, when to take advice and from whom to take it, and when to see himself both as leader and as a team member.

David Sencer directed CDC's growth into the linchpin of the public health system in the United States and a global force for public health. In the former, he ensured that there were balanced relationships at the local, state, and federal levels and that local and state health departments and their officials were held in respect and treated as partners by CDC, their federal counterpart. He recognized the need for CDC to grow beyond the infectious diseases and supported productive, influential, and controversial programs in family planning (now reproductive health) and environmental hazards, opening the way for research of noncommunicable diseases at CDC. He supported WHO as a global counterpart to CDC, assigning experienced CDC staff to WHO leadership roles. His support for the smallpox eradication program makes him as important as anyone assigned to the field or in Geneva for its ultimate success. As Bill Foege described in his recent history of smallpox eradication in West Africa and India ("House on Fire: the Fight to Eradicate Smallpox"), "David Sencer… always found creative ways to provide the needed equipment, people and support. It is said that genius is seeing one's field as a whole. Sencer saw the public health world as a whole." In all these efforts he displayed vision, the courage to make the right decision in politically hazardous situations (whether the topic was family planning and reproductive health, interventions for infected commercial products, or threats of widespread influenza) and the leadership and management skills to ensure that the efforts he started continued and ultimately were successful.

David Sencer was such an integral part of the public health landscape that we all feel the loss of a colleague and friend. Whether we work in public health in health departments, at CDC, universities, foundations, or in industry, we are better off for his lifetime of contributions. Even more important, the people of the United States and the world are healthier because of David Sencer. He was a true giant.

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Fatal Infectious Diseases during Pandemic (H1N1) 2009 Outbreak

To the Editor: Nonpandemic infectious diseases occur with usual incidence during pandemics even though clinical attention is often on the pandemic pathogen. Many of these other infectious diseases share similar clinical signs and symptoms and are sometimes fatal. During the outbreak of pandemic (H1N1) 2009, tissue specimens from case-patients with undiagnosed fatal respiratory illnesses were submitted to the Infectious Diseases Pathology Branch at the Centers for Disease Control and Prevention (Atlanta, Georgia, USA) for evaluation for pandemic (H1N1) 2009 virus infection (*1*).

All respiratory tissue specimens from 450 case-patients received during April 29, 2009–May 5, 2010, were screened by the Centers for Disease Control and Prevention real-time reverse transcription PCR (rRT-PCR) protocol for detection and characterization of swine influenza virus (*2*). Of these, specimens from 250 (56%) tested negative for pandemic (H1N1) 2009 virus and had no other confirmatory or prior influenza testing. Of these case-patients whose specimens tested negative for pandemic (H1N1) 2009 virus, a total of 139 (56%) were male, and the median age was 30 years (range 8 days to 81 years). The median duration from onset of illness to death was 7 days (range 1–40 days). Of the 164 case-patients with available medical records, 127 (77%) had ≥1 underlying or preexisting medical condition.

When compared with case-patients during the same time who had pandemic (H1N1) 2009 virus infection confirmed by rRT-PCR, case-patients who were not infected with pandemic (H1N1) 2009 virus were more likely to be young (<9 years of age; odds ratio [OR] 2.23, 95% confidence interval [CI] 1.38–3.61) and less likely to be obese (OR 0.6, 95% CI 0.36–0.92) or have asthma (OR 0.33, 95% CI 0.16–0.68). Fever, cough, and shortness of breath were less frequently reported in the case-patients without pandemic (H1N1) 2009.

On the basis of the histopathologic features observed in the respiratory tissues of the case-patients who were not infected with pandemic (H1N1) 2009, along with their clinical and epidemiologic information, the specimens were further evaluated by using special histochemical stains, immunohistochemical tests, and molecular assays. At least 1 etiologic agent was identified in specimens from 69 (28%) of the 250 specimens (Table). Bacterial pathogens were identified for 44 case-patients; *Staphylococcus aureus* and *Streptococcus pneumoniae* were the most frequently identified. Immunohistochemical tests and PCRs found evidence of viral agents in samples from 26 case-patients. Most of these were seasonal or unsubtypeable influenza A viruses; in a smaller subset, other respiratory viruses were detected, including respiratory syncytial virus and adenovirus. Multiple fungal pathogens were detected in specimens from 2 case-patients.

For many of the diseases caused by the pathogens subsequently identified, the clinical features are predominantly respiratory, and many nonspecific manifestations are similar to those of influenza. Nonetheless, >50% of the patients in this study who died of suspected influenza had negative test results for pandemic (H1N1) 2009 virus, and for >25% of these, other infectious causes were detected. Infections other than influenza should be considered during a pandemic and during an endemic influenza season to facilitate the diagnosis of illness and treatment of patients with complications or severe respiratory

Table. Infectious agents identified in tissue samples from case-patients without pandemic (H1N1) 2009 virus infection, United States, 2009

Agent	No. case-patients*
Bacterial	44
Staphylococcus aureus	14
Streptococcus pneumoniae	14
Streptococcus pyogenes	4
Streptococcus viridans group	4
Leptospira spp.	2
Clostridium perfringens	1
Capnocytophaga canimorsus	1
Haemophilus influenzae	1
Legionella spp.	1
Neisseria meningitidis	1
Pseudomonas spp.	1
Rickettsia rickettsii	1
Streptococcus agalactiae	1
Viral	25
Influenza A, unsubtypeable†	10
Influenza A, seasonal (H1/H3)	6
Respiratory syncytial virus	3
Dengue virus	2
Enterovirus	2
Adenovirus	1
Human herpes virus 1	1
Fungal	2
Aspergillus spp.	2
Candida spp.	2

*For some case-patients, multiple agents were detected.
†Subtype not determined by 2 assays, including 1 specific for pandemic (H1N1) 2009 (*2*).

infections. Although we did not conduct a case–control study, these findings also support the results of other studies that previously reported the demographic characteristics of patients with pandemic influenza infections and the risk factors for severe or fatal pandemic influenza infections (*3*,*4*), especially with respect to obesity (*5*).

Evaluation of tissues collected during autopsy from patients with a suspected infectious process can provide an etiologic diagnosis that was not available from routine premortem and postmortem testing. Other etiologic agents detected in this study included reportable disease agents (e.g., *Rickettsia rickettsii*, *Legionella pneumophila,* dengue virus), vaccine-preventable diseases (e.g., pneumococcal, meningococcal diseases), and zoonotic agents (*Leptospira* and *Capnocytophaga* spp.). These findings underscore the need for autopsies for diagnosing fatal infectious diseases (*6*). They also confirm the need for coordinated surveillance programs that identify deaths potentially attributable to infectious causes, including the unexplained deaths program (*7*) and medical examiner infectious diseases death surveillance program (*8*). Partnerships of medical examiners and pathologists with local, state, and federal public health departments are crucial for detecting and monitoring pandemic diseases and for assessing the scope and magnitude of infectious agents that continuously affect human populations (*9*). These infections often result in sudden or unexplained death; thus, a standardized approach to death investigations is recommended.

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Epidemic Meningococcal Meningitis, Cameroon

To the Editor: In 2010, the city of Ngaoundéré in Cameroon experienced its first reported epidemic of meningococcal meningitis. Ngaoundéré, with an estimated population of 180,000, is the main city in the Adamaoua region in northern Cameroon. The 2 northernmost regions of Cameroon, North and Far North, are considered to belong to the African meningitis belt (*1*) and are periodically affected by meningococcal meningitis outbreaks. However, the Adamaoua region had been spared because of its altitude, latitude, and low population density in comparison with the North and Far North regions. Fewer than 10 sporadic cases have been reported in the Adamaoua region every year.

During February–April 2010, a total of 126 cases of meningitis (70 cases/100,000 inhabitants) were reported in the Adamaoua region. Of the 126 cases, 34 were confirmed by identification of *Neisseria meningitidis* serogroup A in cerebrospinal fluid (CSF) samples, 46 cases were apparent meningitis in which the patients had turbid CSF, and 46 were clinical cases diagnosed in an epidemic context. The male:female ratio of the patients was 2.7:1. The mean age of patients was 19 years, and median was 17 years.

CSF specimens from 34 patients were sent to the Centre Pasteur du Cameroun in Garoua for testing. Laboratory procedures included assessing CSF turbidity, Gram staining, searching for soluble capsular antigens by using the Pastorex latex agglutination kit (Bio-Rad, Hercules, CA, USA), and testing by the dipstick rapid diagnostic test for *N. meningitidis* serogroups A, C, W135, and Y (provided by the Centre de Recherche Médicale et Sanitaire, Niamey, Niger).

All 34 specimens were positive for serogroup A by agglutination, rapid test, or both. CSF specimens were cultured on blood agar and chocolate agar supplemented with PolyViteX (bioMérieux, Marcy-l'Etoile, France) and incubated at 37°C in an atmosphere of 5% CO_2. Susceptibility to antimicrobial drugs was tested according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (www.sfm.asso.fr). An isolate of *N. meningitidis* was sent to the World Health Organization Collaborating Centre for Reference and Research on Meningococci in Oslo, Norway, for molecular analyses, as described (www.neisseria.org). The result was that the isolate, a *N. meningitidis* serogroup A clone of sequence type 7, was susceptible to β-lactams and chloramphenicol but resistant to trimethoprim/sulfamethoxazole.

This epidemic occurred in an area where the mean annual rainfall for the past 30 years was 1,460 mm (Agency for Aerial Navigation Safety in Africa and Madagascar, unpub. data). This value should exclude Ngaoundéré from the African meningitis belt, for which the southern limit of annual rainfall was classically considered to be the 1,100-mm isohyet (Figure).

This epidemic at the border of the African meningitis belt raises the question of the belt limitation and its potential expansion southward. These topics should be addressed through active and standardized surveillance in countries such as Cameroon, which are not entirely included in the belt (*2*,*3*).

This meningitis epidemic has 2 other noteworthy characteristics. First, 80 (63%) of 126 suspected cases had a lumbar puncture performed at the Ngaoundéré Regional Hospital or at the Norwegian hospital. With the help of the laboratory, an increasing number of cases of meningitis in Cameroon are confirmed cases (*4*). Second, the etiologic agent was serogroup A meningococcus, a serogroup that had not been identified in north Cameroon since 2006 (*5*) but that had been isolated previously (*6*) and in south Cameroon (*7*).

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Figure. Northern regions of Cameroon with mean annual rainfall. Maroua is at the 800 mm isohyet line, Garoua at 1,006 mm, and Ngaoundéré at 1,460 mm. Estimate for Maroua is by the Agency for Aerial Navigation Safety in Africa and Madagascar; recorded rainfall for Garoua and Ngaoundéré are by the Agency for Aerial Navigation Safety in Africa and Madagascar. Eq. Guinea, Equatorial Guinea.

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Foodborne-associated *Shigella sonnei*, India, 2009 and 2010

To the Editor: Infection with *Shigella* spp. is a major cause of foodborne diseases, which have increased considerably during the past decades, but only a small fraction of cases are reported (*1*). *S. dysenteriae* and *S. flexneri* are the predominant species in the tropics; clinically, *S. dysenteriae* serotype 1 is associated with severe disease, large outbreaks, or epidemics. *S. sonnei* occurs more frequently in industrialized than in developing countries and causes milder illness than *S. dysenteriae* and *S. flexneri*. However, occasional foodborne outbreaks by antimicrobial drug–resistant *S. sonnei* have been reported from the United States, Japan, and European countries, mostly among children (*2–5*). During recent years, in Thailand, Vietnam, and Sri Lanka, the predominant species has shifted from *S. flexneri* to *S. sonnei*, a phenomenon possibly linked with country's level of development (*6*,*7*). As a result, *S. sonnei* outbreaks are also being reported from developing countries (*8*). In India, the scenario differed somewhat. Devastating outbreaks of dysentery by multidrug-resistant *S. dysenteriae* type 1, with high case-fatality rates, affected major parts of the country during 1984–1985 (*9*). After a gap of 18 years, during 2002–2003, *S. dysenteriae* type 1 with an altered antimicrobial drug resistance pattern (100% fluoroquinolone resistance) reemerged, causing several dysentery outbreaks in West Bengal (*10*). Although *S. flexneri* was the major species, since 2005, *S. dysenteriae* type 1 has not been isolated (*9*).

We report 2 foodborne outbreaks of *S. sonnei* in India, 1 each from Kerala (southern part) in February 2009 and Maharashtra (western part) in February 2010, which support extension of *S. sonnei* into India. The outbreak isolates were characterized by antimicrobial drug resistance and plasmid and pulsed-field gel electrophoresis profiles.

On February 1, 2009, >300 persons (age range 2–70 years) attended a marriage party at Thiruvananthapuram, Kerala, where they were served local food made of rice, lentils, milk, and water. Within 12 hours after eating, ≈60% of persons had onset of acute diarrhea, vomiting, and abdominal pain and were admitted to local village or district hospitals or the nearest government general hospital for treatment. Illness was more severe in children; because of clinical severity, 10 children (<10 years of age) were admitted to a referral hospital for children in Thiruvananthapuram. One child (7 years of age) was moved to the pediatric intensive care unit because of altered sensorium and drowsiness and was treated with intravenous ceftriaxone and metronidazole. Others were treated with oral cefotaxime until recovery and were discharged. Fecal samples from 15 patients were processed at the local public health laboratory for enteric pathogens; 9 (60%) of 15 samples yielded *S. sonnei* as the sole pathogen. On microscopic examination, 12 (80%) samples had erythrocytes.

The second outbreak occurred on February 11, 2010, at Kolhapur, Maharashtra, among day laborers and their family members who had eaten in 1 madrasa (religious place). Approximately 150 persons reported diarrhea, vomiting, abdominal pain, and fever. They were admitted to the Government Medical College, Kolhapur, and treated with intravenous fluid (lactated Ringer's solution), oral rehydration solution, intravenous ceftriaxone, and metronidazole. All patients were discharged after complete recovery. *S. sonnei* was

isolated as the sole pathogen from 14 (70%) of 20 fecal samples or rectal swab samples from those patients.

Six isolates from outbreak 1 and 11 isolates from outbreak 2 were sent to the National Institute of Cholera and Enteric Diseases (Kolkata, India) for confirmation. We characterized those isolates to determine whether the outbreak isolates of *S. sonnei* predominant in India were clonal in origin.

Antimicrobial drug resistance profiles differed in the 2 outbreaks (Figure) when drug susceptibility was tested by disk diffusion. MICs of antimicrobial drugs by Etest (AB Biodisk, Solna, Sweden) were >32 μg/mL for tetracycline and co-trimoxazole, >256 μg/mL for nalidixic acid, and ≈4 μg/mL for norfloxacin and ciprofloxacin. Plasmid profiles of the isolates showed absence of large plasmids (212 kb) and several smaller plasmids arranged in distinct patterns in each group (data not shown). Because the isolates caused invasive diarrhea (erythrocytes in feces), large plasmids might have been lost through repeated subculture. DNA fingerprinting was performed by pulsed-field gel electrophoresis in a CHEF-DRIII system (Bio-Rad Laboratories, Hercules, CA, USA) after DNA digestion by *Xba*I following standard PulseNet protocol and by using *Salmonella enterica* serovar Braenderup H9812 as control strain. A few sporadic *S. sonnei* isolates from patients of the Infectious Disease Hospital, Kolkata, were included for comparison. The patterns were analyzed by using Dice coefficient, and a dendrogram was generated by using FP Quest Software (Bio-Rad). The isolates with ≈90% similarity threshold were grouped under 1 cluster. Distinct clusters were observed for outbreak 1 (cluster A), outbreak 2 (cluster B), and sporadic 2009 (cluster C) isolates, and patterns in each cluster differed by only a few (*1*,*2*) smaller bands.

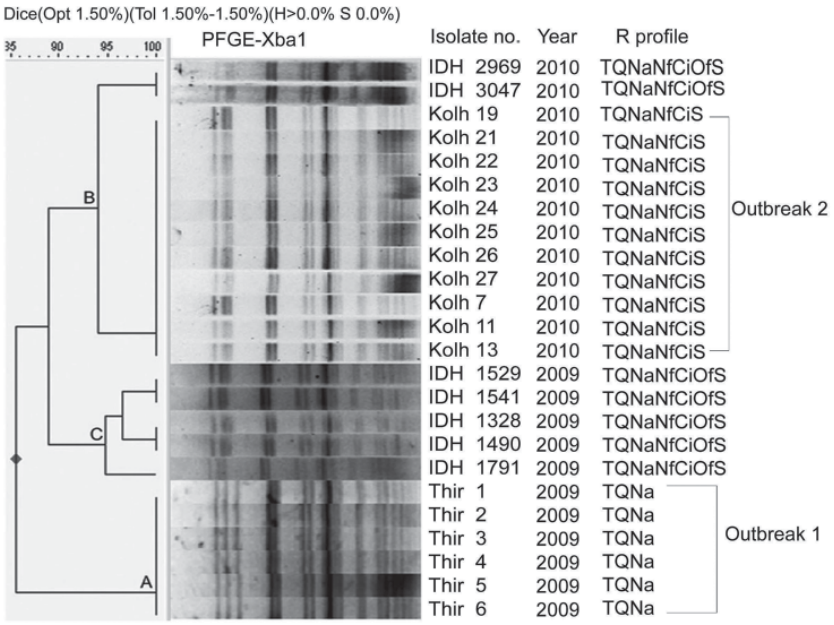

Figure. Digested pulsed-field gel electrophoresis (PFGE) profiles of *Shigella sonnei* outbreak isolates, India (Thiruvananthapuram, Kerala; Kolhapur, Maharashtra), by cluster analysis and comparison with sporadic isolates (IDH). Thir, isolates from Thiruvananthapuram, Kerala; Kolh, isolates from Ispurli, Shiroli Taluk, Kolhapur district, Maharashtra; IDH, isolates from Kolkata, West Bengal; R, resistance; T, tetracycline (30 μg); Q, co-trimoxazole (25 μg); Na, nalidixic acid (30 μg); Nf, norfloxacin (10 μg); Ci, ciprofloxacin (5 μg); Of, ofloxacin (5 μg); S, streptomycin (10 μg).

Therefore, our study supports emergence of *S. sonnei* outbreak clones in India during 2009–2010. These outbreaks may be the forerunners of many more *S. sonnei* outbreaks.

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Mosquito-associated Dengue Virus, Key West, Florida, USA, 2010

To the Editor: Except for sporadic cases along the Texas–Mexico border, local transmission of dengue virus (DENV) has not occurred in the contiguous United States since 1946. In 2009, DENV was diagnosed in a vacationer to Key West, Florida (*1*). Subsequently, 25 other cases were reported that year, transmission was confirmed by detection of DENV serotype 1 (DENV-1) in local mosquitoes, and a random serosurvey showed evidence of recent DENV infection in 5.4% of Key West residents (*1*). Transmission continued in 2010, and an additional 63 cases were confirmed (*2*). We used PCR amplification and sequence analysis of virus identified from mosquito collections during 2010 to identify the closest relatives, probable geographic origin, and divergence time of the Key West DENV.

A total of 1,178 pools of *Aedes aegypti* mosquitoes were collected in Monroe County, Florida, during January 27–December 17, 2010 (online Appendix Figure, panel A, wwwnc.cdc.gov/EID/article/17/11/11-0419-FA1.htm), by using BG-Sentinel (Biogents, Regensburg, Germany) or CDC (Clarke, Roselle, IL, USA) light traps, and stored at −80°C. Reverse transcription PCR was conducted on each pool by using primers designed to amplify all 4 DENV serotypes, followed by seminested PCR with serotype-specific primers (*3*). Results from 2 Key West mosquito pools collected on June 25 and 30 showed a positive first-round reverse transcription PCR and a positive second-round PCR specific for DENV-1. A third Key West pool collected on August 27 showed only a positive second-round PCR specific for DENV-1. No other DENV serotypes were detected.

DENV-1–specific primers (5′-GGGCCTTGAGACACCCAGG-3′ and 5′-CCTCCCATGCCTTCCCAATGGC-3′) were used to amplify products encompassing the envelope (E) gene region and parts of the premembrane and nonstructural 1 genes from the pools collected on June 25 and 30. PCR products were sequenced by using amplification and internal primers to provide double or triple coverage (Functional Biosciences, Madison, WI, USA). The sequences from these 2 pools were identical (GenBank accession no. JF519855). We used ClustalX (*4*) to align the Key West sequences with 175 nonredundant American DENV-1 sequences from the National Center for Biotechnology Information Virus Variation database (*5*) and 9 additional DENV-1 subgenomic E sequences from GenBank, which provided a comprehensive set of American DENV-1 sequences, including several isolates from Hawaii (USA) and Easter Island (Chile) that grouped with Asian DENV-1 clades as an outgroup. Maximum-likelihood phylogenetic analysis of the 1,484-nt E gene region was conducted by using SeaView software (*6*).

The analysis showed that American DENV-1 strains clustered by geography and by year of collection (online Appendix Figure, panel B). This clustering might be the result of lineage replacement that has been described in DENV-1 (*7*). Additionally, clustering might be influenced by serotype prevalence or sampling bias. For example, the sequence database contains few Caribbean isolates from after 2000 and few Central or South American isolates from before 2000. The Key West sequence grouped as a member of a large clade of recent viruses from Central America that was separated from Caribbean and South American viruses with a well-supported bootstrap value (86%) and relatively long branch length. The closest relatives were 2 strains isolated

in Nicaragua in 2006 and 2008. The bootstrap support for this grouping was 100%. Phylogenetic analyses with neighbor-joining, maximum-parsimony, and Bayesian methods gave trees with similar topologies, including clear separation of most recent Central American isolates into 1 clade, as well as grouping of the Key West sequence with the same 2 isolates from Nicaragua (data not shown). No protein coding changes between these strains were identified, which suggests purifying selection for an optimum phenotype. There were 8 synonymous differences over the 1,708-nt amplified region between the Key West and Nicaragua 2008 sequences. Previous molecular clock determinations for DENV-1 provided a range of 2.5–7.0 × 10^{-4} substitutions per nucleotide per year (*8*). This calculation produced an estimate of a 6.7–18.7-year divergence time between the Key West virus and the most closely related Nicaragua strain. When during this time the ancestor of Key West DENV was introduced to Florida is unknown.

Analysis of the entire Key West DENV-1 genome may help pinpoint the origin and address the possibility of selective pressure on other genes or recombination events (*9*). Given the recent reports of DENV in residents of other Florida counties who had no travel histories (*2*), monitoring of Key West and other nearby urban areas for evidence of local DENV transmission should continue.

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Mycobacterium doricum Osteomyelitis and Soft Tissue Infection

To the Editor: Infections with nontuberculous mycobacteria (NTM) are being increasingly identified. Several factors may contribute to this finding, including increased awareness of these organisms as pathogens, improved ability of laboratories to isolate and identify these organisms, and increasing prevalence (*1*). We describe a case of osteomyelitis and soft tissue infection with *Mycobacterium doricum* after trauma in a previously healthy adult.

A 21-year-old man sustained an open right femur fracture with gross contamination of the wound with dirt and gravel. The wound was irrigated and debrided, the fracture was fixed by intramedullary nailing, and the wound was closed.

Sixteen weeks later, pain, swelling, and erythema developed in the right thigh of the patient.

Radiographs showed incomplete union of the fracture and possible loosening of the nailing hardware. Repeat irrigation and debridement of the right thigh was performed. Thirty milliliters of purulent material was drained. Bone was not visible, and the hardware was not removed or replaced. Gram staining of the purulent material showed 1 polymorphonuclear cell per oil-immersion microscopic field and no bacteria. Routine bacterial cultures were negative. Results of acid-fast staining were also negative. Three operative specimens were tested for acid-fast organisms. The patient was discharged and prescribed a 6-week course of vancomycin, 750 mg intravenously every 8 hours, and ciprofloxacin, 500 mg orally 2×/d.

Four weeks later, all 3 operative cultures grew an acid-fast bacillus, which was identified by DNA sequencing and high-performance liquid chromatography as *M. doricum* (Centers for Disease Control and Prevention, Atlanta, GA, USA). While in vitro susceptibility testing results were pending (*2*), recurrent swelling, erythema, and warmth developed in the patient at the previous injury site. A computed tomography scan showed multiple small abscesses adjacent to the nonunited fracture, an irregular periosteal reaction around the fracture site, and lucency surrounding the medullary rod, suggestive of osteomyelitis (online Appendix Figure, wwwnc.cdc.gov/EID/article/17/11/11-0460-FA1.htm).

The patient was treated with irrigation and debridement of the abscesses and exchange of hardware. Two specimens were tested for acid-fast culture, 1 of which grew *M. doricum* after 3 weeks of incubation. The patient was discharged and empirically treated with amikacin, 1,250 mg/d intravenously for 3 weeks, and levofloxacin, 750 mg/d orally for 3 months, as therapy for infection with *M. doricum*, pending susceptibility testing results.

In vitro susceptibility results showed susceptibility to all drugs tested: MIC <1 μg/mL for amikacin, 0.25 μg/mL for ciprofloxacin, 4 μg/mL for clarithromycin, 2 μg/mL for ethambutol, <0.12 μg/mL for rifampin, <0.25 μg/mL for rifabutin, <0.5 μg/mL for streptomycin, and <0.12/2.4 μg/mL for trimethoprim/sulfamethoxazole (Associated Regional and University Pathologists, Salt Lake City, UT, USA). The antimicrobial drug regimen was changed to trimethoprim/sulfamethoxazole (800 mg of trimethoprim and 400 mg of sulfamethoxazole) orally 2×/d, and doxycycline, 100 mg orally 2×/d. After 10 months of therapy, the patient stopped taking these antimicrobial drugs. Six weeks after discontinuation of therapy, he had no signs or symptoms of recurrent infection.

M. doricum was first identified in a cerebrospinal fluid sample from a 50-year-old man with AIDS (CD4+ lymphocyte count 28 cells/mm^3). *Cryptococcus neoformans* was also isolated from the same specimen. This patient was treated for *C. neoformans* infection with amphotericin B and 5-fluorocytosine but died 6 weeks later, before isolation of *M. doricum*. The isolate was susceptible to all antimicrobial drugs tested in vitro, including amikacin, azithromycin, clarithromycin, ciprofloxacin, clofazimine, ethambutol, isoniazid, ofloxacin, rifabutin, rifampin, sparfloxacin, and streptomycin (*3*).

The American Thoracic Society and Infectious Diseases Society of America have published guidelines on the diagnosis, treatment, and prevention of NTM diseases (*1*). However, there are no recommendations for treatment of *M. doricum* infection. At least 2 antimicrobial drugs are recommended for treating infections with NTM, and surgery is indicated for extensive disease, abscess formation, or contraindications/intolerance to medical therapy. When possible, it is recommended that foreign bodies be removed. For skin and soft tissue infections, ≥4 months of therapy is recommended, with extension to 6–12 months for cases of bone involvement (*1*). In this instance, trimethoprim/sulfamethoxazole and doxycycline were chosen because of availability of oral formulations, in vitro susceptibility testing results, and affordability given the patient's lack of medical insurance.

Disease in the patient was eradicated by surgical debridement and prolonged antimicrobial drug therapy. We speculate that the organism was introduced from the soil at the time of the open fracture. Given increased awareness of NTM as pathogens and improvement in the ability of laboratories to isolate and identify these organisms from clinical specimens, this *Mycobacterium* species might be increasingly identified as a cause of disease. Additional reports of treating disease caused by *M. doricum* will be valuable so that in vitro susceptibility testing can be correlated with clinical responses, eventually enabling development of guidelines for therapy.

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Disseminated *Mycobacterium abscessus* Infection and Showerheads, Taiwan

To the Editor: Diseases caused by nontuberculous mycobacteria (NTM) in patients with Sjögren syndrome have rarely been reported (*1,2*). In addition, showerheads in residential bathrooms as a source of *Mycobacterium abscessus*–induced disseminated disease have never been reported (*3–5*).

A 65-year-old woman with Sjögren syndrome sought treatment at National University Taiwan hospital with fever (38.6°C) and a 3-month history of lymphadenopathy over the left neck, left submandibular, and bilateral inguinal areas. Active Sjögren syndrome with lymphadenitis was considered because of progressive hypergammaglobulinemia (IgG 3,030 mg/dL, reference range 700–1,600 mg/dL) and high titers of anti–Sjögren syndrome (SS) A (561 AU/mL) and anti-SSB antibodies (220 AU/mL; positive >120 AU/mL). A chest radiograph obtained 1 month before admission showed no active lung lesions; however, cultures of 3 samples of sputum all yielded *M. abscessus* bacteria (isolate A). Pathologic examination of excised lymph nodes of the bilateral inguinal area showed reactive lymphoid proliferation and granulomatous inflammation with multinucleate giant cell formation, suggestive of mycobacterial disease; however, there was no evidence of caseating necrosis or acid-fast bacilli. ELISA results were negative for antibodies to HIV-1, HIV-2, HTLV-1, and HTLV-2.

Parenteral antimicrobial drugs (imipenem, 500 mg every 8 h) and amikacin (250 mg 2×/d) along with oral clarithromycin (500 mg 2×/d) were administered. Fever subsided 3 days after lymph node excision with concomitant administration of antimycobacterial agents. The patient was treated successfully with intravenous antimicrobial drugs for a total of 14 days, followed by oral clarithromycin (500 mg 2×/d) and doxycycline (100 mg 2×/d) therapy for 4 months. Follow-up blood cultures 10 weeks after initiation of antimycobacterial agents were negative for the organism. *M. abscessus* bacteria grew on cultures of the excised lymph nodes (isolate B) and 2 sets of blood cultures (isolate C).

A total of 6 swab specimens taken from the interior surface of the showerheads from the 6 bathrooms of the patient's 2 houses (3 in each house), 1 in Taichung (central Taiwan) and the other in Taipei (northern Taiwan), and 6 shower water samples of the 6 bathrooms were submitted for mycobacterial cultures. Four of the 6 swab samples (isolates D–G), 2 (isolates D and E) from Taichung and 2 (isolates F and G) from Taipei, grew *M. abscessus* bacteria. Cultures of shower water from the 6 bathrooms were all negative for the organism. These isolates were identified as *M. abscessus* by conventional biochemical methods and confirmed by 16S rRNA gene sequencing analysis and a PCR–restriction fragment length polymorphism–based method targeting a 439-bp fragment of the 65-kDa HSP gene as previously described (*6,7*). Random amplified polymorphic DNA patterns of these isolates (isolates A–G) as determined by means of arbitrarily primed PCR using 3 different random primers were identical (i.e., they shared every band) (Figure). Three unrelated isolates of *M. abscessus* recovered from cutaneous lesions of 3 patients who were treated at the same hospital in

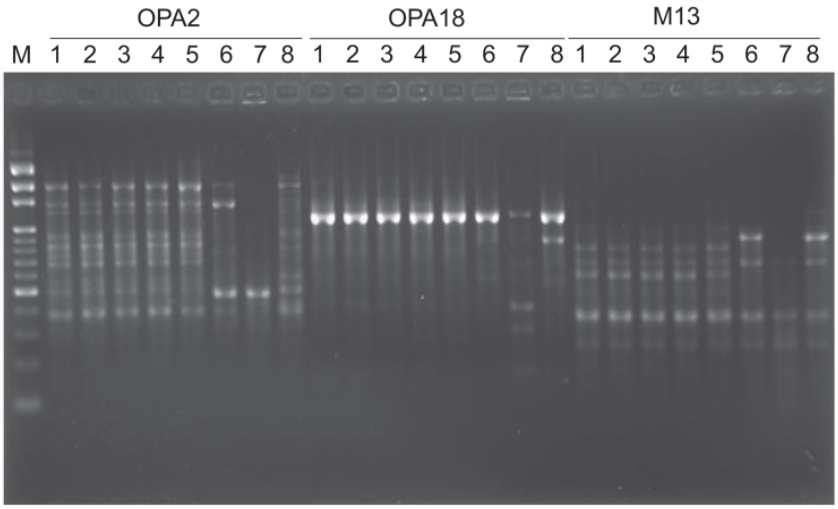

Figure. Random amplified polymorphic DNA patterns of 8 isolates of *Mycobacterium abscessus* generated by arbitrarily primed PCR with the primers OPA2, OPA18, and M13 (Operon Technologies, Inc., Alameda, CA, USA). Lanes: M, molecular size marker (1-kb ladder; Gibco BRL, Gaithersburg,MD, USA); 1, isolate A; 2, isolate B; 3, isolate C; 4, isolate D; 5, isolate F; 6–8, three unrelated isolates of *M. abscessus* recovered from cutaneous lesions of 3 patients who were treated at National Taiwan University hospital in 2010 (see text for designation of isolates).

2010 had distinct random amplified patterns that differed from those generated from isolates A–G (Figure).

A previous study in Taiwan showed that the incidence (no. cases/100,000 inpatients and outpatients) of all pulmonary disease caused by NTM increased significantly from 2.7 (1.26) in 2000 to 10.2 (7.94) in 2008 (*6*). The most common organism in localized pulmonary infection and disseminated infection was *Mycobacteriam avium* cellular complex, and *M. abscessus* predominated in skin and soft tissue infection and lymphadenitis (*6,8*). The rise in pulmonary infections or colonization by NTM over recent decades, particularly among immunocompromised populations, is reported to be partly associated with the increased use of showers (*3–5,9*). Recently, a few studies have shown a link between pulmonary *M. avium* complex infections and home showerhead water microbiology (*3,4*). Although pulmonary disease caused by *M. abscessus* did not develop in the patient reported here, multiple respiratory specimens showed evidence of pulmonary colonization. The fact that cultures of the swabs taken from the interior surface of 4 showerheads were positive for *M. abscessus* but that cultures of the shower water were negative for the organism support previous findings that assemblages of NTM can occur inside biofilm that forms on the interior surface of showerheads (*5*). The same strains of *M. abscessus* isolated from different showerheads suggested the possibility of contamination in the environment by the aerosolized microorganism from respiratory secretions of the patient.

The mechanisms of susceptibility to mycobacterial infection in the patient with Sjögren's syndrome remain unknown (*1,2*). Previous studies suggest that toll-like receptor 2, dectin-1, tumor necrosis factor–α, interferon-γ, leptin, T-cells, and possibly neutrophils are major components in the host defense of HIV-noninfected patients against rapidly growing mycobacterial infections, including those caused by *M. abscessus* (*10*).

In summary, we report a case of bacteremic lymphadenitis caused by *M. abscessus* in a patient with Sjögren syndrome. Our data provide evidence that the interior surface of showerheads may serve as a source of infection by this waterborne and aerosolized microorganism.

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Antimicrobial Drug Resistance in *Corynebacterium diphtheriae mitis*

To the Editor: *Corynebacterium diphtheriae* is the agent of pharyngeal and cutaneous diphtheria. We did a retrospective analysis of the antimicrobial drug susceptibilities of 46 *C. diphtheriae* isolates sent during 1993 through 2010 to the French National Reference Centre of Toxigenic Corynebacteria. The isolates came from metropolitan France and French overseas departments and territories. Only 1 isolate, *C. diphtheriae* biovar *mitis*,

FRC24, expressed the following antimicrobial drug susceptibility profile: susceptible to penicillin, amoxicillin, ciprofloxacin, clindamycin, erythromycin, gentamicin, imipenem, kanamycin, rifampin, tetracycline, and vancomycin and resistant at an uncommonly high level to trimethoprim, sulfamethoxazole, and co-trimoxazole with Etest (bioMérieux, Marcy l'Etoile, France) MICs of >32, >1,024, and >32 mg/L, respectively.

This FRC24 isolate was isolated in 2008 from a cutaneous wound on a vaccinated 11-month-old child in Mayotte, an overseas department located in the Indian Ocean. Cutaneous carriage of *C. diphtheriae* is frequent in tropical countries where cutaneous diphtheria is endemic; cutaneous carriage represents a common mode of transmission of the bacterium. FRC24 was identified by using the API Coryne strip (bioMérieux). FRC24 is a toxigenic isolate; toxigenicity was confirmed by both *tox* gene detection and Elek test (*1*). Multilocus sequence typing was performed, and the sequence type (ST) of the isolate is ST91. This ST contains only this isolate and is part of lineage II, as are all *mitis* and *gravis* biovars (*2*).

To date, resistance to trimethoprim, sulfamethoxazole, or co-trimoxazole seems to be rare among the *C. diphtheriae* species, but few data are available (*3*). As trimethoprim resistance is often encoded by integron-driven *dfr* determinants, we looked for integrons. Integrons are bacterial genetic elements able to capture and express antimicrobial drug resistance gene cassettes (GCs) (*4*). GC movements are catalyzed by an integron-encoded integrase IntI. GCs, mainly promoterless, are usually expressed through a common Pc promoter (*5*). Only rare GCs contain their own promoter (*cmlA, qac, ereA1*). Three main classes of integrons have been described and are involved in the dissemination of antimicrobial drug resistance; class 1 is the most widely found in clinical isolates. Integrons have been mainly described among gram-negative bacteria; only a few studies have reported integrons in *Corynebacterium* spp. (*6,7*).

After bacterial genomic DNA extraction (DNeasy Blood & Tissue Kit; QIAGEN, Courtaboeuf, France), a multiplex Taqman-based quantitative PCR approach able to detect the 3 main classes of integrons was performed (*8*). We found that FRC24 harbored a class 1 integron. Analysis of the GC array showed that this integron harbored 2 GCs: *dfrA16* of 588 bp conferring resistance to trimethoprim and *qacH* of 511 bp conferring resistance to quaternary ammonium compounds (GenBank accession no. FR822749). To our knowledge, this GC array has not been previously reported, even among reports of other gram-negative isolates. Moreover, a *qac* determinant has been found only once in a *Corynebacterium* species, *C. pseudogenitalium* (which harbors a *qacH* variant in the chromosome [GenBank accession no. ABYQ02000013]), but not in an integron background. GC arrays were followed by the *qacEΔ1* (which also confers resistance to quaternary ammonium compounds), *sul1* (resistance to sulfamethoxazole), and *orf5* determinants as found in most class 1 integrons (*4*). In class 1 integrons, 13 Pc variants have been described (*5*). In the FRC24 integron, the *dfrA16* expression was mediated through a strong Pc variant (PcW_{TGN-10}) (*5*) that enables the high-level resistance observed for trimethoprim. As previously demonstrated, the *qacH* GC possessed its own promoter (*9*).

Trimethoprim is a commonly prescribed antimicrobial agent used in combination with sulfamethoxazole (co-trimoxazole) for the treatment of diarrheal diseases. This antimicrobial drug might have selected the emergence of such a strain expressing trimethoprim resistance. Furthermore, the FRC24 integron contains the antiseptic (quaternary ammonium compounds) resistance gene *qacH*. As cutaneous carriage of *C. diphtheriae* is frequent in tropical countries such as Mayotte, this bacterium could be exposed to quaternary ammonium compounds contained in disinfectants, hygienic hand washes, and cosmetic products. These products exert a selective pressure, which might play a role in selecting *qac*-containing strains, as has been suggested for *Staphylococcus* spp. (*10*). For staphylococci, the MICs of quaternary ammonium compounds are ≥2 mg/L. With FRC24, we tested for the MIC of cetyltrimethylammmonium bromide and found a MIC of 4 mg/L, suggesting that *qacH* is expressed in FRC24.

The sequencing of the genetic environment of this integron showed that it was framed by 2 copies of the insertion sequence IS*6100* disrupting at the left-hand side the *intI1* integrase gene (online Appendix Figure, wwwnc.cdc.gov/EID/article/17/11/11-0282-FA1.htm). IS*6100* has been described in a wide spectrum of host organisms, including *Corynebacterium* spp. (*6,7*), thus enabling this integron to be efficiently transferred to various bacteria.

Our findings show that *C. diphtheriae* is able to harbor integrons, which is of clinical relevance. Indeed, this genetic feature would give the isolates the capacity to easily acquire new GCs, such as *ere* GCs encoding resistance to erythromycin, which is one of the antimicrobial drugs recommended for diphtheria treatment.

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Transfusion-transmitted Syphilis in Teaching Hospital, Ghana

To the Editor: Transfusion-transmitted syphilis, which is caused by *Treponema pallidum* subspecies *pallidum*, is one of the oldest recognized infectious risks of blood transfusion (*1*). Routine screening of blood donors and refrigeration of donated blood before its use has resulted in only 3 reported cases of transfusion-transmitted syphilis over the past 4 decades (*2*–*6*).

The World Health Organization recommends screening all donated blood for syphilis (*7*), but doing so is challenging for many developing countries. Many blood banks in low-income countries, including Komfo Anokye Teaching Hospital in Kumasi, Ghana, do not screen donated blood for syphilis.

This study was conducted at Komfo Anokye Teaching Hospital. The purpose of this study was to determine the prevalence of syphilis among blood donors and whether seroconversion occurred in transfusion recipients. The study was approved by the ethics committees in Kumasi, Ghana, and Liverpool, UK.

Pretransfusion plasma samples from 200 conscious transfusion recipients in adult, pediatric, and obstetric inpatient departments and samples of their transfused blood were tested for syphilis. A positive initial result by enzyme immunoassay (EIA) (Bioelisa Syphilis 3.0; Biokit, Barcelona, Spain) was confirmed by using a *T. pallidum* hemagglutination assay (TPHA) (Syphagen; Biokit). A rapid plasma reagin (RPR) assay (RPR Reditest; Biokit) was used to determine whether seropositivity was caused by recent infection. Seronegative recipients who had received seropositive blood were retested 30 days posttransfusion to identify seroconversions. All donors and recipients with recent infections were offered counseling and treatment in accordance with national guidelines.

A total of 145 (73%) blood donors were male, and 109 (57%) units of blood had been stored for <4 days. Sixteen units (8%, 95% confidence interval [CI] 4.3%–11.7%) were seropositive for syphilis by EIA and TPHA. Of these units, 7 (44%) were RPR reactive, which indicated a prevalence of recent infections of 3.5% (95% CI 1.0%–6.0%) (Table). Twenty-six transfusion recipients (13%; 95% CI 8.3%–17.7%) were seropositive by EIA and TPHA. Of these recipients, blood samples from 9 (35%) were RPR reactive, indicating a prevalence of recent infection of 4.5%.

One recipient, an 8-year-old girl with severe malarial anemia (recipient 10), showed seroconversion after receiving an RPR-reactive unit of blood that had been refrigerated for only 1 day before being issued for

Table. Characteristics of 16 recipients of syphilis-positive blood transfusions, Kumasi, Ghana*

Recipient ID	RPR results for transfused blood	Duration of blood storage, d	Blood sample test results						Outcome
			Pretransfusion			Posttransfusion			
			EIA	TPHA	RPR	EIA	TPHA	RPR	
1	R	12	–	ND	ND	NA	NA	NA	Died
2	NR	2	–	ND	ND	NA	NA	NA	Died
3	NR	2	–	ND	ND	NA	NA	NA	Died
4	NR	1	–	ND	ND	NA	NA	NA	Died
5	R	4	–	ND	ND	NA	NA	NA	Lost to follow up
6	NR	1	–	ND	ND	NA	NA	NA	Lost to follow up
7	NR	2	+	+	NR	NA	NA	NA	Not followed up
8	NR	6	+	+	R	NA	NA	NA	Not followed up
9	NR	3	–	ND	ND	–	ND	ND	Well
10	R	1	–	ND	ND	+	+	R	Seroconverted
11	NR	2	–	ND	ND	–	ND	ND	Well
12	R	1	–	ND	ND	–	ND	ND	Well
13	R	3	–	ND	ND	+	–	NR	Well
14	NR	2	–	ND	ND	–	ND	ND	Well
15	R	1	–	ND	ND	–	ND	ND	Well
16	R	4	–	ND	ND	–	ND	ND	Well

*ID, identification; RPR, rapid plasma reagin; EIA, enzyme immunoassay; TPHA, *Treponema pallidum* hemagglutination assay; R, reactive; –, negative; ND, not done; NA, not available; NR, not reactive; +, positive. All results for transfused blood tested by EIA and TPHA were positive.

use. Posttransfusion fever developed in this recipient, who responded to treatment with cefuroxime and gentamicin, although results of blood culture for bacteremia and peripheral blood film for malaria parasites were negative. She had no relevant sexual history, had been febrile after the transfusion, and showed no evidence of mucocutaneous lesions or lymphadenopathy at her follow-up visit 1 month after the transfusion. She was referred to pediatricians for treatment of syphilis.

This recipient who showed seroconversion most likely had a case of transfusion-transmitted syphilis. Other treponemal infections such as yaws cannot be differentiated serologically from syphilis, and a diagnosis of yaws is based on clinico-epidemiologic features (*8*); however, yaws is not endemic to Kumasi, and because this child had no clinical evidence of yaws, this disease is unlikely to be the cause of the seroconversion.

Refrigeration of units of blood for ≥5 days kills *T. pallidum*, but 57% of the donated blood in this study was stored for <4 days before use. This situation prevails across many blood banks in sub-Saharan Africa where, because of inadequate supply and high demand, blood is used as soon as it becomes available. Such short periods of blood storage do not provide an adequate margin of safety against transfusion-transmitted syphilis. Findings from this study have been discussed with the hospital transfusion committee, and new syphilis screening guidelines and testing algorithms are being developed.

The high prevalence of syphilis seropositivity in blood donors and seroconversion of a transfusion recipient shows that in centers where screening is not conducted, recipients of blood transfusions are at risk for contracting transfusion-transmitted syphilis. This finding is likely in blood banks that have a high demand for blood and where blood is stored only for a few days. This study highlights transfusion-transmitted syphilis as a serious public health issue in developing countries and demonstrates that screening of donor blood for syphilis should be conducted.

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Congenital Syphilis, Réunion Island, 2010

To the Editor: Syphilis, caused by the bacterium *Treponema pallidum*, is primarily a sexually transmitted infection, but *T. pallidum* can also be transmitted by infected pregnant women to their children. Every year, at least 500,000 children are born with congenital syphilis (CS); maternal syphilis causes another half million stillbirths and abortions, usually in countries with limited resources (*1*). However, CS has been recently found in industrialized countries such as the United States, where the CS rate increased by 23% during 2005–2008, after a 38% increase in the syphilis rate among US women and girls during an earlier period (2004–2007) (*2*).

Réunion Island, a French overseas territory with 810,000 inhabitants, has a health care system similar to that in continental France. Neither syphilis infection, CS, nor other trepanomatosis (yaws) is notifiable. Since 2006, an increase in early syphilis was documented, first in men who have sex with men infected with HIV and second in the general population.

In 2009, we conducted a retrospective study by using data from 2004–2009 to document the situation of CS on the island. Data from all public (n = 4) and private (n = 2) hospitals on the island with neonatology and obstetrical departments were investigated. Birth deliveries at home were not included. Inclusion criteria were positive specific (*T. pallidum* hemagglutination assay) and nonspecific (Venereal Disease Research Laboratory [VDRL]) test results for *Treponema* spp. among children <2 years of age during 2004–2009. Additionally, hospitalized children coded as having congenital syphilis (International Classification of Diseases [ICD] 10 codes A50.0 to A50.9) in the French national hospital database were included. After reviewing medical files of mothers and their children, cases were classified as confirmed or probable CS according to the case definition of the Centers for Disease Control and Prevention (*2*).

Eighteen children had positive syphilis serologic results by *T. pallidum* hemagglutination assay and VDRL tests, according to the selection criteria. Among these 18 test results, 7 were classified as probable CS (late treatment for mother or symptoms linked to CS), 3 in 2008 and 4 in 2009 (Table). The male:female sex ratio was 0.75. Five case-patients were preterm newborns; 3 of the most premature babies had signs linked to CS, such as hepatosplenomegaly, cutaneous mucosal signs, neurologic signs, radiographic signs of CS in long bones, edema, and biologic anomalies. All were screened for *T. pallidum*–specific IgM by using fluorescent treponemal antibody absorption or IgM capture ELISA from immediately after birth to 15 days old. Two case-patients had positive results; 1 was symptomatic. Six of the 7 children who had probable CS received appropriate penicillin G treatment, except for 1 asymptomatic baby for whom long-term medical supervision was recommended by the pediatrician. Survival rates at 3 months of age reached 100%.

Median age of mothers at delivery was 22 years. All mothers were natives of Réunion Island except 1 who was born in Madagascar and received no antenatal follow-up. Medical history indicated previous genital herpes for 3 women. Social difficulties or alcohol consumption were reported for 3 women. The mean age of gestation at which the first syphilis screening was conducted was 23 weeks (5–33 weeks). Two mothers were symptomatic. Syphilis was diagnosed after delivery for 3 mothers; seroconversion occurred during the pregnancy. Except for missing data on 1 mother, all mothers were HIV negative.

In Réunion Island, in our retrospective review, we found 7 CS cases during 2008–2009 but none during 2004–2007. The incidence rate of probable CS cases was estimated to be 28 cases per 100,000 live births during 2009. However, results may have been underestimated because not all parturients with a positive syphilis test result and fetal deaths were investigated. Meanwhile, a fetal death at 30 weeks was reported during the investigation but not included in the selection criteria. The Centers for Disease Control and Prevention definition of CS based on maternal status can also lead to an overestimation. Late screening of syphilis in mothers, lack of antenatal follow-up, higher VDRL titer, or unknown stage of the disease at time of diagnosis have already been described in other studies (*3*–*5*).

Table. Clinical and biological characteristics of mothers and children with congenital syphilis, Réunion Island, 2010*

Year of diagnosis	Mother					Child	
	Age, y	Time of syphilis screening	Duration of treatment, d	Presence and stage of disease	Positive serologic titer test results	Gestation, wk	Clinical signs
2008	16	≥13 wk gestation	5 d BD	Probable secondary syphilis at first trimester	TPHA, VDRL, FTA-ABS	34	None
2008	25	Unknown	2 d AD	NA	TPHA, VDRL, FTA-ABS	34	None
2008	16	AD	14 d AD	Primary syphilis at third trimester	TPHA, VDRL, FTA-ABS	38	None
2009	26	≥13 wk gestation	17 d BD	NA	TPHA, VDRL, FTA-ABS	31	Present
2009	18	≥13 wk gestation	2 d BD	NA	TPHA, VDRL	32	Present
2009	22	≥13 wk gestation	1 d BD	NA	TPHA, VDRL	32	Present
2009	37	After delivery	1 d AD	NA	TPHA, VDRL	38	None

*BD, before delivery; TPHA, *Treponema pallidum* hemagglutination assay; VDRL, Venereal Disease Research Laboratory; FTA-ABS, fluorescent treponemal antibody absorption; AD, after delivery; NA, not applicable.

Our report highlights an alarming situation in Réunion Island. Reemergence of CS after the increase of early syphilis in women of childbearing age must be considered as a public health alert, especially in countries where health care is supposed to be efficient. CS is easy to prevent with adequate screening of the mother and good follow-up of seropositive parturients.

The results of our study permitted reinforcement of the syphilis mass screening and awareness campaign regarding this sexually transmitted infection in the general population and medical corps. Although it is unrealistic to expect complete eradication of primary and secondary syphilis in communities, a minimal increase of CS rates should trigger reinforcement of these prevention policies.

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Reduced Susceptibility to Vancomycin in *Staphylococcus aureus*

To the Editor: I read with interest the article by Aguado et al. (*1*). I congratulate the authors for their high-quality research and would like to make 2 brief comments.

My first point regards the mechanism by which methicillin-susceptible *Staphylococcus aureus* (MSSA) with reduced susceptibility to vancomycin would also acquire decreased susceptibility to β-lactams. The authors "hypothesize that certain structural modifications might also occur in the cell wall of strains with high vancomycin MIC, including a thicker cell wall as it has been described in MRSA [methicillin-resistant *S. aureus*]." In addition to cell wall thickening, possible

mechanisms could include reduction in autolysis (*2*,*3*) and in the cell wall content of penicillin binding protein 4 (PBP4) (*3*). A study of MSSA isolates has shown a reduction in autolysis and in the bactericidal activity of oxacillin after development of intermediate vancomycin susceptibility (*2*). Lower content of PBP4 and decreased autolysis were reported in MRSA isolates after reduced susceptibility to vancomycin developed after exposure to this antimicrobial drug (*3*). Decreased PBP4 has been associated with reduced methicillin susceptibility in *S. aureus* (*4*).

Second, knowing whether the authors had the exact vancomycin MIC of the isolates by broth microdilution to compare with the Etest results would be interesting. In the article, they only state that "all 99 MSSA strains were susceptible to vancomycin (MIC ≤2 μg/mL) by the broth microdilution method." Although difficult to compare (because Etest dilution progression is arithmetic and broth microdilution is performed with a geometric dilution), some authors have found substantial differences between the vancomycin MIC results given by these 2 methods (*5*). These differences could have major laboratory and clinical implications.

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In Response: We appreciate the comments by M. J. Mimica (*1*). We agree that mechanisms other than increased cell wall thickness, such as decreased autolysis, metabolic changes, and reduction in the cell wall content of penicillin binding protein 4, characterize *Staphylococcus aureus* isolates, and they could explain this reduced susceptibility not only to vancomycin but also to β-lactam antimicrobial drugs. A recently published report by Holmes et al. (*2*) confirms this hypothesis. That study showed that *S. aureus* vancomycin MIC ≥1.5 μg/mL, determined by Etest, was associated with a significantly higher death rate for patients with methicillin-susceptible *S. aureus* bacteremia irrespective of the type of antimicrobial drug used.

Unfortunately, the vancomycin MIC determined by broth microdilution could not be compared with the Etest results because all our isolates had a MIC by broth microdilution that oscillated between 0.5 and 1.0 μg/mL. No isolate had a MIC of 2 μg/mL. In the study by Holmes et al., vancomycin MICs were higher by Etest than by broth microdilution (*1*); however, all isolates were considered vancomycin susceptible by Clinical and Laboratory Standards Institute broth microdilution methods. The authors pointed out that they also found that increased mortality rate was associated with increased broth microdilution MIC (data not shown), although the trend was not so prominent (*2*).

A prospective study would be required to specifically investigate the relationship between vancomycin MIC and outcome of *S. aureus* bacteremia. Our group is performing such a prospective study that we hope will enable us to shed light on this relevant topic.

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Smallpox: The Death of a Disease and House on Fire: The Fight to Eradicate Smallpox

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Smallpox eradication is one of humankind's greatest accomplishments, but like all great successes, it was the result of many individual efforts. For >3,500, years smallpox (variola major strain of the virus) had killed, scared, or blinded most inhabitants of the world. In the past century, it killed more than half a billion persons, and death was dreadful. In large cities, it was a childhood disease infecting almost every child and killing 30% of them. In isolated smaller communities, the disease would strike periodically, killing one third of those who had not previously had smallpox. When introduced into the Western Hemisphere, it killed >80% of the native population. Smallpox changed history, impacted monarchies, and affected outcomes of wars. Its detailed history is told in Smallpox and Its Eradication by Fenner et al., and is available on the World Health Organization (WHO) website (*1*), but the story is now available in more readable forms.

Smallpox: The Death of a Disease, by D.A. Henderson, is the story from the top down by the program director, and House on Fire: The Fight to Eradicate Smallpox by William F. Foege, at that time a medical missionary in Africa, is told from the bottom up. Henderson had recruited Foege because of his prior work at the Centers for Disease Control and Prevention and public health training.

Both books are full of interesting anecdotes. Henderson's stories largely focus on getting around WHO bureaucracy, often by bypassing it functionally, while appearing to follow it carefully. For example, vaccine was supplied immediately after an emergency request was made, but the request was also processed through WHO channels. Two years later, the emergency request was appropriately approved. Dr Foege's anecdotes focus on the challenges in the field, stopping smallpox in eastern Nigeria, and later in India. For example, when relations between eastern and western Nigeria became difficult, and persons in the capital (Lagos) would not send vaccine to continue uninterrupted immunizations, Foege stole vaccine from their warehouse. These 2 books describe dedicated, creative, and capable persons working in many countries and overcoming unpredictable obstacles.

The original plan was for mass vaccination of >80% of the population in any country that had smallpox cases. When Foege was beginning his work in Nigeria, he heard from missionaries communicating by short wave radio that there were outbreaks of smallpox in several villages. He did not have enough vaccine for mass vaccination of the region. Instead, he went to each village whose inhabitants had smallpox infections and vaccinated the inhabitants of only that village and perhaps 1 or 2 nearby villages that had frequent contact. This procedure was called surveillance and containment. As he pursued this approach, smallpox seemed to be cleared from eastern Nigeria. The civil war between the eastern and western regions caused him to evacuate for >1 year. When he returned, eastern Nigeria was still free of smallpox. This amazing result was shared with programs from other nations but met with mixed acceptance. Mass vaccination had been the accepted way.

Foege's final proof of the effectiveness of surveillance and containment came when he was assigned as advisor to the most challenging state in India. The surveillance system there was far more complex than relying on shortwave radio reports of missionaries from isolated communities. In addition, the density and mobility of the population meant that containment had to be more rigorous. As improved surveillance detected more cases, the Indian Minister of Health wanted to return to mass vaccination, but a young, brave health officer pointed out that when a house is on fire, you pour water on that house, not in the neighborhood (thus the book title). That simple argument saved the program. On the basis of the increasing number of reported cases, the Communist Party in India accused the United States of spreading the disease. Foege had to convince Ambassador Daniel Patrick Moynihan to allow the program to continue. The last case of smallpox in India occurred in a homeless beggar on May 18, 1975. She had spent 4 days on the platform of a railroad station potentially infecting thousands. While she was there, 4,500 tickets had been sold to persons traveling to stations throughout India, prompting an intensive widespread search. Fortunately, no additional cases were detected.

Henderson tells the story of the mixed role of the USSR. Few persons realize the key role the USSR played in eradicating smallpox. The USSR was the original force behind the proposal to WHO to eradicate smallpox and provided free vaccine to much of the world. It is unlikely that eradication would have been achieved

without the contributions of the USSR. Conversely, the last chapter in Henderson's book, entitled Smallpox as a Biological Weapon, documents the inhumane posteradication activities of the USSR to produce 50–100 tons of smallpox virus per year for use as a biological weapon. The story of how this information came to light, and the subsequent response of our government to protect our nation, needs to be more widely known.

These 2 books tell the story of humankind's most remarkable achievement, the eradication of the most dreaded infectious disease in history. I have never understood how the Nobel Prize Committee has avoided recognizing this achievement. Two men played the 2 most critical roles. Foege showed that surveillance and containment worked. Henderson helped create and then direct the program and overcame seemingly insurmountable obstacles. Perhaps the greatest obstacle was the "knowledge" that eradication was impossible, but as Foege said, "Something must be believed to be seen." Although hundreds of thousands of persons were involved in the eradication of smallpox, Henderson provided the leadership to get it completed and Foege showed how it should be conducted, even in the most challenging of settings. It is time for their contributions to be recognized!

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Lessons Learned during Public Health Response to Cholera Epidemic in Haiti and the Dominican Republic

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After epidemic cholera emerged in Haiti in October 2010, the disease spread rapidly in a country devastated by an earthquake earlier that year, in a population with a high proportion of infant deaths, poor nutrition, and frequent infectious diseases such as HIV infection, tuberculosis, and malaria. Many nations, multinational agencies, and nongovernmental organizations rapidly mobilized to assist Haiti. The US government provided emergency response through the Office of Foreign Disaster Assistance of the US Agency for International Development and the Centers for Disease Control and Prevention. This report summarizes the participation by the Centers and its partners. The efforts needed to reduce the spread of the epidemic and prevent deaths highlight the need for safe drinking water and basic medical care in such difficult circumstances and the need for rebuilding water, sanitation, and public health systems to prevent future epidemics.

Cholera is a severe intestinal infection caused by strains of the bacteria *Vibrio cholerae* serogroup O1 or O139, which produce cholera toxin. Symptoms and signs can range from asymptomatic carriage to severe diarrhea, vomiting, and profound shock. Untreated cholera is fatal in ≈25% of cases, but with aggressive volume and electrolyte replacement, the number of persons who die of cholera is limited to ≤1%. Since 1817, cholera has spread throughout the world in 7 major pandemic waves; the current and longest pandemic started in 1961 (*1*). This seventh pandemic, caused by the El Tor biotype of *V. cholerae* O1 and O139, began in Indonesia, spread through Asia, and reached Africa in 1971. In 1991, it appeared unexpectedly in Latin America, causing 1 million reported cases and 9,170 deaths in the first 3 years (*2*). The other biotype of *V. cholerae* O1, called the classical biotype, is now rarely seen.

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Cholera is transmitted by water or food that has been contaminated with infective feces. The risk for transmission can be greatly reduced by disinfecting drinking water, separating human sewage from water supplies, and preventing food contamination. Industrialized countries have not experienced epidemic cholera since the late 1800s because of their water and sanitation systems (*3*). The risk for sustained epidemics may be associated with the infant mortality rate (IMR) because many diarrheal illnesses of infants spread through the same route. In Latin America, sustained cholera transmission was seen only in countries with a national IMR >40 per 1,000 live births (*4*). Although cholera persists in Africa and southern Asia, it recently disappeared from Latin America after sustained improvements in sanitation and water purification (*5*,*6*). Although the country was at risk, until the recent outbreak, epidemic cholera had not been reported in Haiti since the 1800s, and Haiti, like other Caribbean nations, was unaffected during the Latin America epidemic (*7*,*8*).

Haiti: A History of Poverty and Poor Health

Haiti has extremely poor health indices. The life expectancy at birth is 61 years (*9*), and the estimated IMR is 64 per 1,000 live births, the highest in the Western Hemisphere. An estimated 87 of every 1,000 children born die by the age of 5 years (*9*), and >25% of surviving children experience chronic undernutrition or stunted growth (*10*). Maternal mortality rate is 630 per 100,000 live births (*10*).

Haitians are at risk of spreading vaccine-preventable diseases, such as polio and measles, because childhood vaccination coverage is low (59%) for polio, measles-

rubella, and diphtheria-tetanus-pertussis vaccines (*9*). Prevalence of adult HIV infection (1.9%) and tuberculosis (312 cases per 100,000 population) in the Western Hemisphere is also highest in Haiti (*11*,*12*), and Hispaniola, which Haiti shares with the Dominican Republic, is the only Caribbean island where malaria remains endemic (*13*).

Only half of the Haitian population has access to health care because of poverty and a shortage of health care professionals (1 physician and 1.8 nurses per 10,000 population), and only one fourth of seriously ill persons are taken to a health facility (*14*). Before the earthquake hit Haiti in January 2010, only 63% of Haiti's population had access to an improved drinking water source (e.g., water from a well or pipe), and only 17% had access to a latrine (*15*).

Aftermath of Earthquake

The earthquake of January 12, 2010, destroyed homes, schools, government buildings, and roads around Port-au-Prince; it killed 230,000 persons and injured 300,000. Two million residents sought temporary shelter, many in internally displaced person (IDP) camps, while an estimated 600,000 persons moved to undamaged locations.

In response, the Haitian government developed strategies for health reform and earthquake response (*16*,*17*) and called on the international community for assistance. The Ministère de la Santé Publique et de la Population (MSPP) requested assistance from the Centers for Disease Control and Prevention (CDC) to strengthen reportable disease surveillance at 51 health facilities that were conducting monitoring and evaluation with support from the US President's Emergency Plan for AIDS Relief (PEPFAR) (*18*) and at health clinics for IDPs (*19*). MSPP also asked CDC to help expand capacity at the Haiti Laboratoire National de Sante Publique to identify reportable pathogens, including *V. cholerae* (*20*,*21*), and help train Haiti's future epidemiologic and laboratory workforce. These actions, supported through new emergency US government (USG) funds to assist Haiti after the earthquake, laid the groundwork for the rapid detection of cholera when it appeared.

Cholera Outbreak

On October 19, 2010, MSPP was notified of a sudden increase in patients with acute watery diarrhea and dehydration in the Artibonite and Plateau Centrale Departments. The Laboratoire National de Sante Publique tested stool cultures collected that same day and confirmed *V. cholerae* serogroup O1, biotype Ogawa, on October 21. The outbreak was publicly announced on October 22 (*22*).

A joint MSPP-CDC investigation team visited 5 hospitals and interviewed 27 patients who resided in communities along the Artibonite River or who worked in nearby rice fields (*23*). Many patients said they drank untreated river water before they became ill, and few had defecated in a latrine. Health authorities quickly advised community members to boil or chlorinate their drinking water and to bury human waste. Because the outbreak was spreading rapidly and the initial case-fatality rate (CFR) was high, MSPP and the USG initially focused on 5 immediate priorities: 1) prevent deaths in health facilities by distributing treatment supplies and providing clinical training; 2) prevent deaths in communities by supplying oral rehydration solution (ORS) sachets to homes and urging ill persons to seek care quickly; 3) prevent disease spread by promoting point-of-use water treatment and safe storage in the home, handwashing, and proper sewage disposal; 4) conduct field investigations to define risk factors and guide prevention strategies; and 5) establish a national cholera surveillance system to monitor spread of disease.

National Surveillance of Rapidly Spreading Epidemic

Health officials needed daily reports (which established reportable disease surveillance systems were not able to provide) to monitor the epidemic spread and to position cholera prevention and treatment resources across the country. In the first week of the outbreak, MSPP's director general collected daily reports by telephone from health facilities and reported results to the press. On November 1, formal national cholera surveillance began, and MSPP began posting reports on its website (www.mspp.gouv.ht). On November 5–6, Hurricane Tomas further complicated surveillance and response efforts, and many persons fled flood-prone areas. By November 19, cholera was laboratory confirmed in all 10 administrative departments and Port-au-Prince, as well as in the Dominican Republic and Florida (*24*,*25*) (Figure 1). Though recently affected departments in Haiti experienced high initial CFRs, by mid December, the CFR for hospitalized case-patients was decreasing in most departments, and fell to 1% in Artibonite Department (*26*). Reported cases decreased substantially in January, and the national CFR of hospitalized case-patients fell below 1% (Figure 2). As of July 31, 2011, a total of 419,511 cases, 222,359 hospitalized case-patients, and 5,968 deaths had been reported.

Field Investigations and Laboratory Studies

To guide the public health response, officials needed to know how cholera was being transmitted, which interventions were most effective, and how well the population was protecting itself. Therefore, CDC collaborated with MSPP and other partners to conduct rapid field investigations and laboratory studies. Central early findings included the following.

First, identifying untreated drinking water as the primary source for cholera reinforced the need to provide

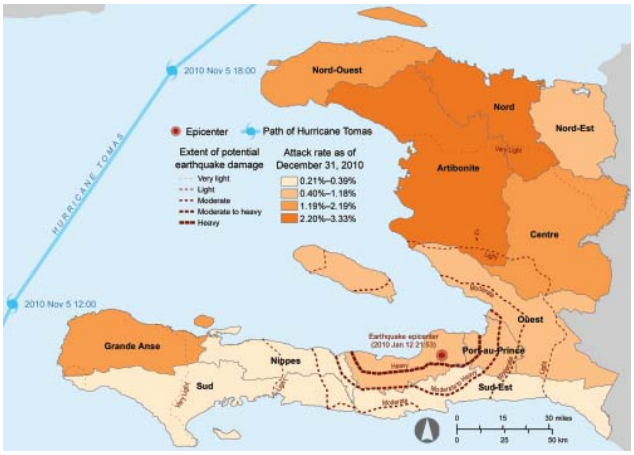

Figure 1. Administrative departments of Haiti affected by the earthquake of January 12, 2010; the path of Hurricane Tomas, November 5–6, 2010; and cumulative cholera incidence by department as of December 28, 2010.

water purification tablets and to teach the population how to use them. Although most of the population had heard messages about treating their drinking water, many lacked the means to do so.

In addition, in Artibonite Department, those with cholera-like illness died at home, after reaching hospitals, and after discharge home, which suggests that persons were unaware of how quickly cholera kills and that the overwhelmed health care system needed more capacity and training to deliver lifesaving care. Also, water and seafood from the harbors at St. Marc and Port-au-Prince were contaminated with *V. cholerae*, which affirmed the need to cook food thoroughly and advise shipmasters to exchange ballast water at sea to avoid contaminating other harbors.

The epidemic strain was resistant to many antimicrobial agents but susceptible to azithromycin and doxycycline. Guidelines were rapidly disseminated to ensure effective antimicrobial drug treatment.

Cholera affected inmates at the national penitentiary in Port-au-Prince in early November, causing ≈100 cases and 12 deaths in the first 4 days. The problem abated after the institution's drinking water was disinfected and inmates were given prophylactic doxycycline.

Finally, investigators found that epidemic *V. cholerae* isolates all shared the same molecular markers, which suggests that a point introduction had occurred. The epidemic strain differed from Latin American epidemic strains and closely resembled a strain that first emerged in Orissa, India, in 2007 and spread throughout southern Asia and parts of Africa (*27*). These hybrid Orissa strains have the biochemical features of an El Tor biotype but the toxin of a classical biotype; the later biotype causes more severe illness and produces more durable immunity (*28*,*29*). A representative isolate was placed in the American Type Culture Collection, and 3 gene sequences were placed in GenBank (*23*).

Training Clinical Caregivers and Community Health Workers

CDC developed training materials (in French and Creole) on cholera treatment and on November 15–16 held a training-of-trainers workshop in Port-au-Prince for locally employed clinical training staff working at PEPFAR sites across all 10 departments. These materials were also posted on the CDC website (www.cdc.gov/haiticholera/traning). The training-of-trainers graduates subsequently led training sessions in their respective departments; 521 persons were trained by early December.

During the initial response ≈10,000 community health workers (CHWs), supported through the Haitian government and other organizations, staffed local first aid clinics, taught health education classes, and led prevention activities in their communities. Training materials for CHWs developed by CDC were distributed at departmental training sessions, shared with other nongovernmental organization (NGO) agencies, and used in a follow-up session for CHWs held on March 1–3, 2011 (see pages 2162–5). The CHW materials discussed treating drinking water by using several water disinfection products; how to triage persons coming to a primary clinic with diarrhea and

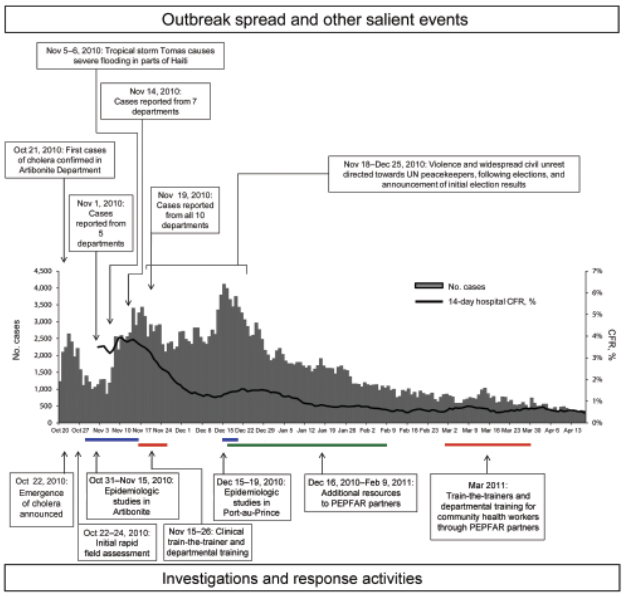

Figure 2. Reported cases of cholera by day, and 14-day smoothed case-fatality rate (CFR) among hospitalized cases, by day, Haiti, October 22, 2010–July 25, 2011. UN, United Nations; CDC, Centers for Disease Control and Prevention; PAHO, Pan American Health Organization; MSPP, Ministère de la Santé Publique et de la Population.

vomiting; making and using ORS; and disinfecting homes, clothing, and cadavers with chlorine bleach solutions. Materials were posted on the CDC website as well.

Working with Partners to Increase Capacity for Cholera Treatment

Supply logistics were daunting as cholera spread rapidly across Haiti. Sudden, unexpected surges in cases could easily deplete local stocks of intravenous rehydration fluids and ORS sachets, and resupplying them could be slow. The national supply chain, called Program on Essential Medicine and Supplies, was managed by MSPP, with technical assistance from the Pan American Health Organization, and received shipments of donated materials and distributed them to clinics.

Early in November the USG provided essential cholera treatment supplies through the US Agency for International Development's Office of Foreign Disaster Assistance (OFDA) to the national warehouse and IDP camps. CDC staff also distributed limited supplies to places with acute needs. To complement efforts by MSPP and aid organizations to establish preventive and treatment services, OFDA provided emergency funding to NGO partners with clinical capacity.

When surveillance and modeling suggested that the spread of cholera across Haiti could outpace the public health response, the USG reached out to additional partners to expand cholera preventive services and treatment capacity. PEPFAR clinicians were authorized to assist with clinical management of cholera patients and participated in clinical training across the country. In December, CDC received additional USG emergency funds and awarded MSPP and 6 additional PEPFAR partners $14 million to further expand cholera treatment and prevention efforts through 4,000 CHWs and workers at 500 community oral rehydration points. Funds were also used to expand cholera treatment sites at 55 health facilities. In addition, CDC established the distribution of essential cholera supplies to PEPFAR partners through an existing HIV commodities supply chain management system.

Improvements in Water, Sanitation, and Hygiene

To increase access to treated water and raise awareness of ways to prevent cholera, a consortium of involved NGOs and agencies, called the water, sanitation, and hygiene cluster, met weekly. Led by Haiti's National Department of Drinking Water and Sanitation and the United Nation's Children's Fund, the members of this cluster targeted all piped water supplies for chlorination and began distributing water purifying tablets for use in homes throughout Haiti. CDC helped the National Department of Drinking Water and Sanitation monitor these early efforts with qualitative and quantitative assessments of knowledge, attitudes, and practices. Emergency measures, especially enhanced chlorination of central water supplies, were expanded in the IDP camps because of the perceived high risk. OFDA and CDC provided water storage vessels, soap, and large quantities of emergency water treatment supplies for households and piped water systems. Distributing water purifying tablet supplies to difficult-to-reach locations remained a challenge.

Figure 3. Educational poster (in Haitian Creole) used by the Haitian Ministère de la Santé Publique et de la Population (MSPP) to graphically present the ways of preventing cholera. DINEPA, Direction Nationale de l'Eau Potable et d'Assainessement; UNICEF, United Nations Children's Fund; ACF, Action Contre la Faim.

Educating the Public

Beginning October 22, MSPP broadcast mass media messages, displayed banners, and sent text messages encouraging the population to boil drinking water and seek care quickly if they became ill. Early investigations affirmed the public's need for 5 basic messages:1) drink only treated water; 2) cook food thoroughly (especially seafood); 3) wash hands; 4) seek care immediately for diarrheal illness; 4) and give ORS to anyone with diarrhea. In mid November, focus group studies in Artibonite indicated that residents were confused about how cholera was spreading

and how to best prevent it, but they understood the need to treat diarrheal illness with ORS, how to prepare ORS, and how to disinfect water with water purification tablets (*30*). Posters provided graphic messages for those who could not read (Figure 3). On November 14, Haitian President René Préval led a 4-hour televised public conference to promote prevention, stressing home water treatment and handwashing, and comedian Tonton Bichat showed how to mix ORS.

Cholera Epidemic in Dominican Republic

Compared with Haiti's experience, the epidemic has been less severe in Dominican Republic. Though the countries share the island, conditions in Dominican Republic are better than in Haiti: the IMR is one third that of Haiti, gross domestic product per capita is 5× greater, and 86% of the population has access to improved sanitation. Within 48 hours of the report of cholera in Haiti, the Ministry of Health in the Dominican Republic and CDC established the capacity for diagnosing cholera at the national laboratory; the first cholera case was confirmed on October 31. Dominican officials quickly planned for cholera treatment centers in at least 70 hospitals, trained staff in primary care clinics and prison dispensaries, and stocked medical supplies sufficient to treat 20,000 cases. By December, 75% of doctors had received training in the management of cholera. Chlorination levels and water quality were monitored in municipal water systems across the country. The border with Haiti was not closed, and no major trade disruptions occurred. Sanitation improvements were instituted in border markets, schools, institutions, and mass gatherings. Public education in the first 3 months included dissemination of 4,300 mass media messages, nearly 3 million flyers, 50,000 classroom booklets for teachers, and a volunteer effort to visit 1 million homes. A survey of the knowledge, attitudes, and practices of residents of Santo Domingo showed that 89% had received cholera prevention messages. Transmission was limited, but sustained, in mid December and continued at low levels through the spring. One large outbreak affected guests at a wedding in January 2011, including some visitors from Venezuela and the United States (see pages 2172–4). From October 21, 2010, through July 30, 2011, a total of 14,598 suspected cases of cholera were reported; 256 persons died (of these, cases in 92 patients were laboratory confirmed) (*31*).

Uncertainties and Challenges of Cholera in the Caribbean

Cholera may increase seasonally in Haiti each year (during the rainy season) as it did in 2011. The lack of a history of cholera in the Caribbean makes prediction a challenge because cholera seasonality varies from place to place. Other unknown factors are what proportion of the population has now been immunized by natural infection and how long this immunity might last. In a setting in which the population has poor access to clean water and sanitation, endemic transmission could persist for years if the epidemic strain finds long-term reservoirs in brackish coastal waters. Antimicrobial drug resistance may emerge in toxigenic *V. cholerae* O1, making continued monitoring of antimicrobial drug susceptibility essential.

Whether the epidemic will spread beyond Hispaniola is also uncertain. With the highest IMR in the Western Hemisphere (reflecting major gaps in sanitation and health care), Haiti is uniquely susceptible. Other countries in the Caribbean region have an IMR less than half that of Haiti (Guatemala is next with an IMR of 33), which suggests less risk for sustained transmission. If shipmasters leaving Haitian ports would exchange their ships' ballast water at sea, they could help prevent the transfer of epidemic cholera from harbor to harbor.

The origin of cholera in Haiti also raises questions. United Nations peacekeeping troops from Nepal may possibly have introduced cholera into Haiti (*32*). Genetic comparison of the Haitian epidemic strain with other strains from around the world suggests that it resembles strains seen in southern Asia and in Nepal (*33*). Although knowing how cholera was introduced into Haiti would not help dampen its spread throughout Hispaniola, the knowledge might help foster disease monitoring and sanitation policies that would prevent such introductions elsewhere (*34*).

A continuing challenge facing Haiti is how to manage cholera treatment with limited resources. Cholera training for doctors and nurses should be added to clinical curricula. By increasing use of ORS and expanding the antimicrobial drug treatment of hospitalized patients, intravenous fluid needs might be decreased, without posing an undue risk for antimicrobial drug resistance. Focusing on supply chain logistics is critical to ensuring the maintenence of tenuous buffer stocks of supplies.

Residents of IDP camps have been largely spared from the outbreak because of safer water supplies and improved sanitation in the camps, but preserving that protection as persons move on to homes without piped water or sewage systems will be a challenge. Encouraging and empowering residents to disinfect drinking water in their homes, schools, and clinics by using chlorine products has been effective in many African and Latin American countries and is a practical interim solution for Haiti (*35*).

The role of oral cholera vaccine in the immediate postepidemic period continues to be evaluated (*36*,*37*). Both the global cholera vaccine supply and Haitian vaccine cold chain are currently insufficient to mount national vaccination campaigns on Hispaniola. A limited vaccination pilot study could increase our global understanding of the

costs, benefits, and practical applicability of using oral cholera vaccine in such circumstances.

Lessons Learned

The existing PEPFAR program that provided support for clinical care delivery and public health infrastructure was a powerful framework that sustained the national cholera response in Haiti. Through additional USG funding for PEPFAR partners, an expanded cadre of Haitian clinicians and CHWs received cholera training, resulting in expanded access to cholera treatment throughout Haiti. In addition, the postearthquake enhancement of diagnostic laboratory testing capacity for reportable diseases enabled health officials to quickly confirm the cholera outbreak and monitor antimicrobial drug susceptibility of the bacterial strains.

The Haitian epidemic shows that as long as cholera exists anywhere in the world, many who drink untreated water and live in areas of poor sanitation are at risk. The epidemic also shows how cholera can emerge where it is least expected. Despite heightened efforts to detect acute watery diarrhea among persons in urban IDP camps, cholera appeared first in rural Haiti, just as in Mexico in the 1990s, where it first emerged unexpectedly in a remote mountainous region (*8*). Therefore, the ability to detect and confirm cholera needs to be broadly available.

The Haitian experience also shows the continued success of the rehydration treatment strategies first developed in Bangladesh and refined over the past 40 years. With training and adequate supplies and treatment facilities, hospitalized case-fatality ratios of <1% were achieved. If the improvements in ORS use in treatment of diarrheal illness are sustained, these actions could reduce childhood deaths permanently.

The more moderate course of the epidemic in the Dominican Republic and the relative sparing of the IDP camps in Haiti illustrate how safer water and better sanitation can prevent transmission. Without these basic public health bulwarks, the risk for recurrent cholera and other major waterborne diseases remains high. In the interim, safe water and handwashing practices should be integrated into household and community settings (*35*).

Investing in Safe Water and Sanitation

Global experience with cholera suggests that the epidemic in Haiti could last for years. Although case counts decreased in early 2011, cases again increased with the onset of the rainy season, and conditions that permit waterborne transmission persist. Improving Haiti's water and sanitation infrastructure is critical to achieving the same profound health gains brought by improved water and sanitation infrastructure elsewhere (*3*,*6*,*38*).

The World Health Organization estimates that meeting the global Millennium Development Goal for improving access to safe water and improved sanitation would have a huge return on investment worldwide (*39*). For each $1 invested, the economic rate of return in regained time at work and school, time saved at home by not hauling water, increased productivity, and reduced health costs would be as much as $8, in addition to the direct health benefits. For Haiti to meet this goal, an estimated 250,000 households would need access to an improved water source, and ≈1 million families would need access to improved sanitation. The Inter-American Development Bank estimated in 2008 that Haiti would require $750 million to achieve this goal (*40*). After the earthquake, the international community pledged >$6 billion to Haiti for relief. A long-term plan to build safe drinking water and sewerage systems is well within the range of the resources pledged.

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Rapid Development and Use of a Nationwide Training Program for Cholera Management, Haiti, 2010

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When epidemic cholera appeared in Haiti in October 2010, the medical community there had virtually no experience with the disease and needed rapid training as the epidemic spread throughout the country. We developed a set of training materials specific to Haiti and launched a cascading training effort. Through a training-of-trainers course in November 14–15, 2010, and department-level training conducted in French and Creole over the following 3 weeks, 521 persons were trained and equipped to further train staff at the institutions where they worked. After the training, the hospitalized cholera patients' case-fatality rate dropped from 4% to <2% by mid-December and was <1% by January 2011. Continuing in-service training, monitoring and evaluation, and integration of cholera management into regular clinical training will help sustain this success.

When toxigenic *Vibrio cholerae* O1 was identified in Haiti on October 21, 2010, it was soon apparent that the epidemic would be severe and clinical training needs great (*1*). Epidemic cholera had never been reported from Haiti, and the clinical community there had virtually no experience with the disease. By November 1, a total of 6,422 hospitalized patients with cholera were reported from 5 of the 10 departments of Haiti (*2*). Of these patients, 244 had died, resulting in a hospitalized case-fatality rate (CFR) of 3.8%. The CFR for untreated clinical cholera is ≥20% (*3*), but with access to care and aggressive appropriate volume replacement, it can be reduced to ≤1% (*4*). In the 1991 Latin American cholera epidemic, transmission was sustained in countries with better water and sanitation and lower infant mortality rates than Haiti, suggesting that the risk for continued transmission in Haiti would be high (*5*,*6*). The unfortunate concurrence in Haiti of an earthquake-ravaged infrastructure; long-standing deficiencies in water, sanitation and transportation; and the limited number of health professionals and their lack of experience with cholera treatment all suggested that further spread was not only likely but would have severe clinical consequences.

In collaboration with the US Centers for Disease Control and Prevention (CDC), the Haiti Ministère de la Santé Publique et de la Population (MSPP) immediately launched a cascading approach to train clinical care providers, using the training-of-trainers approach that has been integral to laboratory and programmatic capacity building in the President's Emergency Program for AIDS Relief (PEPFAR) in many countries (*7*,*8*). Training in cholera treatment supported the MSPP in reinforcing cholera treatment in existing care facilities and in setting up new centers. Many nongovernmental organizations (NGOs) operate in the Haitian health sector, so this training needed to address a range of public and NGO health care providers with varying skills.

After we developed a package of training materials, clinical training occurred in 3 stages. First, a group of master trainers were trained in Port-au-Prince. They then formed 5 teams, each responsible for training health facility staff in 2 departments in the next 2 weeks, supported by department health authorities. This training was followed by on-site training at health facilities. The training package was also provided to primary PEPFAR NGO partners (e.g., Partners in Health, Haitian Group for the Study of Kaposi's

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Sarcoma and Opportunistic Infections, and Catholic Relief Services) for use in their training sessions and was made available by website to all NGOs in Haiti.

To monitor effectiveness of treatment in the short term, we planned to use the hospitalized CFRs from ongoing national surveillance collected by MSPP (*2*). This surveillance provided rapid and consistent information from each department; we thought the hospitalized CFRs would be more complete and would better reflect the clinical treatment outcomes than the overall CFR. We also planned to conduct evaluation of care in health facilities and cholera treatment centers (CTCs) throughout Haiti to identify areas for long-term improvement in diarrheal disease management.

Developing Training Materials

In the 3 weeks following the first report of cholera, a package of modular training materials was developed that supported varied training needs, including information on the basic management, epidemiology, and prevention of cholera, and instruction relevant for conditions in Haiti. The training included management of temporary CTCs, whether freestanding or within existing health centers. The package also included information for use at the community level on cholera prevention and use of oral rehydration solutions (ORS).

Previously developed materials were updated, combined, and translated into French and Creole. Our work was informed by 1) pamphlets and videos developed by CDC with the Pan American Health Organization (PAHO) in response to the 1991 Latin American cholera epidemic (*9*); 2) the Cholera Outbreak Training and Shigellosis Program of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), a package that includes a reference manual, presentations, and pocket information cards specific to each work role (*10*) and that was used in Pakistan earlier in 2010, when cholera appeared after a major flood disaster (*11*); 3) short videos produced by ICDDR,B that illustrated setting up CTCs and treating cholera patients in challenging circumstances (*12*,*13*); 4) standard cholera reference materials developed by the World Health Organization (*14*,*15*); and 5) guidelines of the Médecins Sans Frontières (*16*).

We sought input from other groups with cholera expertise. We reviewed our antimicrobial drug recommendations on the basis of susceptibility testing of Haitian epidemic *V. cholerae* isolates (*17*) with PAHO technical experts. We had favored single-dose doxycyline therapy for children, because the risk of dental staining following a single dose seemed far less than the benefit of treating cholera. However, PAHO experts voiced concern that this recommendation might alter routine prescription practices in the region, leading to frequent treatment of childhood diarrhea with doxycycline. Therefore, other effective treatments for pediatric patients were recommended according to resistance of the pathogen strain and availability of antimicrobial agents. An ICDDR,B expert in cholera clinical management and training joined the development team and participated in the training in Haiti. Médecins Sans Frontières clinicians and logistics experts helped us adapt their materials. Finally, all materials were reviewed and approved by the Haitian MSPP. CDC staff in Haiti worked closely with MSPP to make adjustments to fit the circumstances in Haiti.

Although the primary languages used in preparing materials were French and Creole, some materials were also prepared in English and Spanish for use by those participants whose medical training had been in those languages. The training package was produced as hard copy, placed on thumb drives, and made available on CDC's website (www.cdc.gov/haiticholera/training/hcp_materials.htm).

Training of Trainers, Port-au-Prince, November 2010

The goal of this course was to cover the practical essentials of treatment, epidemiology, and prevention of cholera so that those trained could then immediately train health care providers. A group of 33 master trainers was identified, drawn mainly from CDC locally employed staff and PEPFAR partners with experience in adult learning. Other health officials also attended; 45 persons took the training-of-trainers course.

The first day covered basic clinical concepts of toxigenic *V. cholerae* infection, pathophysiology of the disease, clinical assessment and treatment, and prevention measures. Trainers mastered the different levels of dehydration and learned to tailor care, treatment, and support while taking into account the limited infrastructure, human resources, and supplies. They learned the elements of setting up a CTC, disease reporting, and surveillance. Principal instructors included 3 of the authors (R.V.T., Y.L., and A.K.), with organizational support for the training from CDC/Haiti and the International Training and Education Center for Health, Haiti.

On the second day the trainers formed small groups to develop and then themselves present an aspect of care, treatment, support, infection control, or prevention of cholera. A site visit to a nearby CTC provided an opportunity to observe cholera patients, review clinical management of severe and moderate dehydration, and observe the CTC layout and infection control procedures.

Department Training

By November 15, 2011, MSPP reported confirmed cholera in 7 departments and Port-au-Prince, and a total of

18,383 hospitalizations and 729 hospital–associated deaths had been reported, with a cumulative hospitalized CFR of 4% (*2*). Department-level training was conducted over the next 3 weeks in all 10 departments of the country (Figure).

Each team was assigned 2 departments; equipped with training materials, a projector, and 2 vehicles; and led by CDC regional staff and representatives of the health departments where the training was to be held. Twelve department-level training sessions were conducted, at least 1 in each department. Each team also visited up to 3 functioning CTCs in each department to assess local needs for further training. In departments not yet affected, they visited and assessed facilities proposed as future CTC sites. Critical supplies for first response were provided in some areas to tide centers over while departmental supply logistics were activated.

Nurses, physicians, and pharmacists from all health centers with hospital beds were invited to participate in the department training sessions. These 1-day sessions covered the basic skills needed to care for and treat cholera patients and set up treatment units within their facilities; clarified the need for adequate personnel and supply logistics; and reviewed infection control. The health care providers were also given cards with which to train community health workers on prevention activities, as described by A. Rajasingham et al. (*18*). Personnel in nine departments were trained before civil unrest around the National Election on November 28 complicated travel; by the following week, when department-level training was completed, 521 persons had been trained. One experienced trainer remained in each department to further replicate training and to provide local technical assistance. In each department, further training then began at the health facility level, but the numbers trained were not collected.

Immediately after these sessions, the training teams provided the development team with suggestions for revisions, which were based on questions that arose during the training. The materials were modified to stress even further the primacy of rehydration therapy, to cover the treatment of chronically malnourished patients in more detail, to encourage antimicrobial drug treatment of moderately dehydrated as well as severely dehydrated patients, and to describe more systematically the logistics process for supplies. We also developed a short downloadable synopsis for medical volunteers going to Haiti to staff cholera treatment sites.

Outpatient rehydration and triage of patients with diarrheal illness should reduce the number of cases seeking care at hospitals for severe dehydration. Therefore, community health worker training using another packet of training materials was conducted in early March (*18*).

The training at the department level was enthusiastically received, and trainees reported anecdotally that they would put the knowledge to use immediately. Rapid review in the field of pre-course surveys showed that many trainees entered the training unaware of the basics of cholera treatment but understood the essentials by the end of the course. Regrettably, the assessment forms were then misplaced and were not available for analysis for this synopsis. More objectively, although the number of reported cases increased through December, the CFR rate for hospitalized patients dropped below 2% by mid December and was below 1% by early January (Figure). It has remained there through the end of July, even during a summer increase in cases and even as many NGOs that assisted with the epidemic withdrew. Several factors likely contributed to the decrease in CFR, including expanded support for treatment facilities, improved supply chains, and the growing competence and confidence of caregivers trained in cholera treatment.

Conclusions

Monitoring and evaluation of the outcomes of training are part of continuous improvement (*19*). Trends in the health outcomes of incidence and CFRs for hospitalized

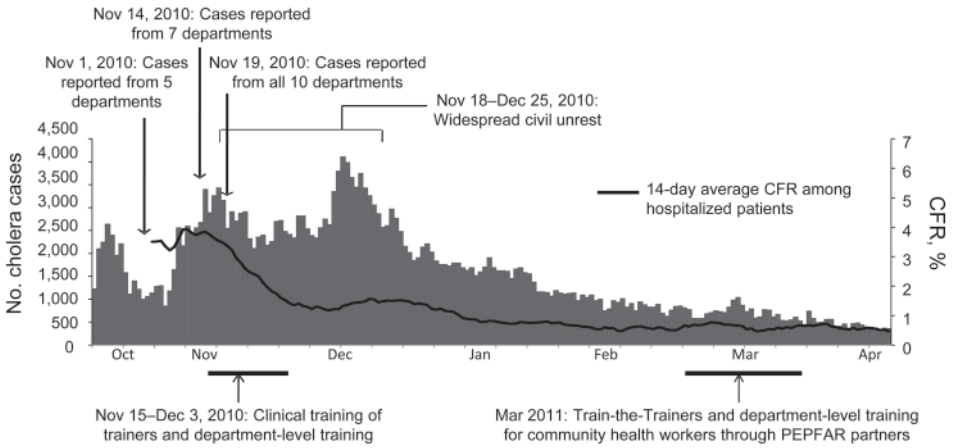

Figure. Major events in training, number of cholera cases reported to Ministère de la Santé Publique et de la Population (MSPP) national surveillance by day, and smoothed 14-day case-fatality rate (CFR) for hospitalized calculated from MSPP surveillance data during the cholera epidemic in Haiti, October 20, 2010–April 20, 2011. The first cases were confirmed in Artibonite Department October 21, 2010; by November 19, cholera was reported in all 10 departments in Haiti. PEPFAR, President's Emergency Program for AIDS Relief.

patients provide the most immediate measure of effect and will need continued monitoring. Longer term evaluation and training are being planned now, including assessing the need for refresher and in-service training. In addition to the CFR for hospitalized patients, the long-term success of training can be measured by its sustained influence on the performance of providers at the department or even health care center level. Measurable hallmarks of good clinical management include efficient triage of patients with diarrhea, rapid diagnosis and assessment by physicians, and swift and appropriate treatment by nursing staff. Other performance measures that the ICDDR,B has found useful include comparing the number of persons treated for cholera in a CTC with the volume of intravenous fluids and ORS used at the facility in the same period of time and tracking the average time it takes to discharge patients. Furthermore, assessing logistical plans may help avert shortages of crucial supplies.

Measuring the number of professionals trained, persons reached, commodities distributed, and service points supported can monitor the increase in capacity, but it will also be vital to assess how much difference training makes in practice (*20*,*21*). Not all parts of a training program are equally effective and relevant. Changes may be needed if, for example, the antimicrobial drug resistance of *V. cholerae* O1 changes.

Cholera may persist in Haiti for years, so cholera training needs to be integrated into the curricula of medical, nursing, and pharmacy schools in Haiti. Practical hands-on training in the assessment of dehydration and the use of oral and intravenous rehydration can help trainees transfer new skills to the clinical setting (*22*). One or more ORS treatment centers maintained in academic settings could provide such practical training, which would be of benefit for the treatment of any dehydrating diarrheal illness, so that clinicians and caregivers continue to be well trained and confident in their skills. Such a center could also train clinicians from other countries in the hemisphere, who might otherwise have little chance to become familiar with cholera and its treatment.

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Cholera—Modern Pandemic Disease of Ancient Lineage

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Cholera has affected humans for at least a millennium and persists as a major cause of illness and death worldwide, with recent epidemics in Zimbabwe (2008–2009) and Haiti (2010). Clinically, evidence exists of increasing severity of disease linked with emergence of atypical *Vibrio cholerae* organisms that have incorporated genetic material from classical biotype strains into an El Tor biotype background. A key element in transmission may be a recently recognized hyperinfectious phase, which persists for hours after passage in diarrheal feces. We propose a model of transmission in which environmental triggers (such as temperature) lead to increases in *V. cholerae* in environmental reservoirs, with spillover into human populations. However, once the microorganism is introduced into a human population, transmission occurs primary by "fast" transmission from person to person (taking advantage of the hyperinfectious state), without returning to the aquatic environment.

Cholera has been an unwanted companion among human civilizations for at least a millennium, with suggestions that it has existed in India "since immemorial times" (*1*). Its impact in Bengal society was sufficient to have resulted in recognition of a goddess of cholera, Oladevi (or Oola Beebee), who required propitiation to protect villages from the disease (*2*). Global pandemic spread of cholera from its ancestral home in Bengal was first documented in 1817 (*1*), the beginning of what has been designated as the first pandemic. In the intervening 2 centuries, cholera has continued to ebb and flow from southern Asia to other parts of the known world, with 6 additional pandemics identified. During the third pandemic, which ravaged London in 1854, John Snow conducted his pioneering epidemiologic studies (and gained fame for removal of a pump handle). We are currently in the throes of the seventh pandemic (caused by *V. cholerae* of the El Tor biotype), which originated almost 50 years ago in the Celebes. In contrast to the earlier 6 pandemics, at no time in these past 50 years has cholera retreated to its southern Asian home. It has instead established endemicity at multiple sites around the globe and continues to trigger major localized epidemics, including the epidemics in Zimbabwe during 2008–2009 (*3*) and Haiti during 2010.

In 2009, the most recent year for which data are available, 221,226 cholera cases were reported to the World Health Organization (WHO) from 45 countries (*4*). This number includes 4,946 deaths, for a case-fatality rate of 2.24%. Although the disease was reported from all continents, 98% of cases reported during 2009 were from Africa, driven in part by large numbers from the latter part of the 2008–2009 Zimbabwe epidemic. However, these numbers should be interpreted with caution because of well-recognized problems with underreporting in the WHO system, particularly because cholera is no longer a notifiable disease and countries can choose whether to report cases. In 2 examples, no cholera cases were included in the annual WHO cholera summary report for 2009 (*4*) from India or Bangladesh, despite anecdotal evidence to the contrary.

Cholera today takes advantage of breakdowns in sanitation and health infrastructure, often in the setting of natural and complex disasters. More notably, cholera has survived the transition from ancient to modern world, with the establishment of endemic foci in virtually every continent. We have learned a great deal about cholera during the past few decades. Major advances have been made in therapy, which has decreased expected case-fatality rates to <0.5%. However, we are just coming to appreciate the evolutionary capabilities of the microorganism and the

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complexity of transmission pathways, an understanding of which is essential to ultimate control of the disease.

Clinical Features and Management

Clinically, cholera is a simple disease. Its manifestations result almost entirely from action of cholera toxin, a protein enterotoxin excreted by the bacterial cell. The A subunit of cholera toxin activates adenylate cyclase, causing increased Cl^- secretion by intestinal crypt cells and decreased NaCl-coupled absorption by villus cells and resulting in a net movement of electrolytes (and water) into the lumen of the intestine (*5*). All manifestations of the disease can be reproduced by administration of cholera toxin: in studies conducted in the 1970s at the University of Maryland (Baltimore, MD, USA), volunteers given 25 μg of pure cholera toxin had >20 L of rice-water feces; ingestion of as little as 5 μg of purified toxin resulted in 1–6 L of diarrhea in 5 of 6 volunteers (*6*).

Severity of illness varies widely. In the most severe form of the disease, cholera gravis, patients can pass ≥1 L of diarrheal feces per hour. Feces are passed effortlessly, with the diarrhea assuming a rice-water appearance. If volumes are not repleted, this diarrhea can result, in as little as 6–8 hours, in circulatory collapse, shock, and death. Shock, even if adequately treated, may precipitate acute renal failure. Severe acidosis results from fecal loss of bicarbonate, exacerbated by hypotension-related lactic acidosis and renal failure.

Although cholera gravis is a devastating disease, studies in the early 1970s suggested that such severe cases accounted for only 11% of total infections among persons infected with strains of the classical biotype (responsible for the sixth cholera pandemic); 59% of infections were asymptomatic or inapparent, and the remainder represented illness of mild to moderate severity. In studies during that same period, only 2% infected with seventh pandemic biotype El Tor strains had severe disease, and 75% of infected persons were asymptomatic (*7*). Although the El Tor biotype has persisted, its relative lack of virulence has not; recent studies have noted substantial increases in the percentage of patients with severe dehydration (*8*), and the percentage of asymptomatic infected patients appears to be much smaller (<50%, in a recent study by Harris et al. [*9*]). As described below, these observations coincide with the appearance of new atypical *V. cholerae* strains that include classical biotype genetic material within an El Tor background (*10*,*11*).

The cornerstone of therapy is replacement of lost fluid. With an infrastructure able to provide adequate rehydration therapy, case-fatality rates should be <1%, ideally <0.5%. In mild to moderate cases, rehydration can generally be successfully accomplished with oral rehydration solution. In patients who are severely dehydrated (loss of 10% of body weight) intravenous rehydration is almost always necessary; limited anecdotal reports suggest that use of intravenous therapy is becoming more frequent in areas where cholera is endemic and epidemic, consistent with concerns about increasing severity of illness. In early placebo-controlled studies, tetracycline reduced duration of diarrhea, total volume of diarrhea, and days of excretion of *V. cholerae* by >50%; more recent studies demonstrated equivalent or better results with ciprofloxacin and azithromycin. However, antimicrobial drug use is also clearly associated with development of resistance, leading to current WHO recommendations that antimicrobial agents be limited to use in patients with severe dehydration. As recently suggested by Nelson et al. (*12*), extending use of antimicrobial drugs to a larger patient group may be reasonable, particularly in light of increasing awareness of direct transmission of the microorganism from person to person, as discussed below. Zinc supplementation also has been recognized as a potentially useful adjunct to therapy; recent studies among children in Bangladesh have shown that its administration resulted in a 12% reduction in duration of diarrhea and 11% reduction in fecal volume in patients compared with controls (*13*).

Genetics/Microbiology

V. cholerae is a diverse species and a natural (and common) inhabitant of estuarine environments around the world. Distribution depends on water temperature (optimal growth at water temperatures >20°C) and salinity (*14*). In contrast to most other *Vibrio* species, it is able to grow in fresh water and is often present in inland rivers and lakes in regions where it is endemic. In areas with seasonal variations in water temperatures, the microorganism shows clear seasonality: environmental counts increase during warmer periods and decline (or become nondetectable) during cold weather. In studies in Peru (*15*), *V. cholerae* counts in the Rimac River (at a site above Lima where sewage contamination was minimal) spiked ≈2 months after an initial summer rise in water temperature but then returned to a nondetectable level within 1–2 months (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-1109-Techapp.pdf). The reason for the sharp drop in counts after the initial spike (a pattern also seen in some ponds in Bangladesh [*16*]) is not clear. One hypothesis is that it is related to rapid increases in the number of *V. cholerae*–specific lytic bacteriophages in the local aquatic environment (*17*,*18*), providing natural predation as a countermeasure to the initial rapid increase in numbers of the microorganism.

V. cholerae can assume a variety of survival forms, including a shift to what has been termed a viable but nonculturable form, which is often associated with biofilms. Strains can also assume a rugose phenotype (identifiable

on culture by a characteristic rough/wrinkled appearance), in which the microorganism produces large quantities of an amorphous exopolysaccharide, leading to formation of a biofilm that is resistant to chlorine, UV light, and other standard disinfectants (*19*). *V. cholerae* has been closely linked with copepods (binding to chitin through the action of a specific chitinase) and with zooplankton (*14*). It has also been found in association with chironomid egg masses and water hyacinth and can be carried by gulls, other birds, and mammals.

Although *V. cholerae* as a species is ubiquitous in the environment, strains responsible for the disease cholera are restricted to a fairly tight subset of strains, as reflected in clustering seen by multilocus sequence typing and sequence analysis. The key gene clusters responsible for the manifestations of cholera are associated with production of cholera toxin located within the *ctx* element (which is part of a filiamentous phage capable of movement among strains [*20*]) and the vibrio pathogenicity island, which includes the TCP (toxin-coregulated pilus) gene, essential for binding of the microorganism to the intestinal mucosa. Other genes common to strains with an epidemic phenotype also have been identified; however, the role of many of these genes in the pathogenesis of cholera remains to be determined (*21*,*22*). Even though virtually all strains that cause cholera produce cholera toxin and have the vibrio pathogenicity island, not all *V. cholerae* that carry 1 or both of these gene complexes cause cholera; several studies have noted the isolation of 1 or both from environmental strains that appear to lack other components of the genetic background essential for virulence in humans and epidemic spread (*22*,*23*).

The *V. cholerae* genome readily undergoes change, with extensive genetic recombination through lateral gene transfer, resulting in what have been termed shifts and drifts in the genome sequence (*21*). This genetic plasticity is reflected in the observation that feces from a single infected patient in an area where cholera is endemic almost always show evidence of infection with multiple genetically distinct *V. cholerae* strains, as defined by variable-number tandem-repeat analysis (*24*). Variability also can be seen in serotype. Traditionally, epidemic disease was thought to be confined to cholera toxin-producing strains in *V. cholerae* O group 1. However, in 1992, a new serotype, O139, was recognized as the cause of a major cholera epidemic on the Indian subcontinent (*25*); emergence was linked to replacement of the O group 1 biosynthesis cassette with a biosynthesis cassette for the O139 antigen (which also encoded material for formation of a capsule). O group 1 strains continue to predominate among epidemic isolates, but serotype clearly does not directly predict virulence; cholera-like illness (albeit without epidemic spread) is now associated with several different serotypes in addition to O1 and O139. These serotypes include O141 and O75 in the United States and O37, O10, O12, O6, and O14 in other parts of the world (*23*,*26*). Changes in serotype, in turn, appear to result from lateral transfer of the gene cassettes responsible for O-antigen biosynthesis (*23*,*26*).

Recent changes or recombinational events also have been seen in the *ctx* gene cluster, with introduction of the classical biotype *ctx* gene into an El Tor background and the appearance of strains containing multiple recombinational events that have modifications in *ctx* as well as other changes that result in loss of traditional El Tor biotype characteristics (*10*,*11*,*27*). Although nomenclature remains in flux (*11*), these new atypical strains have, at this point, entirely supplanted traditional seventh pandemic El Tor strains at a global level (including, most recently, the strain responsible for the Haiti epidemic [*28*]). As discussed above, these strains also appear to have major increases in virulence (potentially because of increased levels of cholera toxin production [*29*]), comparable with (or in excess of) clinical characteristics of the sixth pandemic classical biotype strains.

V. cholerae strains associated with epidemic disease can respond to changes in their immediate environment as they move from environmental reservoirs to humans and back. Of particular relevance, it has been shown that *V. cholerae* passed in human rice-water feces are in a "hyperinfectious" state (*17*,*30*); in animal studies, infectious dose is 1–2 orders of magnitude lower than that for strains grown by using traditional in vitro methods. The hyperinfectious state lasts at least 5 hours after passage of the microorganism from patients. The physiologic basis for this effect is unclear but appears to be associated, at least in part, with changes that include down-regulation of chemotaxis genes (*31*). *V. cholerae* as it is passed from the body also up-regulates a series of genes that are not required for infection but are needed for survival in the environment. Twenty-four hours after the microorganism reaches the aquatic environment, these shifts, potentially combined with lytic phage, result in a dramatic decrease in the ability of *V. cholerae* to cause infection (*18*).

Epidemiology/Transmission

During the 1960s, the scientific consensus was that cholera was transmitted from person to person; a great deal of attention was given to the role of convalescent and chronic carriage in transmission. During the following decades, attention shifted sharply from human carriage to environmental reservoirs, with a focus on the role of environmental factors in persistence of the disease and triggering of epidemics. However, with the advent of increasingly sensitive molecular techniques—and mathematical modeling approaches—there has been movement back toward a transmission model that

recognizes the role of environmental reservoirs and direct (human-to-human) transmission. In this context, we propose the transmission model shown in the Figure.

The aquatic environmental reservoir is critical to long-term maintenance of epidemic *V. cholerae*. These reservoirs constitute complex biological systems, with modulation of *V. cholerae* populations by environmental conditions (the local microenvironment [*15*,*16*] as well as global macroenvironmental factors, such as the El Niño/Southern Oscillation [*32*]); by predatory bacteriophage populations (*18*); and by fluctuations in populations of copepods and zooplankton (which can, in turn, be driven by predation by fish), binding to chironomid egg masses, water hyacinth, carriage by birds and mammals, and a host of other variables. At the same time, our data from Lima (online Technical Appendix) and from Bangladesh show a significant association between a spike in numbers of *ctx*-positive *V. cholerae* in the environment and subsequent occurrence of cholera in the community (*15*,*16*), consistent with the concept of spillover of the microorganism from the environment to human populations.

On the basis of our studies in Lima and Bangladesh, peak environmental counts of *ctx*-positive *V. cholerae* from pristine areas range from 10^1 to 10^2 CFU/mL (*15*,*16*). The infectious dose for *V. cholerae* (classical biotype) ingested by healthy North American volunteers is in the range of 10^8 to 10^{11} CFU/mL, which drops to 10^4–10^8 when the inoculum is given with bicarbonate or food (*5*,*33*). Assuming consumption of a large enough volume of contaminated material; mixing with food (with the potential for further growth in food before consumption (*34*,*35*]); and possible underlying host factors, such as mild hypochlorhydria (which can be associated with *Helicobacter pylori* infection, endemic to many developing countries) and malnutrition, transmission from environmental sources that are not heavily fecally contaminated becomes plausible. However, this infectious dose still would be at the low end of the curve, and the percentage of exposed persons becoming infected at these low levels is likely to be relatively small.

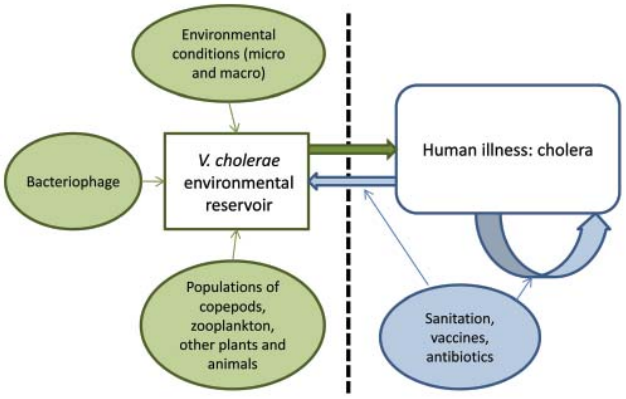

Figure. Proposed model for *Vibrio cholerae* transmission.

The picture changes once the microorganism is introduced into human populations. Rice-water feces contain 10^7–10^9 *V. cholerae* microorganisms per mL. Immediately after passage, these microorganisms are in a hyperinfectious state (further dropping the infectious dose by 1 or 2 logs), generating the opportunity for "fast" transmission of *V. cholerae* to other persons either by direct contact with feces or direct contamination of food or water within the immediate household environment. Microorganisms from feces can also reenter environmental reservoirs by fecal contamination. However, one then has to deal with dilutional effects within the environment and the striking drop in infectivity (noted above) that can occur as the microorganism adjusts to an environmental habitat (*18*). In our studies in Lima at the peak of a cholera epidemic, we found environmental counts of toxigenic *V. cholerae* of $\approx 10^5$/mL in areas with heavy sewage contamination, so the potential for infection from environmental sources clearly increases in settings of poor sanitation during epidemics. Nonetheless, looking at the relative counts from different sources of exposure, these observations are consistent with the hypothesis that a major transmission pathway for *V. cholerae* during an epidemic (particularly at the beginning of an epidemic) is through a "fast" pathway, taking advantage of the short-lived hyperinfectious state to move from person to person, without an intervening "slow" transmission step through the environment.

Data from a variety of sources support this hypothesis. In studies in Bangladesh that used variable-number tandem-repeat analysis (*36*), we found minimal overlap between clinical strain populations circulating in human populations and the *ctx*-positive *V. cholerae* strains that were circulating concurrently in environmental reservoirs. If the environmental reservoir was playing a major role in the ongoing epidemic, one would have expected to see the same genetic types appearing in strains from the environment and strains from patients. In mathematical models, inclusion of a fast (presumed person-to-person or person-to–household environment–to-person) transmission pathway that incorporates the short-lived hyperinfectious state results in a much better match with outbreak data than models that rely solely on a "slow" human-to–aquatic environment–to-human pathway (*37*). In subsequent work we have applied our models to data from the 2008–2009 Zimbabwe epidemic (*3*). When calculated by province, the reproductive number (R_0) for the epidemic ranged from 1.1 to 2.7; our calculations suggest that 47%–94% of this value, dependent on province, was accounted for by fast (hyperinfectious/presumed person-to-person) transmission.

These observations underscore the need to focus prevention efforts on the short window of time after passage of feces when strains are in a hyperinfectious state. This observation translates into the need for an emphasis on

households (and, in particular, an emphasis on households with index cases), where exposure to recently excreted microorganisms is most likely. This finding fits with those from earlier epidemiologic studies from Bangladesh, where risk for illness was linked with the presence of an infected person in the household, not with whether the household used clean tube well water versus potentially contaminated surface water for drinking (*38*). It is also in agreement with work by Deb et al. in Kolkata, India, which highlighted the influence of household transmission during epidemic periods and the associated need to focus on minimizing risk for contamination of water and food sources within the household (*39*).

Summary

V. cholerae is a wily opponent. It can live indefinitely in aquatic reservoirs, making eradication difficult, if not impossible; readily undergoes genetic modification, permitting response to changing environmental (and human) conditions; and shifts patterns of gene expression as it moves from one local environment to another (including a shift to a hyperinfectious state immediately after passage in feces). Several mathematical models (including models developed by the Emerging Pathogens Institute, University of Florida [Gainesville, FL, USA] [*3*]) support the potential value of vaccines for disease control and have outlined potential strategies for their utilization (*40*). Although the environment remains a critical component of transmission, interventions focused increasingly on the household and on blocking transmission immediately after passage of feces are acutely needed. Ultimately, good sanitation (as part of a strong public health infrastructure) is the key to disease control. However, until sanitation is widespread (and the impact of natural and human-made disasters is minimized), ways in which this age-old pathogen causes disease—and ways in which it can be controlled—need to continue to be explored.

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etymologia

Cholera

[käl′ər-ə]

From the Greek *cholē* for bile. Although the term cholera is now used only to refer to disease caused by the bacterium *Vibrio cholerae*, until the late 19th century any diarrheal illness might be referred to as cholera. For many centuries, medicine in Europe was based on Galen's theory of the 4 humors in the body: blood, bile, black bile, and phlegm. Diarrhea and vomiting were interpreted as the body's attempt to restore balance and good health by expelling excess choler, hence, many gastroenterological illnesses were referred to as cholera. A 12th century treatise, the Regimen Sanitatis Salernitanum, described the effects of excess choler thusly, "Your tongue will seem all rough, and oftentimes cause vomits, unaccustomed and hateful, great thirst, your excrements are full of slime, the stomach squeamish, sustenance ungrateful, your appetite will seem in nought delighting."

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Considerations for Oral Cholera Vaccine Use during Outbreak after Earthquake in Haiti, 2010–2011

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Oral cholera vaccines (OCVs) have been recommended in cholera-endemic settings and preemptively during outbreaks and complex emergencies. However, experience and guidelines for reactive use after an outbreak has started are limited. In 2010, after over a century without epidemic cholera, an outbreak was reported in Haiti after an earthquake. As intensive nonvaccine cholera control measures were initiated, the feasibility of OCV use was considered. We reviewed OCV characteristics and recommendations for their use and assessed global vaccine availability and capacity to implement a vaccination campaign. Real-time modeling was conducted to estimate vaccine impact. Ultimately, cholera vaccination was not implemented because of limited vaccine availability, complex logistical and operational challenges of a multidose regimen, and obstacles to conducting a campaign in a setting with population displacement and civil unrest. Use of OCVs is an option for cholera control; guidelines for their appropriate use in epidemic and emergency settings are urgently needed.

After an absence of over a century, cholera was reported in Haiti on October 22, 2010, in the Artibonite River valley (*1*). This happened within 9 months of the January 12 earthquake, which killed >222,000 persons and displaced an estimated 2 million around the capital city of Port-Au-Prince (*2*,*3*). Within 1 month, cholera was confirmed in all 10 Haitian departments, including spread to the earthquake-affected area (*1*,*4*).

Cholera is an acute, watery, diarrheal illness caused by the toxigenic bacterium *Vibrio cholerae* serogroups O1 and O139 and can be rapidly fatal if not promptly treated (*5*). Epidemic cholera is most often caused by fecally contaminated water (*5*). Disruptions in water and sanitation infrastructure after disasters (mainly flooding and cyclones) and overcrowding and precarious conditions caused by large population displacements may create an environment conducive to cholera's rapid spread (*6*–*9*), although 1 report documents epidemic risk to be small after geophysical disasters (*10*). Proven measures for treatment (oral and intravenous rehydration and antimicrobial drugs in severe cases) and prevention (provision of safe water, community education, and improved access to sanitation and hygiene) are prioritized to reduce death and spread during the acute response to epidemic cholera (*11*,*12*). In the long term, increasing population coverage with improved drinking water sources and proper sanitation are the most effective means of preventing outbreaks of cholera and other enteric diseases (*5*).

Cholera vaccination is an additional key option for cholera prevention and control. In cholera-endemic countries, the targeted use of cholera vaccines is increasingly being recognized as a useful complement to improving water, sanitation, and hygiene (*13*). Guidelines for considering the use of cholera vaccines in complex humanitarian emergencies (*14*) and for their preemptive use to protect populations threatened by epidemic cholera have been proposed (*13*). Expert opinions differ on the applicability, feasibility, and impact of cholera reactive vaccination in epidemic situations (*14*–*19*); thus far,

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experience in these situations is limited to small outbreaks in stable populations (*20*,*21*). However, the value of cholera vaccines in controlling ongoing outbreaks through reactive vaccination is not yet established (*13*,*14*,*16*). Furthermore, vaccine use in outbreaks in post-disaster settings poses unique logistic, financial, and human resource challenges. Nonetheless, over the past decade, the occurrence of large, protracted outbreaks (*22*,*23*) and the licensing and marketing of new oral cholera vaccines (OCVs) have focused discussion on the role of vaccination as a supplementary cholera preventive and control measure (*13*). We describe the process used and the evidence reviewed by the US Centers for Disease Control and Prevention (CDC), the Pan American Health Organization (PAHO), and the Ministère de la Santé Publique et de la Population (MSPP) (Haitian Ministry of Public Health and Population), when considering OCV use during the 2010–2011 cholera outbreak in Haiti.

The Study

Decision-making Process and Development of Recommendations for OCV Use in Haiti, October–December 2010

Immediately after notification of the outbreak, an emergency response was launched by MSPP with assistance from CDC, PAHO, and other governmental and nongovernmental organizations; initial discussions regarding the potential role and use of OCVs occurred within days. In subsequent weeks, PAHO and CDC convened working groups and expert advisory committees to review vaccine characteristics, World Health Organization (WHO) position papers and recommendations, published experience with OCV use in complex emergency settings, global vaccine availability, and logistical implications. The most current information regarding vaccine availability was sought from vaccine manufacturers and other partners, and the latest assessments of Haiti's postearthquake vaccine deployment capacity were obtained from agencies working in Haiti. Initial recommendations, presented to MSPP on October 27, 2010, and November 1, 2010, by PAHO and CDC, respectively, did not support cholera vaccination because of pressing needs for priority interventions of safe water provision and cholera treatment measures, and limited immediate vaccine availability (online Technical Appendix 1, wwwnc.cdc.gov/EID/pdfs/11-0822-Techapp1.pdf).

In mid-December 2010, the initial recommendations were revisited for several reasons: 1) clinical training and priority interventions for treatment and improved water quality had been established; 2) rolling 14-day hospital case-fatality rates had decreased from 4% in early November to ≈1.5% by mid-December, suggesting improved access to treatment; 3) relatively few cases were reported from Port-au-Prince, including those in internally displaced persons camps, suggesting that a large population remained at risk; and 4) anecdotal information indicated that additional vaccine supply might soon become available. On December 17, 2010, PAHO convened an ad hoc consultation with international experts and other key stakeholders to reconsider options for OCV use in Haiti, given the situation at that time (*24*).

Real-time modeling was conducted by CDC during the course of the outbreak to develop preliminary estimates of numbers of cases and hospitalizations for planning purposes. Early in the outbreak, an epidemic model fit to the first 7 weeks of cholera surveillance data was created to develop preliminary OCV impact estimations, the details of which are described in online Technical Appendix 2 (wwwnc.cdc.gov/EID/pdfs/11-0822-Techapp2.pdf)

OCV Characteristics and Status

The 2 available OCVs, Dukoral (Crucell, Stockholm, Sweden), and Shanchol (Shantha Biotechnics, Hyderabad, India) are whole-cell, killed vaccines. Key vaccine characteristics are summarized in Table 1. Both vaccines require 2 doses (3 doses of Dukoral are required for children 2–5 years of age) administered about 7–14 days apart (up to 42 days apart for Dukoral). Dukoral doses must be administered with buffer that requires 75–150 mL of clean water; Shanchol does not require buffer. Both vaccines require cold chain maintenance and have packed volumes larger than those of other Expanded Program on Immunization (EPI) vaccines (estimated for Dukoral to be 30× larger than those of the EPI vaccines), indicating the need for greater cold chain capacity (*13*) (see online Technical Appendix 1 for additional references).

At the time, only Dukoral was prequalified by WHO and since 1991 has been licensed in ≈60 countries for persons ≥2 years old. The newer Shanchol vaccine, licensed in India since 2009 for persons ≥1 year of age, was pending WHO prequalification. WHO prequalification is required for vaccine procurement by United Nations agencies, including the PAHO Revolving Fund, the United Nations Children's Fund, and for some donor funding, including the US government.

Both OCVs have been shown to be safe and immunogenic; clinical trials demonstrated protective efficacy of 66%–85% after 2 doses but almost none after a single dose. Protection is achieved ≈7 days following the last dose of Dukoral (estimated to be similar for Shanchol) and persists for ≈2 years. Herd protection has been inferred for Dukoral, according to a reanalysis of the Bangladesh original clinical trial data, and has been suggested to be substantial in cholera-endemic areas. A similar herd-protection effect with Shanchol, although expected, has not yet been studied. Most vaccine effectiveness studies have been conducted in

cholera-endemic settings, where some level of preexisting population immunity can be expected because of recurrent exposure. These study results are in contrast to Haiti, where the population was immunologically naive to cholera until the current epidemic, suggesting a need for higher vaccination coverage with the full series to achieve the suggested herd protection. One study conducted among Peruvian military recruits (an immunologically naive population similar to that in Haiti) shows promise; the vaccine demonstrated an 86% protective efficacy at 4–5 months. Studies have shown these vaccines to be cost-effective in cholera-endemic areas only when herd-protection effects are considered.

Immunity after natural cholera infection is incomplete and, particularly after a first infection and after infection with the El Tor biotype, appears to be of relatively short duration, waning within 6 years in contrast to life-long immunity conferred by viral infections. Nevertheless, the duration of protection with natural infection is longer than that conferred by OCVs.

Global OCV Availability

During the initial weeks of the outbreak, an estimated 100,000–300,000 doses of Dukoral and 150,000 doses of Shanchol were available for immediate shipment, and an estimated 1,000,000 additional doses could have been made available over a 1-year period (International Vaccine Institute, Crucell, PAHO, pers. comm.). In December 2010 and January 2011, both manufacturers indicated that a larger supply (up to 5 million combined doses for both vaccines) (*25*) could be made available gradually over 1–3 years, but firm orders and financial commitments were needed before production capacity could increase.

Previous Experiences with Mass OCV Campaigns in Complex Emergency Settings

Mass OCV campaigns have been conducted in complex emergency settings, with mixed results (*14*,*16*; online Technical Appendix 2) Two such experiences, in Sudan and Indonesia, formed the basis for the 2005 WHO

Table 1. Salient features of oral cholera vaccines available as of December 31, 2010*

Feature	Dukoral†	Shanchol
Composition	Monovalent formalin-based heat-killed whole cells of *Vibrio cholerae* O1 (classical and El Tor, Inaba and Ogawa) + recombinant cholera toxin B subunit	Bivalent, killed whole cells of *V. cholerae* O1 (Inaba and Ogawa, classical and El Tor) and O139
Number of doses for full immunization	2 doses (3 doses in children 2–5 y)	2 doses
Schedule	7–14 d apart (up to 42 d apart)	14 d apart
Age for vaccination per licensure	≥2 y	≥1 y
Administration	Oral with buffer	Oral
Requirement for buffer and water	Yes (adults,150 mL; children 2–5 y, 75 mL)	No (water may be used)
Food and water restrictions before and after vaccination	No food or water 1 h before and after ingestion of vaccine	None
Packaging	3-mL single dose vials (vaccine) + effervescent granules in a sachet (buffer)	1.5-mL single dose vial
Cold-chain and other storage requirements	2–8°C; additional storage space for water (not in cold chain)	2–8°C
Shelf life	3 y at 2–8°C; stable for 1 mo at 37°C	2 y at 2–8°C
WHO prequalified	Yes	No
Cost of vaccine	US $6 per dose ($12–$18 for full series, i.e., for 2–3 doses); price quoted for Haiti in January 2011, $3.64 per dose	$1.85 per dose ($3.70 for full series)
Safety profile	High	High
Earliest onset of protection	7–10 d after full immunization	7–10 d after full immunization per manufacturer
Efficacy and effectiveness	Matlab trial, Bangladesh: 85% at 4–6 mo; 62% at 1 y, 58% at 2 y, 18% at 3 y; in 2–5 y olds: 100% at 4–6 mo, 38% at 1 y; military center, Peru: 86% at 4–5 mo; outskirts of Lima, Peru: 60% at 2 y; Beira, Mozambique: 85% with 2 doses, 78% with ≥1 dose at 1–6 mo	Kolkata, India: 67% at 2-y follow-up with 2 doses
Single dose effectiveness	Low; Matlab trial, Bangladesh: 12% during 3 y (lower limit of 95% confidence interval –29%)	Unknown studies planned
Herd protection	Yes	Expected but not yet demonstrated

*Other oral cholera vaccines not summarized: An injectable vaccine may be available in some countries, but is not recommended by the World Health Organization (WHO) because of its reactogenicity, limited efficacy, and short duration of protection; mORCVAX, similar to Shanchol, is licensed in Vietnam but is not eligible for WHO prequalification, which restricts its global utilization; a single-dose, oral, live attenuated cholera vaccine (CVD 103-HgR: Orochol, Mutachol) by Crucell/Berna Biotech is no longer manufactured. Several new oral cholera vaccines, intended to be administered as a single dose, are in different stages of development and licensure. However, these vaccines in the most advanced stages of development, including Peru-15 (USA and China), *V. cholerae* 638 (Cuba), and VA1.4 (India), are at least a few years away from becoming widely available.
†Includes data from early vaccine trials of whole-cell recombinant beta subunit and whole-cell beta subunit vaccine for Dukoral.

recommendations for cholera vaccine use in complex emergency situations (Table 2). Both were preemptive campaigns; however, the effectiveness of the intervention was not evaluated in either setting.

In 2004, a small-scale mass vaccination campaign in Darfur, Sudan, that focused on 55,000 persons in well-organized refugee camps with limited population movements was deemed feasible because there was strong political and partner commitment, easy access to the intended population, and widespread community mobilization. The campaign lasted ≈2 months, achieved 87% 2-dose vaccination coverage, and cost US$7.10 per fully immunized person, including $6.40 for vaccine purchase and delivery and $0.70 for indirect campaign costs. A prior (1997) OCV campaign targeting 44,000 Sudanese refugees in a similar stable refugee setting in Uganda had also demonstrated feasibility, low indirect campaign costs ($0.53), and high coverage.

In 2005 post-tsunami Aceh, Indonesia, a preemptive vaccination campaign for ≈79,000 persons lasted 6 months, achieved 2-dose coverage of 69%, and cost US$18 per fully immunized person, with >$8.15 being indirect campaign costs. Here, in addition to large cold-chain volume requirements, the need for clean water for administration with the vaccine, 12% vaccine wastage, and difficulty reaching persons for the second dose, other obstacles included infrastructure destruction, disaster-related loss of critical human resources, and high population movements, conditions similar to those in Haiti in 2010 when the cholera outbreak began.

Current WHO Position on OCV Use

In March 2010, WHO issued a revised position statement regarding OCV use in disease-endemic and outbreak settings (*13*). WHO recommends OCV use in endemic settings, in conjunction with other prevention and control strategies, but the organization's position on OCV use in epidemic cholera settings is less conclusive. In outbreak situations and during complex emergencies, WHO states that preemptive vaccination, in areas determined to be at imminent risk for infection, should be considered after taking into account the local epidemiologic context and capacity to mount a vaccination campaign. However, given the limited experience, WHO states that reactive vaccination could be considered in affected areas. To guide health authorities regarding OCV use during complex emergencies, WHO proposes a 3-step predictive risk assessment approach, which considers 1) the risk for cholera outbreak, 2) outbreak containment capacity, and 3) the feasibility of conducting a mass vaccination campaign (*14*).

Table 2. WHO recommendations for cholera vaccination in complex emergencies, 2005*

- The relevance of cholera vaccination should be examined in light of other public health priorities. If vaccination is deemed necessary, water and sanitation programs should be implemented before or concurrently with vaccination.
- A high level commitment by all stakeholders and national authorities is critical.
- Vaccination with the current prequalified vaccine is not recommended by WHO once an outbreak has started, because of logistic and operational challenges.
- Vaccination campaign should not interfere with other critical public health interventions.
- Other exclusions for vaccination would include these criteria: high mortality from other causes; basic unmet needs of water, food and shelter; an ongoing outbreak of other disease; untenable security situation.

*WHO, World Health Organization.

Situation and Vaccine Deployment Capacity in Haiti

Haiti is the third largest and third most populous country in the Caribbean, with a population of ≈10 million persons living in 10 administrative departments, and has long been the poorest country in the Americas with remarkably low socioeconomic and health indicators compared with the rest of the region (*26*). Poor access to basic health care services has been evident from recently reported 2009 routine EPI (www.who.int/immunization_delivery/en) coverage of 68% for third dose of diptheria-pertussis-tetanus vaccine and 60% for the first dose of measles-containing vaccine (*27*). In 2008, only 63% of the Haitian population had access to improved water sources (such as a protected well or piped water) with only 12% receiving treated, piped water and only 17% having access to adequate sanitation (*28*). Diarrhea has been the leading cause of death among Haitian children <5 years of age (*29*), and, given the limited access to clean water and sanitation, rapid, sustained cholera transmission after introduction of the disease is not surprising (*30*). Postearthquake loss of infrastructure and human capacity, coupled with massive population displacement, worsened these preexisting conditions.

The enormous destruction caused by the earthquake disrupted the ability to initiate large-scale interventions in Haiti. A postearthquake immunization campaign using measles–rubella and diphtheria–tetanus toxoid vaccines to address the immediate threat of vaccine-preventable diseases encountered challenges that included difficulty reaching a large target population, time needed for completion (≈4 months), and achieving adequate coverage (*31*), problems that could also hamper a cholera vaccine campaign.

An assessment of vaccine deployment capacity indicated lack of adequate cold-chain capacity and a critical shortage of human resources. Furthermore, identification of a well-defined target population, a major prerequisite for

OCV campaigns (*14*), was difficult because the outbreak had spread rapidly to all 10 departments within ≈1 month (*4*); >1,000 cases were confirmed in each department by January 16, 2011 (Figure 1). Age-group and gender-specific attack rates suggested that both sexes and all age groups were at similar risk (*4*). Complicating the post-disaster situation, Tropical Storm Tomas caused severe flooding in parts of the country on November 5–6, 2010 (*32*). In addition, violence and widespread civil unrest directed toward United Nations peacekeepers, who were perceived as having introduced cholera to Haiti, occurred on November 18 (*33*). Additional protests erupted before the presidential election on November 28 and after announcements of the initial results on December 8, disrupting communications and travel for several days (*34*). The timeline of the cholera epidemic, with salient outbreak, meteorologic, social, and political events, and vaccination-related events is depicted in Figure 2. Results of the CDC Preliminary Real Time Modeling program for Projected Vaccine Impact are included in online Technical Appendix 2.

Cholera Vaccination Recommendations for Haiti

During the early phase of the outbreak, CDC and PAHO did not recommend cholera vaccination because of the severe challenges in preventing death among case-patients and controlling the rapid spread of the epidemic, constraints on available vaccine supply and on vaccine delivery resources, and the time needed from vaccine administration to development of protective immunity. In mid-December, when deaths among case-patients had decreased and vaccination recommendations were revisited, complex logistical and operational considerations and ongoing vaccine supply limitations led to recommendations to consider pilot intervention studies by some groups. On January 18, 2011, MSPP decided that cholera vaccination would be considered for Haiti only if sufficient vaccine (>1 million doses) were available to immunize a large proportion of the population with a goal to eventually reach 6 million persons (*35*; online Technical Appendix 1).

Discussion and Conclusions

Our effort highlights the in-depth consideration given to the possibility of using cholera vaccination for controlling the outbreak in Haiti, which was also considered multiple times by many partners. To date, although small-scale demonstration projects have been proposed and larger scale campaigns have been called for, cholera vaccine has not yet been used in Haiti.

Numerous challenges were identified, and efforts were made to assess them. Obstacles to vaccine use included limited resources to address the acute need for medical treatment and basic prevention services during the early epidemic phase, a limited supply of available vaccine and of WHO-prequalified vaccine, the complex planning and logistics that the 2-dose vaccine requires, and political opposition to anything less than a nationwide immunization campaign. Furthermore, identification of an equitable and politically acceptable target population for this limited vaccine supply was not possible in the heat of the epidemic, resulting in MSPP's rejection of proposals for small-scale demonstration projects.

The logistics of organizing a multidose vaccination campaign in a setting characterized by shortages of human resources, cold-chain capacity, and health system infrastructure and by a large, displaced, highly mobile population were also limitations. Additional vaccine-

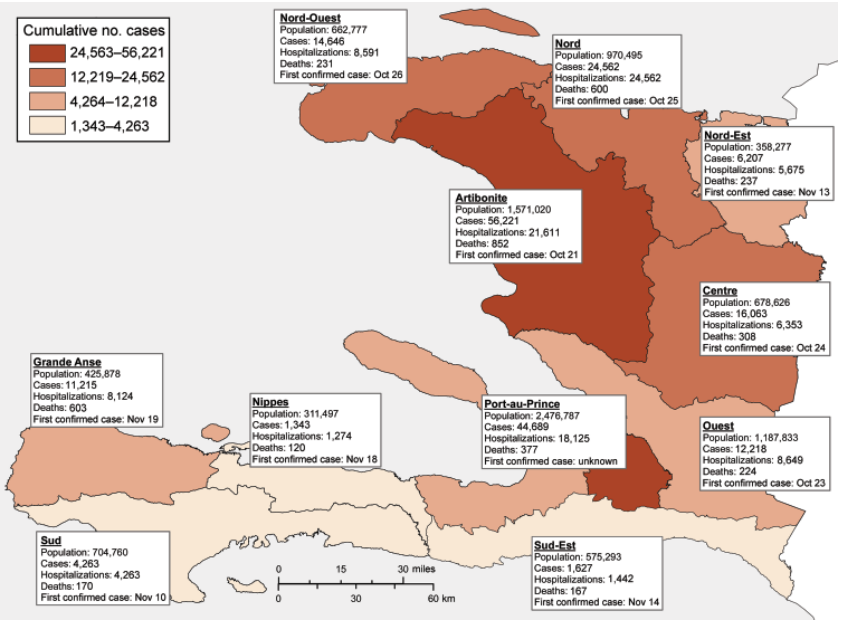

Figure 1. Distribution of cases of cholera among departments in Haiti, October 2010–January 16, 2011. Department population, earliest known date of confirmed case, and number of hospitalizations and deaths are indicated. Totals for Haiti: population, 9,923,243; cholera cases, 194,095; hospitalizations, 109,015; deaths: 3,889. Port-au-Prince includes the following communes: Carrefour, Cité Soleil, Delmas, Kenscoff, Petion-Ville, Port-au-Prince, and Tabarre. Data sources: Ministère de la Santé Publique et de la Population, Institut Haitien de Statistique et d'Informatique, Centre National de l'Information Géo Spatiale, and Laboratoire National De Santé Publique.

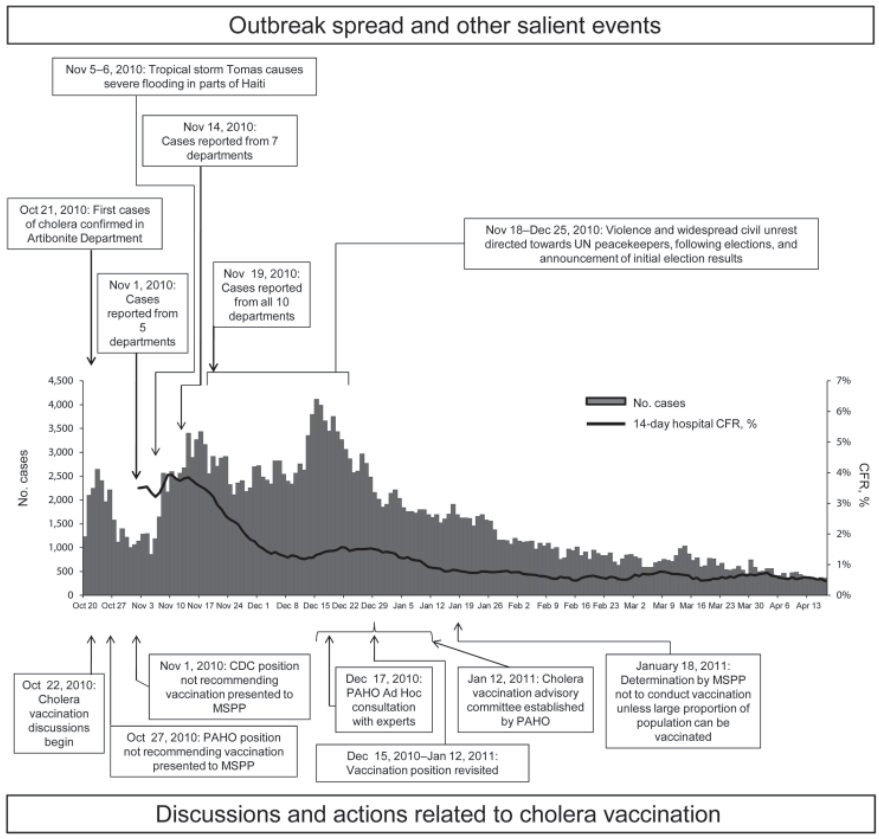

Figure 2. Events and actions related to considerations for cholera vaccination, Haiti, October 2010–April 2011. The full epicurve after January 18 is shown for reference only. Events and discussions regarding vaccination or other events after that date are not depicted. UN, United Nations; CFR, case-fatality rate; CDC, Centers for Disease Control and Prevention; MSPP, Haiti Ministère de Santé Publique et de la Population; PAHO, Pan American Health Organization.

related issues included the need for clean water for Dukoral administration and the relatively long interval after vaccination before immunity develops. A single-dose vaccine that can be administered without water would be much easier to deliver. Promising data from a Shanchol immunogenicity study in cholera-endemic Kolkata, India, found that vibriocidal antibody responses after 1 dose were equivalent to those seen after 2 doses (*36*); however, actual efficacy trials in populations previously unexposed to cholera are needed before 1dose of Shanchol could be considered for epidemic control in Haiti.

Lack of WHO prequalification was an additional impediment to use of the Shanchol vaccine in Haiti. Haiti procures vaccines through the United Nations Children's Fund, which only purchases WHO-prequalified vaccines. A decision to use a nonprequalified vaccine would require direct vaccine procurement by the Haitian government or donation by manufacturers or donors. In December 2009, the WHO Strategic Advisory Group of Experts on Immunization recommended that Shanchol be prioritized for prequalification (online Technical Appendix 1). Accelerated prequalification of cholera vaccines will be helpful for large-scale manufacturing and will reduce delays in obtaining vaccine for wider global use.

A well-defined public communications strategy in advance of a vaccine campaign would have been helpful in Haiti because the local population had no previous experience with cholera, and it was widely believed to have been introduced by external aid agencies. Active and timely monitoring of and to Adverse Events Following Immunization during a campaign would be essential; however, instituting such a monitoring system would have been problematic because of the volatile political situation with widespread unrest, which created insecurities with field operations.

Preliminary CDC real-time modeling estimates in December 2010, using data from the first 7 weeks of the outbreak, predicted only a marginal impact for outbreak control with the immediately available vaccine supply. This model had several limitations (described in detail in online Technical Appendix 2). It was a real-time effort conducted during the early response phase for planning, resource allocation, and preliminary decision making, when sparse outbreak data were available, according to conservative assumptions, which may have underestimated the impact of vaccination. In contrast, other recently published disease models using additional outbreak data and different assumptions suggest that substantial health gains could be achieved by reactive cholera vaccination. Although promising, these models may not be fully applicable to the Haitian situation: some use data from cholera-endemic countries or assumptions that may not have been consistent

with the situation in Haiti. Modeling may be useful for identifying appropriate indications for reactive OCV use in the future, particularly if precise and detailed surveillance data, which accurately reflect field conditions, are available for modeling early in an epidemic.

The careful consideration of cholera vaccination for outbreak control in Haiti yielded valuable lessons. For example, inadequate stocks of prequalified cholera vaccine prompted discussion of the establishment of a global cholera vaccine stockpile (online Technical Appendix 2) to help reduce the projected high costs of mass vaccination and overcome the inability of manufacturers to produce large stocks without a firm demand. Earlier considerations regarding the utility and relevance of a cholera vaccine stockpile indicated the need for precise guidelines for its establishment and management, accurate vaccine demand projections, and cost-effectiveness estimates (online Technical Appendix 2). The issue of equitable vaccine distribution of available global OCV supplies is essential, especially in the context of simultaneous multinational outbreaks and vaccine demands. For example, cholera outbreaks were reported in several countries coincident with the outbreak in Haiti (online Technical Appendix 2). Creating a stockpile for cholera vaccines will, therefore, require engagement of the broader global community and development of practical guidelines and strategies for its use.

WHO guidelines on control of cholera outbreaks note a potential role for pre-emptive and reactive vaccination as part of comprehensive public health intervention measures (*13*). However, as the situation in Haiti demonstrates, additional guidelines are needed on the relative priority for cholera vaccine use as outbreaks rapidly evolve in a variety of epidemiologic situations. The 3-step decision-making tool originally developed in 2005 (*14*) provides general guidance for decision making on the use of cholera vaccine before an outbreak but was not easily applicable to the complexities of the specific situation in Haiti. Modeling and field experience can help inform revisions of the WHO decision-making tool.

OCVs remain an option for cholera control globally and in Haiti, where OCVs could potentially be used to dampen the recurrence of cholera in the years to come. However, cholera vaccination should be considered in the context of the introduction of other new and underutilized vaccines and must take into account the potentially competing resource needs of the routine national immunization program. If cholera becomes endemic to Haiti, the projected preventable disease prevalence and cost-effectiveness are critical issues that will inform cholera vaccine introduction (*37*). The Strategic Advisory Group of Experts on Immunization and the Global Alliance for Vaccines and Immunization have recommended the preparation of an investment case for potential donors and national and international organizations, to provide critical information regarding OCVs and to highlight potential demand and funding gaps (*38*,*39*).

Cholera vaccination must be synergistic with other cholera prevention and control measures, and studies are ongoing to evaluate this effect (*40*). But these cohesive efforts are challenging in the context of rapidly expanding epidemics in complex post-disaster situations, where resources for essential surveillance, treatment, and other nonvaccine control measures quickly become depleted. Reactive OCV use in the setting of an outbreak requires consideration of multiple issues unique to each situation. The feasibility of OCV use has been demonstrated in other stable refugee settings (*16*) and 1 small-scale outbreak setting (*21*). Successful efforts by national and international agencies to introduce and expand the use of cholera vaccines in outbreak and post-disaster settings will depend on clear, well-informed, and specific guidelines to help countries and donors make appropriate decisions regarding reactive OCV use.

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Comparative Genomics of *Vibrio cholerae* from Haiti, Asia, and Africa

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Cholera was absent from the island of Hispaniola at least a century before an outbreak that began in Haiti in the fall of 2010. Pulsed-field gel electrophoresis (PFGE) analysis of clinical isolates from the Haiti outbreak and recent global travelers returning to the United States showed indistinguishable PFGE fingerprints. To better explore the genetic ancestry of the Haiti outbreak strain, we acquired 23 whole-genome *Vibrio cholerae* sequences: 9 isolates obtained in Haiti or the Dominican Republic, 12 PFGE pattern-matched isolates linked to Asia or Africa, and 2 nonmatched outliers from the Western Hemisphere. Phylogenies for whole-genome sequences and core genome single-nucleotide polymorphisms showed that the Haiti outbreak strain is genetically related to strains originating in India and Cameroon. However, because no identical genetic match was found among sequenced contemporary isolates, a definitive genetic origin for the outbreak in Haiti remains speculative.

The current (seventh) cholera pandemic was caused by serogroup O1 El Tor biotypes of *Vibrio cholerae*. This biotype first emerged on the Indonesian island of Sulawesi in 1961, then subsequently spread throughout Asia and Africa, where endemic and epidemic disease persists today (*1*,*2*). Seventh cholera pandemic biotypes were introduced into Peru in 1991 and subsequently spread across South and Central America, but these biotypes never reached the island of Hispaniola. Recent endemic and epidemic cases in Asia and Africa are increasingly attributed to genetically atypical El Tor variants that share characteristics of classical and El Tor strains (*1*,*3*,*4*).

After the 2010 earthquake in Haiti, an outbreak of cholera emerged that resulted in >385,000 infections and 5,800 deaths as of July 7, 2011 (*5*). The outbreak strain quickly spread to the neighboring Dominican Republic and globally as travelers returned home from affected regions (*6*,*7*). Concurrent cholera cases in the United States, linked by travel to cholera-endemic regions in Asia and Africa, were identified by national surveillance activities of PulseNet USA (Centers for Disease Control and Prevention [CDC], Atlanta, GA, USA.)

Serotyping, biotyping, and pulsed-field gel electrophoresis (PFGE) fingerprinting investigations suggested that the travel-associated cases could be genetically related to the Haiti outbreak strain (*8*). Because of the historical absence of cholera in Haiti before the 2010 earthquake, speculation abounds that the outbreak strain was imported into Haiti. Although clonality of the Haiti outbreak strain has been inferred by phenotypic characterization and

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genotypic subtyping, thereby supporting a single foreign source hypothesis (*6*,*8*), definitive evidence, e.g., by whole-genome sequencing for the genetic ancestry of the Haitian strain is lacking.

Preliminary comparative analysis of whole-genome sequences from two 2010 Haiti outbreak isolates with genomes from historical cholera cases resulted in speculation that the outbreak originated in southern Asia (*9*). However, this study lacked recent, globally distributed cholera case isolates and particularly lacked studied genomes from Africa, to which cholera is endemic. We selected contemporary *V. cholerae* isolates from clinical infections, attributed to geographically distinct locations and sharing PFGE fingerprints with Haiti outbreak strains, from the PulseNet USA database for comparative whole-genome analysis. Although detailed epidemiologic investigations are essential for unequivocally attributing geographic origin(s) and means of cholera introduction into Haiti, genome sequences of these 23 contemporary isolates showed details related to genetic content and diversity that were otherwise missed with lower-resolution PFGE subtyping, thereby providing useful genetic ancestry information for interpreting the outbreak in Haiti.

Methods

Patients and Isolates

V. cholerae isolates and travel histories from cholera case-patients in the United States were referred to CDC. A strain from an outbreak in Cameroon in 2010, isolated from a specimen received at CDC, and an isolate from South Africa likely linked to an outbreak in Zimbabwe in 2009 were also included in this study (*10*). Isolates C6706 and 3569–08 were acquired during the outbreak in Latin America in 1991 and from the US Gulf Coast in 2008, respectively. All strains were characterized as *V. cholerae* O1 on the basis of standard biochemical, cholera toxin, and serologic testing performed as described (*11*,*12*). PFGE was performed according to the PulseNet standardized protocol with restriction enzymes *Sfi*I and *Not*I; PFGE patterns were designated by using BioNumerics version 5.10 (Applied Maths Inc., Sint-Martins-Latem, Belgium) and compared by unweighted pair group method with arithmetic mean analysis (DICE coefficient 1.5% tolerance and optimization). Strain designations and other information are shown in Table 1.

Whole-Genome Data Acquisition, Assembly, and Annotation

Single-end pyrosequencing reads (GS FLX-Titanium; Roche Diagnostics, Indianapolis, IN, USA) and single-end 36-bp or 76-bp Illumina reads (GAIIe sequencer; Illumina, San Diego, CA, USA) were acquired and yielded >99% genome coverage and 32× and 240× average coverage depths, respectively (Table 2). Pyrosequencing reads were first assembled de novo by using Newbler version 2.5.3 (Roche Diagnostics). To correct potential base-calling errors attributed to homopolymers, Illumina GAIIe reads (average 14 million reads/genome) were mapped to the Newbler contigs by using CLC Genomics Workbench version 4.5 (www.clcbio.com/index.php?id=1042) and yielded an average combined coverage depth of 270× per genome.

Both chromosomes of Haiti outbreak isolate 2010EL-1786 were sequenced to full closure by using PCR and Sanger sequence-based bridging of contigs and a fosmid library of templates. Optical mapping also supported the contig ordering derived for 2010EL-1786. For all remaining isolates, Illumina-supplemented, homopolymer-corrected, Newbler-assembled contigs were prepared as pseudogenomes by first linking contigs with a linker sequence containing stop codons in all 6 translation reading frames. These high-coverage pseudogenomes were used for downstream analyses. Identification of coding sequences was achieved by using Glimmer3 (*14*). Genome annotation was achieved by using an automated, in-house, modified version of GenDB version 2.2 (*15*) and manual curation for regions of interest.

Comparative Genomics

Whole-Genome Alignment and Core Genome Phylogeny

Whole-genome alignments of all study isolates and 5 available reference *V. cholerae* genomes (Table 1) were performed by using Progressive Mauve (*16*) and visualized by using PhyML 3.0 (*17*). To determine vertical inheritance patterns, study genomes were analyzed with historical *V. cholerae* genomes (isolates M66–2, MJ-1236, CIRS101, and N16961) by using phylogenetic analysis of high-quality single-nucleotide polymorphisms (hqSNPs) contained in core genes. Coding region predictions were analyzed by using parallelized BLASTn (http://blast.ncbi.nlm.gov/Blast.cgi) to identify highly similar orthologs in all strains. Highly similar orthologs were defined as those containing a high-scoring segment pair >400 bp and identity ≥97%. Each orthologous loci set was multiply aligned by using ClustalW (*18*). Multiple alignments were manually inspected to remove erroneously aligned regions; indel-associated SNPs and loci containing ≥30 SNPs were also excluded. Each SNP column from each multiple nucleotide alignment was analyzed for hqSNPs, defined as those containing no gaps or ambiguous basecalls, and having an adjusted quality score >90 (of a maximum score of 93). A total of 4,376 hqSNPs were identified from 632 orthologous loci and extracted from the alignments to prepare a compressed

pseudoalignment composed of hqSNPs (online Technical Appendix 1, www.cdc.gov/eid-static/spreadsheets/11-0794-Techapp1.xls). This pseudoalignment was used to build a maximum-likelihood phylogenetic tree by using PhyML 3.0 (*17*). Branch confidences were estimated by using the approximate likelihood-ratio test (*19*).

BLAST Atlases

A circular BLAST atlas was generated for each chromosome by using Haiti isolate 2010EL-1786 as mapping reference. Glimmer3 was used to predict coding sequences contained on pseudogenomes for the remaining isolates sequenced in this study and for 4 available genomes (*14*). Reference isolate 2010EL-1786 was mapped against the resulting translated coding sequences by using BLASTx with a percentage identity cutoff value of 70% and an expected cutoff value of 1×10^{-10} for high-scoring segment pairs >100 aa. The results were visualized by using GView (*20*). Sequence accession numbers are shown in Table 1.

Results

*Sfi*I and *Not*I PFGE Patterns of Recent Global Cholera Isolates

Nine *V. cholerae* isolates directly associated with the outbreak on Hispaniola were examined, 7 of which had

Table 1. Characteristics of *Vibrio cholerae* isolates from Haiti, Asia, Africa, and the United States*

Isolate	Serotype	*ctxB* allele†	PFGE patterns		Date of collection	Origin of infection	Comment or GenBank accession no. (reference)
			*Sfi*I	*Not*I			
2010EL-1961	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2010 Oct 17	Haiti	Earliest Haiti outbreak case
2010EL-1786	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2010	Artibonite, Haiti	None
2010EL-1792	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2010	Artibonite, Haiti	None
2010EL-1798	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2010	Haiti	None
2010EL-2010N	Ogawa	B-7	KZGS12.0160	KZGN11.0134	2010	Haiti	Nonhemolytic
2010EL-2010H	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2010	Haiti	Hemolytic
2011EL-1089	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2010 Nov 27	South Department, Haiti	None
2011EL-1133	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2011 Jan 26	Northwest Department, Haiti	Travel associated
2011V-1021	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2011	Dominican Republic	Travel associated
2009V-1085	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2009	Sri Lanka/India	Travel associated
2009V-1096	Inaba	B-7	KZGS12.0088	KZGN11.0092	2009	India	Travel associated
2010EL-1749	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2010	Cameroon	Outbreak
2009V-1131	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2009	India	Travel associated
3554–08	Ogawa	B-7	KZGS12.0088	KZGN11.0092	2008	Nepal	Travel associated
2011EL-1137	Ogawa	B-1	KZGS12.0089	KZGN11.0092	2009	South Africa	Outbreak
2009V-1046	Ogawa	B-1	KZGS12.0088	KZGN11.0092	2009	Pakistan	Travel associated
2009V-1116	Ogawa	B-1	KZGS12.0088	KZGN11.0092	2009	Pakistan	Travel associated
2010V-1014	Ogawa	B-1	KZGS12.0088	KZGN11.0092	2010	Pakistan	Travel associated
3582–05	Inaba	B-1	KZGS12.0088	KZGN11.0092	2005	Pakistan	Travel associated
3500–05	Inaba	B-1	KZGS12.0088	KZGN11.0092	2005	India	Travel associated
3546–06	Inaba	B-1	KZGS12.0088	KZGN11.0092	2006	India	Travel associated
3569–08	Inaba	B-1	KZGS12.0055	KZGN11.0029	2008	US Gulf Coast	Environmental isolate
C6706	Inaba	B-3	KZGS12.0114	KZGN11.0033	1991	Peru	Latin America outbreak
CIRS101‡	Inaba	B-1	Unknown	Unknown	2002	Dhaka, Bangladesh	NZ_ACVW00000000 (*4*)
MJ-1236‡	Inaba	B-1	Unknown	Unknown	1994	Matlab, Bangladesh	NC_012667, NC_012668 (*4*)
O395‡	Ogawa	B-1	Unknown	Unknown	1965	India	NC_009456, NC_009457 (*1*)
N16961‡	Inaba	B-3	Unknown	Unknown	1970s	Bangladesh	NC_002505, NC_002506 (*13*)
M66–2‡	Unknown	§	Unknown	Unknown	1937	Makassar, Indonesia	NC_012578, NC_012580 (*1*)

**ctxB*, cholera toxin subunit B; PFGE, pulsed-field gel electrophoresis.
†B-7, classical allele, Orissa variant; B-1, classical allele; B-3, El Tor allele (*2*).
‡These isolates have been sequenced by others investigators and sequences have been deposited in GenBank. PFGE was not performed on these isolates.
§This isolate does not contain *ctxB*.

Table 2. Next-generation sequence average coverage and number of mapped reads for *Vibrio cholerae* isolates from Haiti, Asia, and Africa

Isolate	No. mapped Illumina reads*	Average Illumina coverage*	No. 454 aligned reads†	Average 454 coverage†
2009V-1046	12,100,798	167.5	288,870	28
2009V-1085	13,679,291	187.8	365,484	33
2009V-1096	14,818,679	205.2	649,798	60
2009V-1116	13,486,955	181.8	264,833	23
2009V-1131	1,370,5972	185.9	273,608	24
2010EL-1749	16,654,195	189.7	735,029	51
2010EL-1786	26,312,006	343.8	216,539	17
2010EL-1792	23,073,959	295.9	239,940	19
2010EL-1798	27,914,201	369.9	270,493	21
2010V-1014	15,247,545	195.5	501,200	44
3500–05	10,962,437	268.6	279,246	27
3546–06	14,625,431	331.0	238,176	22
3569–08	15,920,777	201.4	228,302	18
3582–05	12,181,066	302.0	621,605	62
C6706	15,578,468	349.4	363,226	35
2010EL-1961	9,077,044	229.0	415,643	40
2011EL-1089	10,841,303	263.2	194,828	17
2011EL-1133	12,544,418	283.8	112,039	10
2011EL-1137	11,703,624	285.2	505,482	48
2011EL-2010N	12,178,627	323.5	409,268	41
2011V-1021	11,274,787	282.8	213,312	20
2010EL-2010H	11,366,854	291.4	422,937	40
3554–08	16,149,256	373.9	498,131	45

*Determined by using GAIIe Sequencer; Illumina, San Diego, CA, USA.
†Determined by using 454 Sequencer; 454 Life Sciences, Branford, CT, USA.

indistinguishable *Sfi*I and *Not*I PFGE patterns designated PulseNet USA patterns KZGS12.0088 and KZGN11.0092, respectively (Table 1). Also sequenced were a hemolytic variant and a nonhemolytic variant that harbored a minor variation of the main Haiti outbreak PFGE pattern and were derived from an isolate from 1 patient in Haiti (Table 1). Twelve contemporary *V. cholerae* isolates from global sources with matched PFGE fingerprints were also sequenced. Infections for these 12 contemporary isolates originated (by documented patient travel) from regions of Pakistan, India, or Nepal. Two additional isolates were from patients in outbreaks in Cameroon and South Africa likely connected to the cholera outbreak in Zimbabwe in 2009 (*21*). Although all sequenced clinical isolates were serogroup O1, Inaba and Ogawa serotypes were observed among PFGE pattern-matched isolates (Table 1). All strains were biotype El Tor and all produced cholera toxin.

Phylogenetics of Strains

Haiti outbreak isolates and 12 global PFGE pattern-matched *V. cholerae* isolates belong to phylogroup 1 of the seventh pandemic clade. The phylogenetic tree based on whole-genome sequencing showed clustering of the 9 Hispaniola isolates (8 from Haiti and 1 related isolate from the Dominican Republic) with 12 other PFGE pattern-matched isolates. All 21 isolates were in 1 cluster relative to non-PFGE–pattern-matched outliers (Figure 1). When compared with historical reference genomes, the closest ancestors for Haiti genome sequences (2010–2011; derived herein) were isolates CIRS101 from Dhaka, Bangladesh (2002) and MJ-1236 from Matlab, Bangladesh (1994). These data confirm the genetic relatedness also inferred by PFGE subtyping and further support inclusion of the Haiti outbreak isolates in phylogroup 1 of the seventh pandemic clade (Figure 1). The whole-genome sequencing dataset showed that additional underlying genetic diversity was present across PFGE pattern-matched isolates (including 9 isolated from Hispaniola) not observed by PFGE subtyping.

Common Mobile Elements and Genes of Haiti Outbreak Strain and PFGE Pattern-matched Isolates

V. cholerae macrodiversity is commonly attributed to presence or absence of mobile genetic elements (*22*). The contiguous genome derived for Haiti isolate 2010EL-1786 was used as the outbreak type strain and harbored 2 circular chromosomes of 3.03 Mbp (chromosome I) and 1.05 Mbp (chromosome II), which encoded 2,920 and 1,051 predicted coding sequences, respectively. Pairwise comparisons of all coding sequences from each study genome with all coding sequences from reference isolate 2010EL-1786 (all vs. all comparison) showed congruent gene content and low overall diversity on larger chromosome I (Figure 2). One noteworthy exception was the absence of *Vibrio* pathogenicity island 1 in the 2005 isolate 3582–05 from Pakistan. This island contains essential cholera virulence factors, including the *tcp* gene cluster, which encodes toxin-

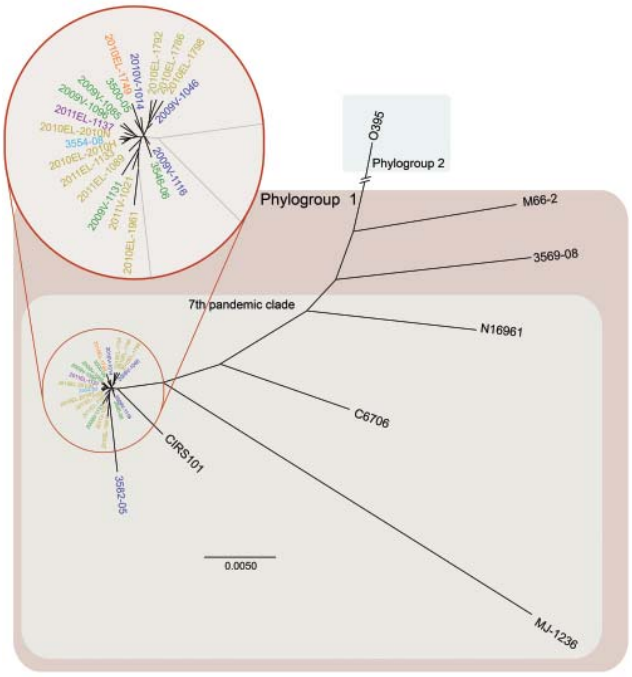

Figure 1. Whole-genome neighbor-joining tree of *Vibrio cholerae* isolate from cholera outbreak in Haiti, fall 2010; concurrent clinical isolates with pulsed-field gel electrophoresis pattern-matched combinations; reference isolates sequenced in this study; and available reference sequences. Sequence alignments of quality draft or complete genomes were performed by using Progressive Mauve (*16*) and visualized by using PhyML version 3.0 (*17*). Whole-genome relationship of Haiti isolates with closest genetic relatives is shown in the inset. Scale bar indicates nucleotide substitutions per site.

coregulated pilus involved in *V. cholerae* colonization of the human intestine and necessary for horizontal transfer of the cholera toxin bacteriophage. This finding was the only macroscopic difference observed between isolate 3582–05 and PFGE matches. All Haiti outbreak and PFGE pattern-matched isolates contain an integrated conjugative element belonging to the SXT/R391 family (SXT-ICE) that carries genes conferring antimicrobial drug resistance. No macroscopic differences were observed in SXT-ICE among Haiti outbreak and PFGE pattern-matched isolates (Figure 2; online Technical Appendix 2 Figure 1, panel A, wwwnc.cdc.gov/EID/pdfs/11-0794-Techapp2.pdf).

Smaller chromosome II was more content variable and divergent across study strains. These findings were largely attributable to the hypervariable superintegron region, an ≈120-kb gene capture system predominantly encoding hypothetical proteins (Figure 2; online Technical Appendix 2 Figure 1, panel B) (*13*). Gene polymorphisms observed in the 9 sequenced isolates from Hispaniola also localized primarily within the superintegron region. Despite these observed differences, no major deletions in the superintegron were observed among PFGE pattern-matched isolates (Figure 2; online Technical Appendix 2 Figure 1). Thus, phylogeny derived from *V. cholerae* whole-genome sequencing (Figure 1) showed genetic diversity within PFGE pattern-matched isolates. However, binary (present or absent) gene content assessment failed to pinpoint extensive contiguous diversity outside the superintegron region.

Shared Ancestry between Isolates from Haiti, India, and Cameroon

A core genome phylogeny was constructed on the basis of 4,376 hqSNPs found within 632 orthologous core genes (0.81 Mbp) that were universally present in all 27 study and reference genomes (online Technical Appendix 1; online Technical Appendix 2 Figure 2). Among 9 sequences from Hispaniola isolates, 0–2 SNPs were observed (online Technical Appendix 2 Figure 2). Hispaniola isolates differed from PFGE pattern-matched genomes from other locations by 4–25 SNPs, and genomes with nonmatched PFGE patterns differed from the outbreak isolates by 13–3,361 SNPs. Notably, phylogeny based on hqSNPs showed clustering of the Haiti strain with 3 epidemiologically unrelated clinical isolates, which represented isolates from 2 travelers from the United States to India in 2009 and a patient in Cameroon in 2010. Isolates 2009V-1085 (India, 2009), 2009V-1096 (India, 2009), and 2010EL-1749 (Cameroon, 2010) were most related to the Haiti isolates. These 3 isolates had 4–7 core hqSNPs when compared with the outbreak strain, and the derived sequence for a 2008 clinical isolate from Nepal differed from outbreak isolates by 7–8 core hqSNPs (online Technical Appendix 1; online Technical Appendix 2 Figure 2).

Conversely, historical isolates (1970–2005) from Pakistan, Bangladesh, the US Gulf Coast, and South America, and recent clinical isolates (2009–2010) from cases linked to Pakistan or South Africa independently clustered away from Haiti outbreak isolates (online Technical Appendix 2 Figure 2). Clade analysis of outbreak isolates and highly related isolates 2009V-1085, 2009V-1096, and 2010EL-1749, identified 25 hqSNPs in 24 conserved loci that distinguish members of this clade (online Technical Appendix 2 Figure 3; online Appendix Table, wwwnc.cdc/gov/EID/article/17/11/11-0794-TA1.htm). Resulting distances suggest that the outbreak isolates have a closer genetic relationship with 2009V-1085 and 2009V-1096 from India (7–10 hqSNPs) than with 2010EL-1749 from Cameroon (10–13 hqSNPs).

Comparison of Haiti Outbreak Genomes

Across the 18 described hypervariable *V. cholerae* mobile genetic elements sequences (representing >300 kb

of the total genome), no macroscopic differences were observed among the 9 Hispaniola isolate sequences (Figure 2; online Technical Appendix 2 Figure 1), and as stated, only 2 hqSNPs were identified in the core genome. Pairwise alignment of the complete genome of study reference 2010EL-1786 with available genome data for 2 sequenced Haiti 2010 outbreak isolates, designated H1 and H2 (*9*), showed only 3 polymorphisms across the entire genome. However, because the available H1 and H2 consensus sequences contain ambiguous basecalls, these nucleotides were excluded from our comparative analyses. Nonetheless, these data confirm the clonal nature of the Haiti outbreak strain.

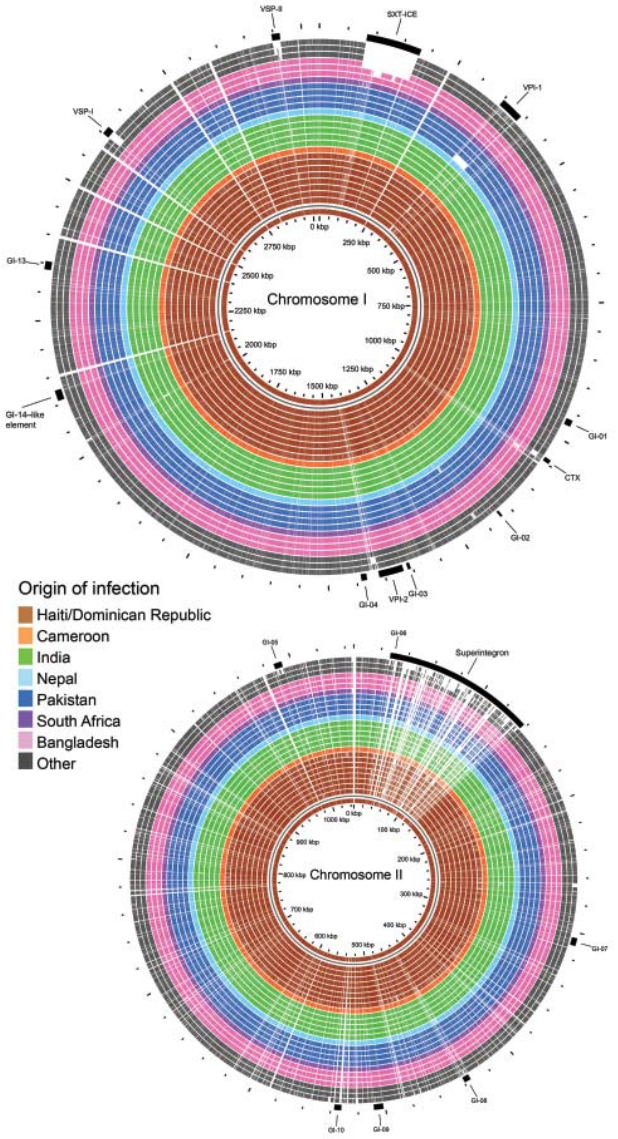

Figure 2. BLAST (http://blast.ncbi.nlm.gov/Blast.cgi) atlas of predicted protein homologies mapped against the closed genome of Haiti *Vibrio cholerae* outbreak type isolate 2010EL-1786, fall 2010. Full color saturation represents 100% sequence homology, and gaps indicate regions of divergence. Gaps in the innermost (red) circle for reference isolate 2010EL-1786 represent gaps between coding sequences, not genetic diversity. A) Chromosome I; B) chromosome II. From center: Haiti/Dominican Republic isolates 2010EL-1786, 2010EL-1961, 2011EL-1089, 2010EL-2010N, 2010EL-2010H, 2011V-1021, 2010EL-1798, 2010EL-1792, and 2011EL-1133; Cameroon isolate 2010EL-1749; India isolates 2009V-1085, 2009V-1096, 2009V-1131, 3546–06, and 3500–05; Nepal isolate 3554–08; Pakistan isolates 3582–05, 2009V-1046, 2010-V1014, and 2009V-1116; South Africa isolate 2011EL-1137; Bangladesh isolates CIRS101, MJ-1236 and N16961; and other isolates C6706, M66–2, and 3569–08.

Structural and Alleleic Profiles of Isolates Carrying a Hybrid Cholera Toxin Prophage

Structure and allelic profiles of the CTXϕ prophage have been used for *V. cholerae* lineage analysis (*23*). Chromosome I of Haiti isolate 2010EL-1786 harbors 1 hybrid CTXϕ characterized by a 1-nt variant of the classical *ctxB* allele (*ctx*B-7) and El Tor *rstR* flanked by a toxin-linked cryptic element and El Tor–type RS1 element with an intact *rstC* locus (Figure 3). The SNP at *ctxB* codon19 results in replacement of the classical cholera toxin B histidine residue with asparagine, and this *ctxB*-7 allele was observed among all Hispaniola isolates (Table 1). Five of the 12 PFGE pattern-matched isolates from other locations (2008–2010) also shared this variant *ctxB* allele. The remaining 7 PFGE pattern-matched isolates encoded classical *ctxB* alleles.

Discussion

Public health investigators use PFGE, the current standard technique for subtyping most bacterial enteric pathogens, to link patients infected with a particular pathogen to a specific infection source(s) by fingerprint matching to pathogens isolated from environmental samples. Whole-genome sequencing has recently emerged as an enhanced laboratory tool for high-resolution analysis of microbial diversity and has been successfully used to investigate bacterial disease outbreaks (*24–26*). Because whole-genome sequencing can provide pathogen genetic fingerprints at single-nucleotide resolution, it should revolutionize the diagnosis, surveillance, and control of microbial diseases.

For molecular epidemiologic investigations using whole-genome sequencing, an expansive number of isolates from an outbreak would ideally be selected to ensure broad coverage for possible genotype variants within that population that might otherwise be masked with lower-resolution typing methods. In addition, outlier isolates from different locations that are indistinguishable or related by several diverse subtyping methods should also be subjected to whole-genome sequencing to contextualize the diversity seen within the outbreak population and to find other clonal relationships In this study, a temporal and

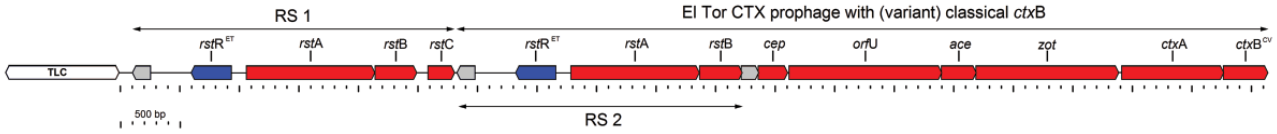

Figure 3. Genetic structure of cholera toxin (CTX) prophage and associated elements in Haiti cholera outbreak *Vibrio cholerae* isolate 2010EL-1786, fall 2010. The toxin-linked cryptic (TLC) element is not drawn to scale. Black arrows indicate the direction of transcription for each coding region. Red, forward transcription; blue, reverse transcription; gray, predicted open reading frame with no experimental evidence.

geographic distribution of outbreak isolates was selected to confirm clonality of the outbreak strain and to gain insight into the microevolution of *V. cholerae* during an outbreak. Additionally, minor PFGE and nonhemolytic variants observed among outbreak isolates were also sequenced to confirm their clonal relationships with isolates exhibiting the main outbreak pattern and phenotype.

The PulseNet USA database substantially contributed to this work by identifying genetically related (using PFGE typing) and epidemiologically relevant isolates for whole-genome sequencing analyses. Notably, one 2008 isolate from a traveler from the United States to Nepal was identified and included in this study, although we acknowledge that the evolutionary relationship of the Haiti strain to strain(s) circulating in Nepal during 2010 may not be ideally represented by this 2008 isolate. Microbial evolution will have occurred during 2008–2010, and global travel may have introduced additional strains into Nepal in the interim, such that the 2008 isolate from Nepal may differ substantially from a strain circulating in Nepal in 2010, the suggested progenitor of the outbreak strain. Unfortunately, 2010 isolates from Nepal were not available for analysis.

Also identified in the PulseNet USA database was 1 PFGE pattern-matched isolate from western Africa. The close genetic relationship of this isolate from Cameroon to the Haiti strain suggests that a potential link between western Africa and the Haiti outbreak cannot be ignored. Further studies on additional isolates from western Africa are required to confirm or refute this possibility. Similarity of whole-genome sequences for Haiti isolates, PFGE pattern-matched isolates, and other seventh pandemic strains confirmed the clonal nature of the 2010–2011 cholera outbreak strain and the close genetic relationships for the studied strains initially suggested by PFGE subtyping (Figure 1). Previous *V. cholerae* studies have reported that seventh pandemic strains are clonal, sharing near identical gene content on a highly related genome backbone but containing variable mobile genetic elements or gene cassettes (*27*). Despite dynamic horizontal gene transfer (*22*), we identified only a few nucleotide differences among mobile sequences of the 9 sequenced 2010–2011 outbreak-related Hispaniola isolates and the 12 recent PFGE pattern-matched clinical isolates (Figure 2).

Extensive recombination in *V. cholerae* genomes may confound evolutionary relationship analyses as strains and lineages undergo reassortment (*1*). However, base substitutions acquired horizontally as recombination segments generally occur with localized density (*28*). Although we cannot guarantee that recombinant segments were absent from the core genome phylogeny (online Technical Appendix 2 Figure 2), the even spatial and genome-wide distribution of core genome hqSNPs suggests that they were vertically inherited. We have derived a useful phylogenetic approximation of isolate relatedness on the basis of hqSNPs, which supports shared ancestry for the Haiti outbreak isolates and 12 recent clinical isolates sharing PFGE patterns (online Technical Appendix 2 Figure 2). Sequenced isolates from India and Cameroon (2009–2010) were shown to be the closest genetic relatives among the non-Hispaniola isolates (isolated in 1991–2010; this study) and 4 other available reference *V. cholerae* genomes (isolated in 1937–2002). The c*txB* allele variant (*ctxB*-7) of the Haiti strain (and its genetic relatives) was first observed among isolates from a cholera outbreak in Orissa, India, in 2007 (*29*), but the *ctxB*-7 allele has since also been observed in isolates from southern Asia and more recently from western Africa (*8*,*30*).

The genetic makeup of the Haiti outbreak strain will likely have substantial public health implications for Haiti and other susceptible locations. Our reasoning is that the atypical O1 El Tor *V. cholerae* strains (CIRS101 and CIRS101-like variants) have already emerged as the predominant clone causing cholera in Asia and Africa and have displaced prototypical O1 El Tor strains (*3*,*4*,*29*). Unfortunately, atypical O1 El Tor *V. cholerae* strains appear to have retained the relative environmental fitness of their prototypical O1 El Tor ancestors while acquiring enhanced virulence traits, such as classical or hybrid CTX prophage and SXT-ICE (*4*). Thus, with higher relative fitness and virulent and antimicrobial drug–resistant phenotypes, the Haiti outbreak strain harbors infectivity and ecologic persistence advantages over other seventh pandemic strains. Consequently, the Haiti outbreak strain

(or its genetic ancestor) may easily replace current El Tor *V. cholerae* strains circulating in the Western Hemisphere to become endemic (like other atypical El Tor strains) and will likely cause future outbreaks. Such dire predictions warrant enhanced epidemiologic surveillance and renewed priorities aimed at cholera prevention.

Absence of cholera in Haiti over the past century; the clonal nature of the outbreak strain; and a massive influx of international travelers, aid workers, and supplies after the 2010 earthquake suggest an outside infection source for the 2010–2011 outbreak. Our core genome phylogeny (online Technical Appendix 2 Figure 2) suggests that the Haiti outbreak strain most likely derived from an ancestor related to isolates from within or near the Indian subcontinent. However, concurrent identification of a 2010 isolate from Cameroon as a close genetic relative of the Haiti outbreak strain illustrates that whole-genome sequencing on such a relatively small number (n = 27) of *V. cholerae* isolates is insufficient to exclude other plausible ancestral geographic locations.

Our study results are consistent with recent findings of Chin et al. (*9*), who concluded that two 2010 Haiti outbreak isolates shared ancestry with variant O1 El Tor strains isolated in Bangladesh in 2002 and 2008 and a more distant relationship with an isolate from an outbreak in Latin American in 1991. The vertical inheritance pattern of hqSNPs in our study provide unequivocal genetic evidence for introduction of the outbreak strain into Haiti from an external source as opposed to local aquatic emergence. However, the specific geographic source and mode of entry of the outbreak strain into Haiti cannot be proven by microbiological investigations. Only large-scale epidemiologic studies and microbiological data can provide conclusive evidence of how cholera was introduced into Haiti. This whole-genome sequencing study provides expanded evidence that variant O1 El Tor *V. cholerae* appeared in Haiti by importation and has generated a whole-genome sequencing dataset for future study.

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Characterization of Toxigenic *Vibrio cholerae* from Haiti, 2010–2011

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In October 2010, the US Centers for Disease Control and Prevention received reports of cases of severe watery diarrhea in Haiti. The cause was confirmed to be toxigenic *Vibrio cholerae*, serogroup O1, serotype Ogawa, biotype El Tor. We characterized 122 isolates from Haiti and compared them with isolates from other countries. Antimicrobial drug susceptibility was tested by disk diffusion and broth microdilution. Analyses included identification of *rstR* and VC2346 genes, sequencing of *ctxAB* and *tcpA* genes, and pulsed-field gel electrophoresis with *Sfi*I and *Not*I enzymes. All isolates were susceptible to doxycycline and azithromycin. One pulsed-field gel electrophoresis pattern predominated, and *ctxB* sequence of all isolates matched the B-7 allele. We identified the *tcpET*^{CIRS} allele, which is also present in Bangladesh strain CIRS 101. These data show that the isolates from Haiti are clonally and genetically similar to isolates originating in Africa and southern Asia and that *ctxB*-7 and *tcpET*^{CIRS} alleles are undergoing global dissemination.

Vibrio cholerae has caused epidemics around the world for centuries. Cholera has long been a companion of devastation and poverty, and epidemics occur in areas without clean water, sanitation, or accessible health care. The collapse of Haiti's infrastructure after the January 2010 earthquake created conditions suitable for cholera to affect the country's vulnerable population.

The first clinical cases of *V. cholerae* infection in Haiti in >100 years were seen on October 17, 2010. Cholera, primarily a waterborne disease, quickly spread from its origin along a main river in the Artibonite Department north of Port-au-Prince to all 10 departments in Haiti and to the Dominican Republic. Earlier that year, in anticipation of outbreaks after the devastation of the January 2010 earthquake, the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) had collaborated with local scientists to tactically position rapid diagnostic tests and had provided training in their use. These tests enabled early recognition of the 2010 cholera outbreak in Haiti. By October 21, 2010, scientists at the Haiti National Public Laboratory (Laboratoire National de Santé Publique) cultured *V. cholerae* and sent isolates to CDC (*1*).

Our objectives were to confirm the identification of *V. cholerae*, characterize the isolates by using multiple genetic and phenotypic methods, evaluate the clonality of the isolates from Haiti, and attempt to explore the genetic origin of the strain. Isolates from Haiti were compared with isolates from western and eastern Africa, southern Asia, Latin America, the Middle East, and the Gulf Coast of the United States.

Methods

Bacterial Strains

A total of 122 *V. cholerae* isolates from all 10 departments in Haiti were characterized. We also included 25 *V. cholerae* isolates that showed hemolytic and nonhemolytic phenotypes on sheep blood agar and 2

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chloramphenicol-resistant colonies that grew inside the zone of inhibition, for a total of 149 isolates from Haiti. The isolates arrived in 4 groups that were received on October 26, 2010 (16 isolates), November 26, 2010 (92 isolates), January 27, 2011 (30 isolates), and February 9, 2011 (11 isolates). We also studied 51 *V. cholerae* strains from other countries; they were obtained during past outbreaks and from sporadic cases and ongoing CDC surveillance of travelers entering the United States. Additional strains were provided by collaborators at CDC-Kenya Medical Research Institute (Kisumu, Kenya); Naval Medical Research Unit 3 (Cairo, Egypt); and the National Institute of Communicable Diseases (Johannesburg, South Africa). The origins of the strains evaluated in this study are shown in Table 1. Isolate CDC 2010EL-1786 from Haiti was deposited in the American Type Culture Collection (ATCC; BAA-2163).

Table 1. Strains used in characterization study of *Vibrio cholerae* from Haiti, 2010–2011*

Location	No. isolates	Year(s) collected
Afghanistan	2	2008
Cameroon	1	2010
Djibouti	2	2007
Ethiopia	1	2009
Haiti	149	2010–2011
India	6	2005–2009
Kenya	14	2007–2009
Nepal	1	2008
Nigeria	2	2008
Oman	1	2007
Pakistan	6	2005–2010
Peru	2	1991, 1998
Somalia	2	2008
South Africa	2	2009
Sri Lanka	1	2007
Sudan	5	2007
Togo	2	2009
US Gulf Coast	1	2007
Total	200	

*Isolates collected during 1991–2011.

Confirmation and Characterization of *V. cholerae*

All isolates were confirmed positive by using standard methods (*2*). Serogroup and serotype were determined by using specific antisera (Lee Laboratories, Franklin Lakes, NJ, USA). Saline controls were included to detect autoagglutination. Biotypes were determined by PCR of specific regions of the biotyping (*tcpA*) gene (*3*).

Antimicrobial Drug–Susceptibility Testing

Pure cultures were tested by disk diffusion on Mueller-Hinton agar without blood with amoxicillin/clavulanate, ampicillin, chloramphenicol, ciprofloxacin, furazolidone, nalidixic acid, streptomycin, sulfisoxazole, tetracycline (as a marker for doxycycline), and trimethoprim/sulfamethoxazole (Becton Dickinson, Franklin Lakes, NJ, USA). ATCC (Manassas, VA, USA) strains 25922 (*Escherichia coli*), 29213 (*Staphylococcus aureus*), and 27853 (*Pseudomonas aeruginosa*) served as internal quality controls. Results were interpreted according to Clinical and Laboratory Standards Institute guidelines (*4*).

Broth microdilution was run on 122 isolates from Haiti and on all isolates from non-Haiti locations. Broth microdilution methods were performed according to the manufacturer's instructions by using CAMPY and CMV1AGNF Sensititer Plates (both from Trek Diagnostics, Cleveland, OH, USA) with 2 modifications: we transferred 50-μL (CAMPY plates) and 20-μL (Sensititer plates) volumes from the suspension to the broth and used Mueller-Hinton broth without blood for the CAMPY panel. Antimicrobial drug sensitivity results from Sensititer plate testing were available for amoxicillin/clavulanate, ampicillin, azithromycin, chloramphenicol, ciprofloxacin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. Both panels were inoculated at concentrations of 5×10^4 and 5×10^5 CFU/mL. Internal quality controls included those used for disk-diffusion testing plus ATCC 29212 (*Enterococcus faecalis*). Where available, specific interpretive criteria for *V. cholerae* were used (*5*). For drugs with no criteria, interpretation was guided by using Clinical and Laboratory Standards Institute criteria for *Enterobacteriaceae* or consensus breakpoints used by the National Antimicrobial Resistance Monitoring System (*6*).

Pulsed-Field Gel Electrophoresis

Isolates were analyzed by using a PulseNet standardized pulsed-field gel electrophoresis (PFGE) protocol for *V. cholerae* (*7*) with *Sfi*I and *Not*I restriction enzymes (Roche Molecular Biochemicals, Indianapolis, IN, USA). Images of restriction patterns were analyzed by using BioNumerics software (Applied Maths, Inc., Austin, TX, USA). Gel patterns were compared with others in the National PulseNet *V. cholerae* database (www.cdc.gov/pulsenet/whatis.htm) and the PulseNet International *V. cholerae* database (www.pulsenetinternational.org/protocols/Pages/vcholeraedatabase.aspx).

Detection of Virulence and Species-specific Genes, PCR, and Sequencing

We amplified DNA from boiled lysates for 30 cycles in a multiplex PCR to detect cholera toxin gene subunit A (*ctxA*) (*8*) sequences, biotyping genes (*tcpA*) (*3*), and species-specific genes *ompW* (*9*), and *toxR* (*10*) by using the primers and methods described. Primer pair *smp*-F and *smp*-R (*11*) was used to amplify the seventh pandemic–specific gene VC2346 at cycling conditions of 93°C for 15 min; 35 cycles of 92°C for 40 s, 52°C for 1 min, and 72°C for 1.5 min; followed by 72°C for 7 min.

The primers and cycling conditions for PCR amplification of biotype-specific repeat sequence transcriptional regulator (*rstR*) alleles were used as described (*12*). We sequenced 13 isolates from Haiti and from the strain from the US Gulf Coast by using the same primer set to verify results.

The complete coding region of the cholera toxin gene *ctxAB* was amplified with flanking primer pair primers S86 ($ctxAB_1$) and S87 ($ctxAB_2$) (*13*,*14*). A step-down PCR to avoid nonspecific amplification was run as follows: 15 min at 93°C; 11 cycles of 92°C for 40 s, decrementing by 1°C from 60°C to 50°C for 1 min, 72°C for 1.5 min; followed by 30 cycles of 92°C for 40 s, 50°C for 1 min, 72°C for 1.5 min; with a final extension at 72°C for 7 min. Sequences were determined with amplification primers and 2 internal primers, CTX93-F and CTX618-R (*15*). The complete *tcpA* gene was amplified and sequenced with primer pair *tcpH*1 and *tcpA*4 (*16*). PCR cycling conditions were 93°C for 15 min followed by 35 cycles of 92°C for 40 s, 52°C for 1 min, and 72°C for 1.5 min, with a final extension at 72°C for 7 min.

We purified *ctxAB*, *tcpA*, and *rstR* amplicons by using the QIAquick PCR Purification Kit (QIAGEN, Inc., Valencia, CA, USA). Sequencing was performed on the Applied Biosystems 3730 DNA analyzer with POP-7 polymer and a 50-cm capillary array (all from Life Technologies, Carlsbad, CA, USA) following the manufacturer's instructions. Chromatograms were assembled by using Lasergene SeqMan Pro version 8.0.2 (www.dnastar.com). Sequences were aligned with other *V. cholerae* sequences by using ClustalW (www.clustal.org) in MEGA4 software (*17*) and trimmed in-frame for analyses. The full genome of 1 *V. cholerae* strain from Haiti (2010EL-1786 [ATCC BAA-2163]) and partial genomic regions (integrated conjugative elements and cholera toxin phage) of other isolates were sequenced as described (*18*).

Results

Isolates

We identified all 149 isolates from Haiti as *V. cholerae*, serogroup O1, serotype Ogawa, biotype El Tor, containing species-specific genes *ompW* and *toxR*. All contained the cholera toxin gene *ctxAB*. All isolates from countries other than Haiti selected for comparison were confirmed as *V. cholerae*, serogroup O1, biotype El Tor, *ctxAB* positive. Serotypes varied among geographic regions (Table 2).

Table 2. Serotype distribution among *Vibrio cholerae* isolates collected during 1991–2011

Location	No. strains typed	Serotype	
		Ogawa	Inaba
Haiti	149	149	0
Afghanistan	2	2	0
Cameroon	1	1	0
Djibouti	2	0	2
Ethiopia	1	0	1
India	6	2	4
Kenya	14	0	14
Nepal	1	1	0
Nigeria	2	2	0
Oman	1	0	1
Pakistan	6	5	1
Peru	2	1	1
Somalia	2	0	2
South Africa	2	2	0
Sri Lanka	1	0	1
Sudan	5	0	5
Togo	2	2	0
US Gulf Coast	1	0	1
Total	200	167	33

Antimicrobial Drug–Susceptibility

Disk-diffusion testing demonstrated that all strains from Haiti were resistant to furazolidone, nalidixic acid, streptomycin, sulfisoxazole, and trimethoprim/sulfamethoxazole. Strains were susceptible to tetracycline and either susceptible or intermediately susceptible to ampicillin and chloramphenicol. Broth microdilution testing of the isolates from Haiti showed similar results (furazolidone was not tested by broth microdilution) plus decreased susceptibility to ciprofloxacin (MIC range 0.25–1.0 μg/mL). These isolates were susceptible to ampicillin, azithromycin, and chloramphenicol, except for 2 isolates that gave intermediate results for chloramphenicol. Antimicrobial drug susceptibility among strains tested from countries other than Haiti varied; 19 showed the same antimicrobial drug resistance as the Haiti outbreak strains, including isolates from Nepal, Cameroon, South Africa, Oman, and India. Two isolates were similar to the strains from Haiti, but they did not display decreased susceptibility to ciprofloxacin. Twenty-seven isolates from Africa and the Middle East showed the outbreak resistance pattern, but they were susceptible to nalidixic acid and fully susceptible to ciprofloxacin. Two isolates from Peru and the isolate from the US Gulf Coast were susceptible to all antimicrobial drugs tested.

PFGE Genotypes

Two-enzyme PFGE analyses with *Sfi*I and *Not*I identified a predominant Haiti outbreak pattern combination, KZGS12.0088/KZGN11.0092, in 123 (82.6%) of 149 isolates tested (Table 3). This primary pattern combination was detected in isolates from all 10 departments in Haiti, in isolates from the Dominican Republic, and in isolates from travelers returning to the United States from Hispaniola (data not shown). There were 10 PFGE pattern variations, defined as ≥1 band difference in number or size from the

Table 3. *Sfi*I and *Not*I PFGE patterns among toxigenic *Vibrio cholerae* isolates from Haiti, by department and collection date, 2010–2011*

	No. (%) isolates									
						Artibonite only		West Department only		
PFGE types	All	2010 Oct 26	2010 Nov 26	2011 Jan 27	2011 Feb 9	2010 Oct 26	2010 Nov 26	2010 Nov 26	2011 Jan 27	2011 Feb 9
*Sfi*I types										
KZGS12.0088	130 (87.2)	16 (100)	76 (82.6)	27 (90.0)	11 (100)	16 (100)	16 (84.2)	35 (81.4)	21 (87.5)	8 (100)
KZGS12.0089	8 (5.4)	0	8 (8.7)	0	0	0	2 (10.5)	3 (7.0)	0	0
KZGS12.0097†	7 (4.7)	0	4 (4.3)	3 (10.0)	0	0	0	3 (7.0)	3 (12.5)	0
KZGS12.0063†	1 (0.7)	0	1 (1.1)	0	0	0	0	0	0	0
KZGS12.0158†	1 (0.7)	0	1 (1.1)	0	0	0	1 (5.3)	0	0	0
KZGS12.0159†	1 (0.7)	0	1 (1.1)	0	0	0	0	1 (2.3)	0	0
KZGS12.0160†	1 (0.7)	0	1 (1.1)	0	0	0	0	1 (2.3)	0	0
*Not*I types										
KZGN11.0092	137 (91.9)	15 (93.8)	85 (92.4)	26 (86.7)	11 (100)	15 (93.8)	19 (100)	36 (83.7)	21 (87.5)	8 (100)
KZGN11.0034†	5 (3.4)	1 (6.3)	4 (4.3)	0	0	1 (6.3)	0	4 (9.3)	0	0
KZGN11.0142†	4 (2.7)	0	0	4 (13.3)	0	0	0	0	3 (12.5)	0
KZGN11.0124†	2 (1.3)	0	2 (2.2)	0	0	0	0	2 (4.7)	0	0
KZGN11.0134†	1 (0.7)	0	1 (1.1)	0	0	0	0	1 (2.3)	0	0
*Sfi*I/*Not*I combinations										
KZGS12.0088/KZGN11.0092	123 (82.6)	15 (93.8)	73 (79.3)	24 (80.0)	11 (100)	15 (93.8)	16 (84.2)	32 (74.4)	19 (79.2)	8 (100)
KZGS12.0089/KZGN11.0092	8 (5.4)	0	8 (8.7)	0	0	0	2 (10.5)	3 (7.0)	0	0
KZGS12.0097/KZGN11.0092†	4 (2.7)	0	2 (2.2)	2 (6.7)	0	0	0	1 (2.3)	2 (8.3)	0
KZGS12.0088/KZGN11.0142†	3 (2.0)	0	0	3 (10.0)	0	0	0	0	2 (8.3)	0
KZGS12.0088/KZGN11.0034†	4 (2.7)	1 (6.3)	3 (3.3)	0	0	1 (6.3)	0	3 (7.0)	0	0
KZGS12.0097/KZGN11.0124†	2 (1.3)	0	2 (2.2)	0	0	0	0	2 (4.7)	0	0
KZGS12.0063/KZGN11.0092†	1 (0.7)	0	1 (1.1)	0	0	0	0	0	0	0
KZGS12.0097/KZGN11.0142†	1 (0.7)	0	0	1 (3.3)	0	0	0	0	1 (4.2)	0
KZGS12.0158/KZGN11.0092†	1 (0.7)	0	1 (1.1)	0	0	0	1 (5.3)	0	0	0
KZGS12.0159/KZGN11.0034†	1 (0.7)	0	1 (1.1)	0	0	0	0	1 (2.3)	0	0
KZGS12.0160/KZGN11.0134†	1 (0.7)	0	1 (1.1)	0	0	0	0	1 (2.3)	0	0

*PFGE, pulsed-field gel electrophoresis.
†Unique PFGE patterns first seen in this outbreak.

primary pattern (Figure; Table 3). Variant patterns were detected in isolates from 8 Haiti departments. Of the 10 variant 2-enzyme combinations in the 10 PFGE patterns, the most common was KZGS12.0089/KZGN11.0092, which was found in 8 (5.4%) of 149 isolates. Four new *Not*I and 5 new *Sfi*I restriction patterns and 9 new *Sfi*I/*Not*I PFGE pattern combinations were seen among isolates from this outbreak in Haiti (Table 3).

All 16 initial isolates (received October 26, 2010) were from Artibonite Department, the source of the outbreak in Haiti, and demonstrated little PFGE diversity. In contrast, 76 additional isolates (received on November 26, 2010) represented all 10 departments and demonstrated substantial variation, including PFGE types never observed (Table 3). The isolates received on January 27, 2011, and February 6, 2011, were primarily from West Department (including Port-au-Prince) and thus did not enable continuing analysis of isolates from other departments. However, enough isolates were available to roughly compare PFGE pattern distributions in 2 departments over time: Artibonite (16 isolates received October 26, 2010, and 19 isolates received November 26, 2010) and West (43 isolates received November 26, 2010, another 24 isolates received January 27, 2011, and 8 isolates received February 6, 2011) (Table 3). In neither department was there an unambiguous trend toward more diversity over time, but the diversity already present in the November 26, 2010, West Department isolates is striking.

Isolates (n = 17) from Afghanistan, Cameroon, India, Nepal, Oman, Pakistan, and South Africa shared the primary PFGE pattern of isolates from Haiti (Table 4). An isolate from South Africa, which was obtained from an outbreak possibly related to the Zimbabwe outbreak in 2009 (*19*), had the most common Haiti variant (KGZS.0089/KGZN11.0092) (Table 4).

Of 25 cultures with colonies having hemolytic and nonhemolytic phenotypes, only 3 displayed nonidentical PFGE patterns. There was no apparent association of PFGE pattern with hemolysis; 20 (80%) of 25 hemolytic and 22 (88%) of 25 nonhemolytic isolates had the main

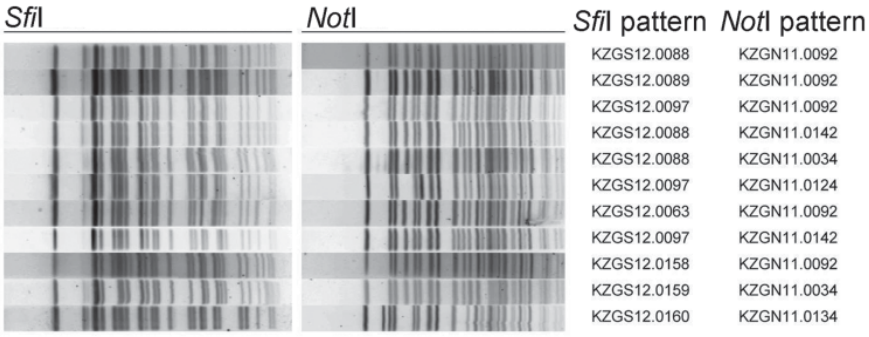

Figure. Pulsed-field gel electrophoresis patterns for *Vibrio cholerae* isolates from Haiti, 2010–2011.

combination pattern seen in isolates from Haiti. One colony from inside the chloramphenicol zone of inhibition also displayed a variant pattern (data not shown).

Virulence and Species-specific Genes, PCR Results, and Sequences

The sequencing results for *ctxB* and *tcpA* and PCR results for *rstR* and the VC2346 gene are shown in Table 5. We sequenced the complete 1,148 bp of the *ctxAB* operon for 107 strains. The *ctxB* gene sequence of all 56 isolates from Haiti that we tested matched the B-7 allele first seen in an outbreak in 2008 in Orissa, India (*20*) (Table 5). The B-7 allele was also contained in 1 isolate from Cameroon, 3 of 6 from India, and 1 from Nepal. The B-7 allele has a single-nucleotide polymorphism (SNP) at nt 58 relative to the classical B-1 allele ("genotype 1" [*21*]), resulting in substitution of asparagine for histidine at aa 20, which is adjacent to the B subunit proteolytic cleavage site between aa 21 and aa 22. The remaining isolates from non-Haiti locations carried the B-1 allele, with the exception of the strains from Peru, which carried the B-3 allele (genotype 3, reported by Olsvik et al. [*21*]) that historically has been associated with the El Tor biotype.

Table 4. *Vibrio cholerae* isolates from various countries with the *SfiI*/*NotI* PFGE pattern combinations most commonly found in outbreak isolates obtained from Haiti in 2010–2011*

Location	No. isolates	*SfiI*/*NotI* PFGE pattern, no. (%) KZGS12.0088/ KZGN11.0092	KZGS12.0089/ KZGN11.0092
Haiti	149	123 (82.6)	8 (5.4)
Afghanistan	2	2 (100)	0
Cameroon	1	1 (100)	0
Djibouti	2	0	0
Ethiopia	1	0	0
India	6	6 (100)	0
Kenya	14	0	0
Nepal	1	1 (100)	0
Nigeria	2	0	0
Oman	1	1 (100)	0
Pakistan	6	6 (100)	0
Peru	2	0	0
Somalia	2	0	0
South Africa	2	1 (50.0)	1 (50.0)
Sri Lanka	1	0	0
Sudan	5	0	0
Togo	2	0	0
US Gulf Coast	1	0	0
Total	200	141	9

*Isolates were collected during 1991–2011. PFGE, pulsed-field gel electrophoresis.

A 1,234-bp sequence, which included 675 bp of the entire *tcpA* gene, matched sequences from CIRS 101, a *V. cholerae* serogroup O1, biotype El Tor strain (*22*) that has an integrated El Tor type phage (CTXΦ)$^{\text{El Tor}}$ but expresses the classical *ctxB*-1 allele. This combination is considered to be an altered or atypical El Tor. The *tcpA* sequence of CIRS 101 has a novel SNP at nt position 266 (aa 89), which differentiates it from typical El Tor strains (*22*). All 56 tested isolates from Haiti had this novel SNP in the *tcpA* sequence, which we have designated the *tcpET*^{CIRS} allele. Isolates from Africa and southern Asia also had the *tcpET*^{CIRS} allele (Table 5).

The *rstR*$^{\text{El Tor}}$ allele was found in all isolates tested except the strain from the Gulf Coast of the United States. All isolates tested, except the strain isolate from the US Gulf Coast, contained the VC2346 gene and are therefore confirmed as seventh pandemic *V. cholerae* (*11*). As shown by whole genome sequencing, isolates with the PFGE pattern KGZS12.0088/KGZN11.0092 (containing the *ctxB*-7/*tcpET*^{CIRS} alleles from Cameroon, India, and Nepal) are most closely related to the strain from Haiti. Details of whole genome sequencing are provided elsewhere (*18*).

Discussion

We characterized the strains in this study by using basic phenotypic markers and genotypic tests that in other studies have been shown to be useful for characterizing *V. cholerae* outbreak strains. Epidemic cholera strains cause human illness by expression of specific genes that enable *V. cholerae* to exist in the environment long enough to be ingested, overcome host immunity, colonize the intestinal tract, and produce cholera toxin in the host (*13*,*23*–*25*). The horizontal acquisition of genes and expression of the transcriptional co-regulated pilus (located on *Vibrio* pathogenicity island 1) and CTXAB (carried on CTXΦ)

Table 5. Sequencing and PCR results for *Vibrio cholerae* isolates collected during 1991–2011*

Location of origin	Sequencing results				PCR results			
	No. sequenced for *ctxB*	*ctxB* allele	No. sequenced for *tcpA*	*tcpA* allele	No. tested for *rstR*	*rstR* allele	No. tested for VC2346 gene	VC2346 gene
Haiti	56	B-7	56	ET^{CIRS}	56	ET	56	Pos
Afghanistan	2	B-1	2	ET, ET^{CIRS}	2	ET	2	Pos
Cameroon†	1	B-7	1	ET^{CIRS}	1	ET	1	Pos
Djibouti	2	B-1	2	ET	2	ET	2	Pos
Ethiopia	1	B-1	1	ET	1	ET	1	Pos
India†	6	B-1 (3), B-7 (3)	6	ET^{CIRS}	6	ET	6	Pos
Kenya	14	B-1	14	ET	2	ET	14	Pos
Nepal†	1	B-7	1	ET^{CIRS}	1	ET	1	Pos
Nigeria	2	B-1	2	ET, ET^{CIRS}	2	ET	2	Pos
Oman	1	B-1	1	ET	1	ET	1	Pos
Pakistan	6	B-1	6	ET^{CIRS}	6	ET	6	Pos
Peru	2	B-3	2	ET	2	ET	2	Pos
Somalia	2	B-1	2	ET	2	ET	2	Pos
South Africa	2	B-1	2	ET^{CIRS}	2	ET	2	Pos
Sri Lanka	1	B-1	1	ET^{CIRS}	1	ET	1	Pos
Sudan	5	B-1	5	ET	2	ET	2	Pos
Togo	2	B-1	2	ET	2	ET	2	Pos
US Gulf Coast	1	B-1	1	ET	1	Classic	1	Neg
Total	107		107		92		104	

**ctxB*, cholera toxin subunit B; *tcpA*, biotyping gene; *rstR*, biotype-specific repeat sequence transcriptional regulator; Pos, positive; Neg, negative.
†By pulsed-field gel electrophoresis and *ctxB* and *tcpA* sequencing, the isolates from Cameroon and Nepal and 3 isolates from India are most closely related to the isolates from Haiti.

determine primary virulence (*9*,*23*,*24*). CTXΦ has 2 regions, a core that includes *ctxAB* and the RS2 region that carries phage replication genes, such as *rstR*. Allelic variations in *ctxB*, *tcpA*, and *rstR* can be useful markers to characterize CTXΦ types and track cholera strains. Whereas nucleotide sequence analysis of the acquired virulence genes *ctxAB*, *rstR*, and *tcpA* differentiates among *V. cholerae* O1, the VC2346 gene is part of the nontransferable genomic backbone that identifies seventh pandemic *V. cholerae* O1 El Tor strains currently in circulation.

The adaptability of *V. cholerae* as a pathogen is facilitated by extensive genetic diversity driven by acquisition and recombination of various genetic elements. Community defense mechanisms, mediated by cellular signaling, such as quorum sensing in biofilms, gives the species additional resiliency against changing environmental conditions (*26*). All *V. cholerae* have these attributes, but only O1 and O139 serogroups currently have pandemic potential. The El Tor biotype was first identified 100 years ago, and 50 years ago it emerged from Indonesia to begin the ongoing seventh pandemic, displacing the classical biotype of the fifth and sixth pandemics because of its superior ability to survive in the environment, increased frequency of asymptomatic carriers, and more efficient transmissibility (*27*).

Genes encoding hemolysis, such as *hlyA*, may be a virulence factor in some *Vibrio* spp., and in the past, hemolysis patterns were used to distinguish biotypes (*28*). Classical biotype strains carry deletion mutations in the *hlyA* locus, and many El Tor strains produce no detectable hemolysis on blood agar plates. Our findings confirm observations that hemolysis is not a reliable marker for strain discrimination. Of interest, hemolytic colonies were often observed within a streak predominated by nonhemolytic colonies.

The predominant KZGS12.0088/KZGN11.0092 *Sfi*I/*Not*I PFGE pattern in the strains from Haiti is a relatively new subtype that was first seen in 2005 in the PulseNet USA database in isolates from travelers returning from India. Although the isolates from Haiti show diversity in their PFGE subtyping patterns, the constancy of the main pattern coupled with virulence genotyping results indicate a high clonality of the outbreak strains, which is consistent with a point-source introduction. Such PFGE diversity, similar to what we observed, has been noticed during outbreaks in the Bengal region (T. Ramamurthy, pers. comm.). This primary KZGS12.0088/KZGN11.0092 pattern and its close variant, KZGS12.0089/KZGN11.0092, were found in strains from Afghanistan, Cameroon, India, Nepal, Oman, Pakistan, and South Africa. Among the strains from Haiti, we identified 4 new *Not*I restriction patterns, 5 new *Sfi*I patterns, and 9 new *Sfi*I/*Not*I combinations, a finding suggestive of continuing evolution of the outbreak strain. The PFGE pattern combination KZGS12.0019/KZGN.0092 was commonly seen in serotype Inaba strains originating from East Africa and the Middle East and was recently seen in isolates from Togo; in this study, the pattern was associated with the *ctxB*-1 allele.

Although the strains from Haiti are genetically an El Tor biotype, they contain the classical *ctxB*-7 allele. This allele was first identified in 2007 in strains from an outbreak in Orissa, India (*20*). The appearance of a classical *ctx*B gene in El Tor strains is not unprecedented. In the early 2000s, hybrid El Tor strains emerged carrying CTXΦ$^{\text{Classical}}$ with the *ctxB* gene of the classical biotype, which is thought to cause more severe clinical disease (*29*). These hybrid strains most likely arose through horizontal transmission of CTXΦ$^{\text{Classical}}$, and the resulting genotypes with the classical *ctx*B1 allele have spread to Asia and Africa. In this study, we show that *V. cholerae* carrying the *ctxB*-7 allele are also disseminating globally.

The transcriptional co-regulated pilus serves a dual role as the major intestinal colonization factor and CTXΦ receptor. All tested isolates from Haiti had *tcpA* sequences with an SNP at nt 266 (*tcpET*^{CIRS}), an allele previously reported in strain CIRS 101 from Bangladesh (*22*), which is an El Tor biotype that produces a classical toxin yet carries CTXΦ$^{\text{El Tor}}$; this SNP produces a distinct allele that distinguishes it from classical and typical El Tor. In the present study, isolates from Haiti, Africa, and southern Asia carried *tcpET*^{CIRS} (Table 5). Our results show that the *tcpET*^{CIRS} allele is also spreading globally, although not in tandem with the *ctxB*-7 allele because the *tcpET*^{CIRS} allele was also found in isolates with the *ctxB*-1 allele from Afghanistan, India, Pakistan, South Africa, and Sri Lanka (Table 5). Our findings agree with those from a recent study suggesting a close relationship between 2 isolates from Haiti and isolates from Southeast Asia (*30*). However, we also observed a relationship between isolates from Haiti and Africa.

The finding of these *ctxB*-7/*tcpET*^{CIRS} isolates in Cameroon, India, Nepal, and now Hispaniola is not surprising, given the ease of international travel; we are unable to identify the origin of the Haiti strains because of geographic and temporal limitations in our culture collection. In particular, our findings do not rule out the presence of *ctxB*-7/*tcpET*^{CIRS} isolates in countries not represented in our collection. Whole-genome sequencing results confirmed the genetic relationship of these isolates from Haiti, Cameroon, India, and Nepal (*18*). More extensive whole-genome sequencing studies and other subtyping methods, such as multiple-locus variable-number tandem repeat analysis, hold promise for providing a better understanding of the relationships between isolates.

The strain from Haiti is distinct from the isolate from the US Gulf Coast. The isolate from the Gulf Coast was characterized as KZGS12.0055/KZGN11.0029, *ctxB*-1, *tcpET*, *rstR*$^{\text{Classical}}$ and negative for VC2346. The isolate from Haiti is also not related to the isolates from Peru from the 1991 Latin America outbreaks, which were characterized as KZGS12.0114/KZGN11.0033, *ctxB*-3, *tcpET*, and *rstR*$^{\text{El Tor}}$.

The strain from Haiti has the core characteristics of the seventh pandemic El Tor clone. Our findings support the widespread observation that the typical El Tor strain, which started the seventh pandemic, is gradually being replaced by El Tor isolates with classical cholera toxin subunits.

The isolates from Haiti and those from other regions displayed a consistent resistance phenotype, with resistance to the clinically relevant antimicrobial drugs trimethoprim/sulfamethoxazole and sulfisoxazole but susceptibility to other primary antimicrobial drug options, including doxycycline and azithromycin. A discussion of integrating conjugative and other mobile genetic elements that can potentially mediate transfer of antimicrobial drug resistance is provided elsewhere in this issue (*31*). Development of additional antimicrobial drug resistance, particularly to doxycycline and macrolides, remains a serious clinical threat, and Laboratoire National de Santé Publique and CDC continue to monitor for the emergence of such resistance.

The strains from Haiti are fully virulent and contain all the genes necessary for orchestrating the expression of *Vibrio* spp. virulence factors. These strain characteristics, coupled with the sudden and explosive course of the 2010 outbreak, are consistent with an introduction of this strain into a vulnerable population at a single point in time.

The adaptive immunity of the local population as well as climate variations will further drive bacterial evolution; for example, it will not be surprising to observe a gradual switch over time from the Ogawa to the Inaba serotype as population immunity to Ogawa rises, as has been reported during several outbreaks (*23*). The primary PFGE patterns continue to diverge as the infections continue, likely reflecting interactions with the host immune system and between environmental and epidemic populations of bacteria networking in complex ways. Regardless, rapid diagnosis and continuing public health control of the current outbreak in Haiti as well as future outbreaks is paramount for limiting sickness and death, and intensive studies using a variety of basic science, diagnostic, and epidemiologic tools will remain useful for reducing the overall global impact of cholera.

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Cholera in Haiti and Other Caribbean Regions, 19th Century

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Medical journals and other sources do not show evidence that cholera occurred in Haiti before 2010, despite the devastating effect of this disease in the Caribbean region in the 19th century. Cholera occurred in Cuba in 1833–1834; in Jamaica, Cuba, Puerto Rico, St. Thomas, St. Lucia, St. Kitts, Nevis, Trinidad, the Bahamas, St. Vincent, Granada, Anguilla, St. John, Tortola, the Turks and Caicos, the Grenadines (Carriacou and Petite Martinique), and possibly Antigua in 1850–1856; and in Guadeloupe, Cuba, St. Thomas, the Dominican Republic, Dominica, Martinique, and Marie Galante in 1865–1872. Conditions associated with slavery and colonial military control were absent in independent Haiti. Clustered populations, regular influx of new persons, and close quarters of barracks living contributed to spread of cholera in other Caribbean locations. We provide historical accounts of the presence and spread of cholera epidemics in Caribbean islands.

Research indicates that "*Vibrio cholerae* was newly introduced into Haiti" in 2010 (*1*). Yet, as recently as July 2011, the Boston Globe claimed that "Cholera appeared in Haiti last year for the first time since the 1960s" (*2*), implying that cholera had occurred in Haiti in the 20th century. The uncertainty in newspapers and nongovernmental organizations over whether Haiti had experienced cholera during the 20th century, a period when there was no epidemic cholera in the Caribbean islands, reflects the historically uneven documentation of cholera epidemiology in different regions.

Almost 2 centuries of cholera research such as John Snow's famous environmental detective work on cholera in mid 19th century England (*3*) helped to found the field of epidemiology. However, until 2011, there was no comprehensive historical record of cholera in Africa (*4*), despite its crucial status as a piece of the global disease puzzle. Epidemiologic frameworks must nevertheless confront the transnational influences and fluid biosocial networks that influence the global routes of epidemic pathologies (*5*). The cultural and linguistic heterogeneity of the Caribbean island states, many of which were ruled by consecutive or simultaneous European colonial dominions, amid dwindling Native American populations and huge forced influxes of Africans of diverse ethnicities, is paradigmatic of the challenge for epidemiology of searching beyond the narratives privileged by a given institutional structure, such as the colonial medical research apparatus.

Historical Context for Cholera in Haiti

Although it is difficult to confirm the absence of a disease in an earlier era, this report explores textual evidence for the immunologic status of persons to cholera in Haiti reported in scientific journals and lay journalism sources from the 19th century. It also considers the reasons that epidemic cholera failed to occur in Haiti in the 19th century even though it did affect many neighboring environments, including the Dominican Republic.

There is no published source that identifies all Caribbean islands affected by cholera epidemics in the 19th century. In the field of 20th-century and 21st-century research, an article in 1985 by Kiple comes the closest to describing the effect of cholera across the 19th-century Caribbean. In assessing Cuba, Jamaica, Puerto Rico, and St. Thomas, Kiple proposed that 200,000 deaths from

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cholera in the Caribbean would not be unrealistic, although a total figure would have to be "considerably higher as a number of islands have not been included in this survey" (*6*). Higman notes that basic public health measures were prompted by cholera epidemics in the British Caribbean in the 19th century but that they emerged from "a maze of environmental mystery" (*7*). Vega Lugo provides a recent study of cholera in 19th-century Puerto Rico (*8*).

However, the 3 major overviews of cholera in the 20th century and 21st century, the book Cholera by Pollitzer in 1959 (*9*), the foundational work with the same title by Barua and Greenough in 1992 (*10*), and the Encyclopedia of Pestilence, Pandemics, and Plagues by Byrne in 2008 (*11*), do not compare the timeframe of the totality of Caribbean epidemics in the 19th century with those of global pandemics or their historically debated environmental influences such as hurricanes and drought. When cholera occurred in Peru in 1991 but did not spread from Latin America to Caribbean islands, researchers may have asked different questions than they would have otherwise (*12*), although their concluding assessment and prescriptions remained accurate: "The lack of reported cases in Uruguay and the Caribbean may reflect a low risk for ongoing transmission..." (*13*). From the time of the earliest medical commentaries on epidemics in the Caribbean, researchers had noted that proportions of infections and deaths were "much higher than in all European cholera epidemics" (*14*).

In the 19th century, there was abundant discussion of Caribbean cholera in international medical journals such as Lancet or Gazette Médicale de Paris, and in colonial medical and political reports to European colonial dominions. However, cultural and political heterogeneity in the Caribbean discouraged 19th-century researchers from providing a comparative overview of Caribbean cholera epidemics. Furthermore, their reigning hypotheses, such as the noncontagion theory, and their motivations, such as proslavery or antislavery arguments, differed from those of medical and research establishments today. Considerable variation in accounts of the numbers of the sick and the dead was the rule rather than the exception.

Fortunately, where no complete narrative exists, searchable databases (e.g., Digital Collections of the US National Library of Medicine or Google Books for medical sources, and America's Historical Newspapers, World Newspaper Archives, or Gallica for journalism sources) now provide the capacity to research many fragments. In the 19th century Caribbean context, it is useful to compare and contrast medical and lay sources. Journalism in the 19th century attempted to ensure successful navigation of culturally diverse environments through scoops from traveling merchants and maritime personnel.

These unwitting citizen participants in epidemiologic journalism about the Caribbean had the simple but compelling goal of preserving maritime commerce from contamination, quarantines, and disease-devastated economies through direct testimony about epidemics. In a typical example, readers learned of cholera in Puerto Rico through the testimony of a Newburyport captain who was thwarted in his effort to do business in the harbor.

> "Capt. Reed, of the schooner Life Boat, at Newburyport, reports that the cholera was raging fearfully at Mayaguez, carrying off from 40 to 50 persons daily. The epidemic broke out about the 5th of August, and when Capt. R. left was rather on the decrease. It had stopped business entirely, so that very few sales or purchases could be made, and laborers could with difficulty be obtained. Capt. R. carried out spars on deck, and says that the men employed to float them ashore were all dead within 4 hours of the job being completed" (*15*).

The Caribbean region experienced cholera in 3 major waves, mostly overlapping the timeframes of Barua and Greenough (*10*) for the second (1829–1851), third (1852–1859), and fourth (1864–1879) cholera pandemics. The 3 periods of cholera in the Caribbean that we have identified are 1833–1834 (with, according to Kiple [*6*], possible lingering cholera in outlying areas until late 1837 or early 1838) in Cuba; 1850–1856 in Jamaica, Cuba, Puerto Rico, St. Thomas, St. Lucia, St. Kitts, Nevis, Trinidad, the Bahamas, St. Vincent, Granada, Anguilla, St. John, Tortola, the Turks and Caicos, the Grenadines (Carriacou and Petite Martinique), and possibly Antigua; and 1865–1872 in Guadeloupe, Cuba, St. Thomas, the Dominican Republic, Dominica, Martinique, and Marie Galante.

Cholera in the Caribbean Region

Cholera reached the Western Hemisphere during the second pandemic, although the term cholera morbus was in use much earlier to designate a range of conditions including dysentery and inflammation and bleeding in the digestive tract. Thus, an 1816 public health report in Baltimore read, "There died in Baltimore, in the year 1815, 1349 persons, among them 218 of consumptions; 167 of Cholera Morbus; 108 of pleurisy; 98 of fits; 83 of old age; 74 of worms; … 3 of cancer" (*16*). A 1783 obituary in Boston read, "[H]e made a collection of Toad-stools, under the notion of Mushrooms, which, having fryed they eat the following evening; but … their supper proved a poison to them, operating much like a Cholera Morbus, of which said Kreamer expired" (*17*). Although researchers have remained cautious about distinguishing between such earlier uses of cholera morbus and the cholera associated with pandemics, lay journalism sources in June 1832 in Quebec, Montreal, and New York had no qualms about

distinguishing ordinary cholera, common at this season of the year, from the ravages of the Asiatic cholera (*17*). Asiatic cholera, associated with "large numbers of Irish and other emigrants," a "class of persons particularly exposed and carrying the disease wherever they go," killed considerable numbers of persons ≤6 hours from onset of symptoms. Rosenberg noted that in the context of cholera in the United States, "the newly arrived immigrant found all doors closed to him" (*18*).

Cholera arrived in the Caribbean in late February (24 or 25), 1833 (*4*,*19*). The first reported death was of a Catalan man named José Soler who lived in the Lazaro neighborhood of Havana, Cuba, in the poor port section outside the city walls. A neighboring mulatta woman then died, followed by the nearly mass extinction of barracks of newly arrived slaves from Africa, leading to the rumor that they had brought cholera with them. However, this rumor was likely not true because cholera did not arrive in West Africa until 1868 in Senegal and 1869 in the Gambia (*4*). Day by day, cholera spread through different neighborhoods of Havana. Cholera spread from Havana to the Matanzas region in mid-March and continued to rage there until mid-June. After an apparent pause in large-scale epidemic activity, cholera then flared up in Havana and Trinidad (Cuba), in the summer and fall of 1834.

The severe death toll in 1833–34 was a first harbinger of a disastrous relationship in the Caribbean between cholera epidemics and the 2 major imported populations typically housed in barracks, often weakened in advance of their arrival by shipboard conditions and after their arrival by the encounter with yellow fever and plague: slaves and colonial soldiers. We read that in Matanzas, "Two cargoes of slaves (over 1000), arrived a few days since; … all of whom died" (*20*). In August, another large group of newly arrived slaves heard such stories, "which drove the newcomers to desperation, and thinking that they might as well die in one way as another, they rose upon their keepers and murdered them" (*21*).

Even when cholera briefly waned Cuba in late June 1833, the community was wary of what the future held. They asked "Where is our guarantee that the disease is not located permanently in the island? How is confidence utterly lost and ruined to be restored?" (*22*).

After the first wave of Caribbean cholera, the epidemic reappeared in Cuba in February 1850 but not as severe as in 1833. According to a European medical bulletin, the Health Office in Havana had first confirmed the epidemic in a specific location (the military hospital). However, outside the military community, the effects seemed mild: the Daily National Intelligencer noted that among citizens, after the first 3 weeks of the epidemic, "panic has in great measure subsided" (*23*). In an intriguing but indirect description of what may have reflected effects of partial local immunity resulting from recent outbreaks on the severity of the disease, this article claimed that "for cholera to become fatal, the patient must have an elective affinity for the disease."

In the first cholera epidemic in Jamaica in the fall of 1850, there was no necessary elective affinity for patients to court death. Some medical observers claimed that it had taken the lives of 10% of the total population by the time it subsided in Kingston in early January 1851 (*24*). Unpredictable movements from area to area kept the epidemic in Jamaica going for 2 years. Although there was some overlap of affected communities in 2 most recent of these regional epidemics, new influxes of soldiers may have accounted for regional epidemics, as they apparently did for flare-ups in Kingston: "The soldiers in camp at Kingston had suffered severely" (*25*). Cholera would occur again in Jamaica during April–September 1854, mostly in regions (Mont Diablo, Sturgetown, Salem, and St. Ann's Bay) not devastated in previous epidemics.

During February–March 1851, Cuba experienced a pattern of sporadic and mild cases in Havana. However, during late September–December 1852, Santiago was affected with an appalling intensity. This regional extremity of the island had not been struck by cholera in the 1830s, and its population was therefore still immunologically naive: "The town of St. Jago de Cuba experienced its first and only visitation in 1852" (*26*).

Beginning in late September 1852, cholera devastated Nassau in the Bahamas. The Daily Atlas described "Vessels in the harbor being crowded with persons fleeing from the scourge" (*27*).

In 1853, cholera reappeared in Cuba in specific demographic groups. One journalist spoke of the toll among newly arrived slaves and soldiers: "The thousands of recently introduced Africans have brought with them a terrible kind of diarrhea, which is carrying off vast numbers of victims…. In Havana the troops are said to be dying like rotten sheep" (*28*).

These cholera episodes were only a prelude to 1854, when cholera epidemics exploded across the Caribbean. This was a period in which "immigrant vessels" from Europe were identified as catalysts. However, an east-to-west pattern for the movement of the epidemics, especially along the chain of the Windward Islands, may be indicative of more local transmission. Island after island saw its already small population reduced. The Albany Evening Journal recounted, "The arrival of the steamer Curlew, from St. Thomas, brings detailed accounts of the ravages of the cholera in those islands. Of the 14,000 inhabitants of the island of St. Thomas, 1,600 died. At Tortola, there were not enough survivors to bury the dead. At Nevis, out of 5,000, there have been 550 deaths" (*29*). In Barbados, which had a total population of 126,000, cholera exacted

an appalling toll. Accounts from Bridgetown described the disposal of bodies as resembling heaps of merchandise consigned to the grave.

Although many communities appeared to recover from the epidemics, this was not always the case. In Trinidad in 1853, cholera has been blamed for the disappearance of Native Americans on the northern coast of this island (*30*). (Unfortunately, there is little information about this aspect of the epidemic because a curious phenomenon, that of hundreds of monkeys falling dead from the trees of what was believed to be cholera, dominated journalistic and medical commentary [*31*].)

In Puerto Rico, awareness of a surrounding Caribbean world destabilized by cholera led to a host of preventative measures ranging from quarantines to spraying the mail with vinegar (*6*). However, in November 1855, the disease belatedly arrived.

After the end of the Puerto Rico epidemic in 1856, there was a calm of almost a decade. However, in 1865, cholera was reimported by a ship from cholera-stricken Marseille to Guadeloupe, launching an epidemic that would exact a toll of ≥11,000 deaths in a population of 150,000. Martinique, Marie Galante, and Dominica were also affected, but sparsely.

In 1868, cholera returned to its Caribbean starting point, which was now a major battlefield of the Cuban revolution. For a time, cholera and yellow fever brought the hostilities to a halt, in a kind of pathologic truce. In Nuevitas in early August 1869, cholera reportedly caused the deaths of 200 Spanish troops a day. It had waned in Puerto Principe in late August, leading the Spanish leader to call for a general public thanksgiving. One journalist commented that because the rebels had left the area to avoid being infected, "Perhaps, in offering thanksgiving for the disappearance of cholera, the Spaniards may be celebrating the occupation of their district by the Rebels" (*32*). In 1870, there was cholera in Santiago and Havana; in 1871, cholera was reported in urban slums and among Spanish troops. A final possible epidemic was reported in 1872.

The Exception in Haiti

In the 3 pandemics that involved the Caribbean in the 19th century, we found no medical or lay reports of cholera in Haiti, the Netherlands Antilles of Aruba, Bonaire, and Curaçao; the Cayman Islands; and St. Martin, St. Barthélemy, or Montserrat. (Martinique is a borderline case because despite reports of 1 or 2 cases of cholera in 1835, and sparse epidemic effect in 1865 and 1866, cholera never caused large-scale mortality rates there.) Of these sites, Haiti is the largest and most densely populated and was a close neighbor of hard-hit Cuba and Jamaica. Journalistic coverage in Haiti of cholera epidemics elsewhere in the official paper La Feuille du Commerce make it unlikely that epidemic cholera occurred there but went undocumented.

In the decades leading into the Caribbean era of cholera, the cultural situation in Haiti was unique. The Haitian revolution, beginning with the slave insurrection of 1791, led to the defeat of French colonialism and the founding of the first independent nation of former slaves in 1804 (*33*). (The course of the Haitian Revolution was influenced by epidemic yellow fever, a disease in relation to which Haiti was cited in a broad array in medical texts in the 19th century.) After 1804, Haiti continued to be a major trade partner with the United States and many other nations, even during embargo periods, as attested by lists of international ships in the harbor of Port-au-Prince on the front page of every issue of La Feuille du Commerce. However, key elements of integration of diverse world populations in substandard living conditions were absent in Haiti because of the lack of plantation slavery and colonial military troops.

Government records in Haiti from the 19th century show early recognition of the cholera threat posed by maritime traffic with Europe and the United States. In August 1832, even before the arrival of cholera in the Caribbean, Haitian President Boyer urged the adoption of a *cordon sanitaire* in port communities:

> "Prudence dictates that we take all necessary means to prevent, to the extent that it is possible, the invasion of Haiti by cholera morbus, which has already traversed Europe and penetrated the United States. I urge you all… to take the most appropriate local measures with regards to ships arriving from ports in the United States, whose geographic proximity with Haiti makes the danger of epidemic spread particularly acute" (*34*).

Even in the fourth pandemic and in the most recent epidemics of cholera in the 19th century, when cholera did not spread into the Caribbean, the government in Haiti took measures to prevent introduction of cholera. In an 1852 English medical report (*35*), glancing allegation of cholera in Haiti was made in a long list of worldwide outbreaks. However, this reference appears to be inaccurate because an 1851 epidemic of malignant gastritis in Haiti was characterized by rotting of the stomach, a symptom that is not characteristic of cholera. A contemporary US newspaper article also discredited the rumor of cholera in Haiti from 1851: "From Aux Cayes [Haiti]. The schooner Panama from Aux Cayes reports that port as healthy. The rumor that cholera was raging proves false" (*36*).

Haiti did act when the neighboring Dominican Republic on the island of Hispaniola had a cholera epidemic, apparently imported from St. Thomas, beginning in late 1867. On March 14, 1868, the New York Herald

reported "The Puerto Rican health boards have declared all Dominican ports foul on account of cholera. No vessels coming from thence will be allowed entry" (*37*), but Haiti had already established a policy of quarantining ships from the eastern side of the island. On April 1, 1868, a *Te Deum* was sung in Santo Domingo in thanks for the disappearance of the cholera that had been decimating the population, but there was never any sign of spread into Haiti (*38*).

Haiti did not participate in a system of centralized international public health reporting on infectious epidemics until 1881, when Haitian delegate Stephen Preston, Envoy and Minister Plenipotentiary at Washington, voted for the following resolution at the international Sanitary Conference.

> "A centralized international system of sanitary notification being deemed indispensable to the successful carrying out of measures for preventing the introduction of disease, it is advisable to create international organizations to be charged with the duty of collecting information in regard to the outbreak, spread, and disappearance of cholera, pest, yellow fever, etc., and of conveying such information to the parties interested" (*39*).

However, Haiti historians had already been engaged in reporting the striking absence of cholera there, as in this late 1850s comment by the eminent Haitian historian Thomas Madiou:

> "It must be observed that cholera has never entered in Haiti, even when it raged all around our island, in St. Thomas, Puerto Rico, Jamaica, and Cuba, in the Lesser Antilles and the Greater Antilles alike. Could this be due to some emanations of our soil that don't allow choleric toxins to survive, or to some condition of our atmosphere?" (*40*).

The absence of earlier epidemic cholera in Haiti contrasts with the presence of epidemic cholera in the Caribbean in the 19th century. A digital map (www.caribbeancholera.org) shows how major cholera outbreaks unfolded over time in the Caribbean region. The 3 key clusters during 1833–1834, 1850–1856, and 1865–1872 are documented with newspaper and other historical accounts of the spread of cholera among and within countries in the Caribbean in the 19th century. Haiti did not have any cholera outbreaks in the 19th century, even when this disease was raging in surrounding regions. At that time, Haiti did not have the risk factors of imported new populations of slaves and colonial soldiers, which had been devastating in Cuba. Cuba, with its repeated outbreaks over the 19th century, was the last country in the Caribbean to abolish slavery (in 1879) and one of the last to overthrow European colonial dominion. In the contemporary epidemic in Haiti, influxes of international soldiers from cholera-endemic areas, who lived in barracks-style housing, present an unexpected parallel to the 19th-century risk factor of new colonial enslaved or military populations living in crowded conditions.

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Risk Factors Early in the 2010 Cholera Epidemic, Haiti

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During the early weeks of the cholera outbreak that began in Haiti in October 2010, we conducted a case–control study to identify risk factors. Drinking treated water was strongly protective against illness. Our results highlight the effectiveness of safe water in cholera control.

On October 19, 2010, the Haitian Ministry of Public Health and Population (MSPP) was notified of increased cases of acute watery diarrhea resulting in death among adults in Artibonite Department. Within 2 days, MSPP's Laboratoire National de la Santé Publique had identified toxigenic *Vibrio cholerae* O1, serotype Ogawa, biotype El Tor in stool specimens (*1*). The first reports of illness consistent with cholera occurred on October 16, and, by November 19, cholera had reached all 10 Haitian administrative departments (*2*).

Because the first cases were in persons who worked near the Artibonite River, contaminated river water was suspected as the initial source. In a proactive effort to protect the population, MSPP rapidly implemented a cholera prevention campaign that began on October 22, 2010, to discourage the population from drinking river water, distribute water treatment products, and promote water treatment, handwashing, sanitation, and safe food preparation. To inform further prevention activities, we conducted a case–control study during the second and third weeks of the outbreak to identify risk factors for symptomatic cholera.

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The Study

This study was conducted in Artibonite Department close to where the first cases were identified. On the basis of detailed hypothesis-generating interviews with patients and known risk factors associated with cholera in other investigations in the Americas, we created a questionnaire to assess multiple exposures, including river and other water-related exposures, sanitation and hygiene practices, foods, and other factors. We enrolled and interviewed participants from October 31 through November 13, 2010, with a 4-day break during November 5–8 because of Hurricane Tomas. To rapidly generate relevant information to guide outbreak response, we set a goal of enrolling 50 case-patients and 100 controls, a sample size that, although limited, was in line with that of previous successful emergency investigations.

Eligible case-patients were persons ≥5 years of age who were hospitalized between October 22 and November 9 for acute watery diarrhea at the Médecins Sans Frontières cholera treatment unit in Petite Rivière, a town in a densely populated rural region near the Artibonite River. Only case-patients with the first case of acute watery diarrhea in their household since October 16 were eligible. Case-patients were interviewed about exposures during the 3 days before illness onset. Within 72 hours of the interview, we visited case-patients at home, where we observed household drinking water sources and storage containers, presence of water treatment products, access to toilet facilities, and the case-patient's handwashing technique. Drinking water was tested for free chlorine as an objective measure of chlorine treatment. Matching by neighborhood (through a systematic door-to-door search from the case-patient's house) and age group (5–15, 16–30, 31–45, and ≥46 years), we enrolled 2 controls per case-patient at the time of the visit to case-patients' homes from households with no diarrhea since October 16. We interviewed controls about exposures during the same 3 days as the matched case-patient and made the same household observations.

The term "improved drinking water source" indicated it met the World Health Organization definition, which describes technologies that protect water from outside contamination (*3*). "Lacking safe water storage" referred to water stored in an open container or bucket without a tap. "Proper handwashing technique" was defined as observed use of soap and thorough lathering.

We performed descriptive statistical analysis and exact conditional logistic regression to compute the most likely estimate or, when small cell sizes required, the median unbiased estimate of matched odds ratios (mORs) with 95% confidence intervals (CIs). Demographic and household poverty indicators were assessed for effect modification and confounding. Matched ORs adjusting for sex and the

presence of a mud floor in the household are presented in the Table. As part of the public health response to the outbreak, this investigation did not require human subjects review. Informed consent was obtained.

We enrolled 49 case-patients and 98 controls; 16 (33%) case-patients and 53 (58%) controls were female. The median age was 23 years for case-patients (range 6–63 years) and controls (range 5–75 years) (Table).

Few case-patients (15/49 [31%]) or controls (23/98 [23%]) had an improved drinking water source. The most common water source was an unimproved well (30/49 [61%] of case-patients, 59/98 [60%] of controls). Similar percentages of case-patients (33/42 [79%]) and controls (69/93 [74%]) lacked safe water storage, and many case-patients (28/46 [61%]) and controls (40/84 [48%]) practiced open defecation.

Although comparable percentages of case-patients (25/48 [52%]) and controls (48/95 [51%]) reported treating their drinking water before the outbreak, case-patients were significantly less likely than controls to report treating their drinking water during the outbreak (59% vs. 85%, mOR 0.2, 95% CI 0.1–0.7). Water treatment products were found in homes of 31 (69%) of 45 case-patients and 73 (75%) of 98 controls. A lower, though not significant, percentage of case-patient households than control households (13/44 [30%] vs. 37/90 [41%]) had ≥0.1 mg/L of free chlorine in stored water. Among 50 foods examined, only sugar cane juice was associated with illness (9% vs.1%, mOR 9.1, CI 1.0–∞; data for other foods not shown).

Conclusions

This study, conducted early in the cholera epidemic in Haiti in one of the first populations to be affected, demonstrated that treating drinking water was strongly protective. This finding is not unexpected, because most cholera outbreaks are spread through contaminated water, but it provides compelling specific evidence that safe drinking water is a critical need in Haiti. The disparity between the high percentage of homes with water treatment products and the lower percentage of homes with detectable chlorine in stored drinking water suggested that the communication strategy that accompanied product delivery needed modification.

The low proportions of participants with an improved water source, adequate water storage, and sanitary facilities were typical of rural Haiti (*4*). Nevertheless, the increase in reported frequency of treating drinking water during the outbreak, particularly among controls, suggested that MSPP's cholera prevention message effectively reached at least part of the population. This campaign may have prevented the epidemic from causing even more illness and death. The association with sugar cane juice also emphasized that cholera can be transmitted by multiple routes. In the study area, sugar cane juice is typically produced by squeezing cane through a press; it is not

Table. Exposures of case-patients with cholera and matched controls, Artibonite Department, Haiti, October–November 2010*

Variable	No. (%) case-patients exposed, n = 49	No. (%) controls exposed, n = 98	mOR (95% CI)
Participant completed primary school†	7 (23)	18 (31)	1.0 (0.2–3.8)
Drinking water source			
Improved water source	15 (31)	23 (23)	3.5 (0.6–40.8)
Well	30 (61)	59 (60)	0.3 (0.1–2.5)
Water storage			
Lacked safe water storage	33 (79)‡	69 (74)‡	1.3 (0.5–4.0)
Bucket (unsafe storage)	31 (72)‡	67 (70)‡	1.1 (0.4–2.8)
Plastic bottle (safe storage)	7 (16)‡	19 (20)‡	0.6 (0.2–2.0)
Water treatment			
Treating drinking water before the outbreak	25 (52)‡	48 (51)‡	0.9 (0.4– 2.3)
Treating drinking water 3 d before illness onset (during outbreak)	29 (59)	82 (85)	0.2 (0.1–0.7)
Water treatment product in home	31 (69)‡	73 (75)	0.8 (0.3–2.4)
Drinking water test			
Residual chlorine presence in home drinking water ≥0.1 mg/L	13 (30)‡	37 (41)‡	0.4 (0.1–1.3)
Residual chlorine presence in home drinking water ≥0.5 mg/L	8 (16)‡	18 (18)‡	0.4 (0.1–1.8)
Contact with river water	17 (35)	26 (27)	1.1 (0.4–3.1)
Sanitation and hygiene			
Open defecation	28 (61)	40 (48)‡	2.2 (0.7–7.8)
Handwashing with soap and lather	29 (59)	20 (41)	0.6 (0.3–1.5)
Household characteristics: electricity	8 (16)	29 (30)	0.6 (0.1–2.3)
Food exposure: sugar cane juice	4 (9)‡	1 (1)‡	9.1§ (1.0–∞)

*Exposures adjusted by sex and mud floor in home. Median age of case-patients was 23 y (range 6–63 y); median age of controls was 23 y (range 5–75 y). mOR, matched odds ratio; CI, confidence interval.
†Among those >15 y of age.
‡Denominators may be lower than the total number of participants because of missing data.
§Median unbiased estimate.

typically made or served with water or ice, though we do not know how the juice consumed by participants was produced. After being contaminated with *V. cholerae*, however, it provides a hospitable environment for bacterial growth (*5*). These findings highlight the central importance of safe water in cholera control and the need to prevent both foodborne and waterborne transmission.

The cholera epidemic should galvanize both governmental and nongovernmental organizations to address Haitians' need for safe water and sanitation. Experience in other cholera epidemics has shown that the benefits will likely go beyond preventing the spread of cholera; other serious public health problems, such as typhoid fever and other enteric infections, have improved substantially with effective measures to control cholera in other settings (*6*).

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Rapid Assessment of Cholera-related Deaths, Artibonite Department, Haiti, 2010

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We evaluated a high (6%) cholera case-fatality rate in Haiti. Of 39 community decedents, only 23% consumed oral rehydration salts at home, and 59% did not seek care, whereas 54% of 48 health facility decedents died after overnight admission. Early in the cholera epidemic, care was inadequate or nonexistent.

Epidemic cholera remains a public health problem in developing countries. In 2009, a total of 45 countries reported 221,226 cases and 4,946 deaths; for both, >98% occurred in sub-Saharan Africa (*1*).

On October 21, 2010, an outbreak of acute watery diarrhea in Artibonite and Centre Departments, Haiti, a country with no history of epidemic cholera, was confirmed as cholera when fecal specimens yielded toxigenic *Vibrio cholerae* O1 (*2*). Within 1 month, cholera spread to all 10 departments (*2*).

With prompt treatment, the cholera case-fatality rate (CFR) should remain <1% (*3*). However, by November 13, 2010, the Ministère de la Santé Publique et de la Population (MSPP) had reported 16,111 persons hospitalized with suspected cholera and 992 cholera-related deaths, for a CFR of 6.2% (*4*) (Figure 1). To determine reasons for the high CFR, we conducted a rapid cholera mortality assessment in Artibonite Department during November 12–16.

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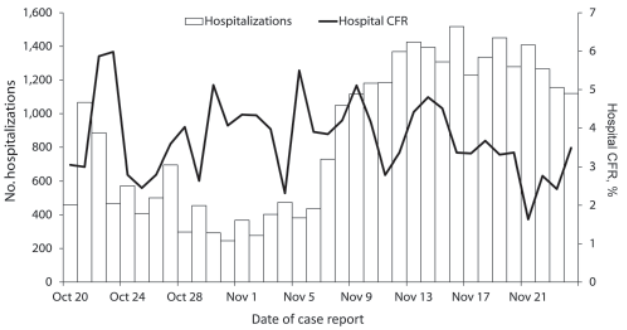

Figure 1. Number of and case-fatality rate (CFR) for persons hospitalized with cholera, Artibonite Department, Haiti, October 20–November 24, 2010.

The Study

We defined cholera decedents as persons who died of suspected cholera (acute watery diarrhea in persons ≥5 years of age [*5*]) with illness onset after October 16, 2010, three days before the first case-patients were seen at the hospital (reflecting the 3-day average incubation period [*6*]). To locate decedents, we obtained reports of cholera-related deaths from 2 sources: admission records from 2 hospitals in Artibonite that had cholera treatment centers (Hôpital Albert Schweitzer and Hôpital Charles Colimon) and verbal reports from community health workers (CHWs). We attempted to locate households of all decedents from hospital records and verbal reports. Logistic and time constraints limited case finding to communities within 2 hours' travel from the hospitals. We visited decedents' households; obtained informed consent; and interviewed families about demographics, symptoms, health-seeking behavior, treatment, type of health facility, and knowledge about cholera. We also asked decedents' household members and local CHWs about other cholera-related deaths. If additional decedents were identified, we visited their homes and interviewed household members. The Centers for Disease Control and Prevention Institutional Review Board (Atlanta, GA, USA) and MSPP determined that this emergency response activity was nonresearch.

We enrolled 87 decedents. Of 28 decedents identified from hospital records, we found homes of 22 (79%); homes of 6 decedents could not be located or were too remote for inclusion. Illness onset ranged from October 16 through November 14; a total of 29 (33%) persons died during the first week of the epidemic (Figure 2). Median age of decedents was 50 years (range 5–100 years); 58 (67%) were

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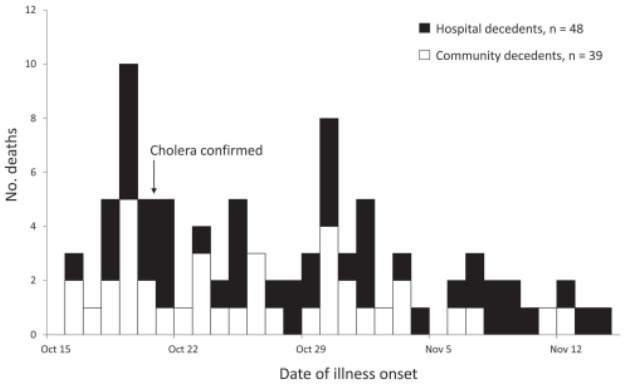

Figure 2. Number of persons who died of cholera, Artibonite Department, Haiti, October 16–November 14, 2010.

male. Forty-eight (55%) decedents died in a health facility (health facility decedents) and 39 (45%) died at home or en route to a facility (community decedents). We identified 17 (35%) health facility decedents from hospital records and 31 (65%) from community interviews; we identified 5 (13%) community decedents from hospital records and 34 (87%) from community interviews.

Twenty-three (48%) health facility decedents and 9 (23%) community decedents had used oral rehydration solution (ORS) at home before seeking care (Table 1). ORS use at home was lower for persons who died during the first week of the outbreak (7 [27%]) than during the second (8 [40%]) or third (17 [46%]) weeks. We observed ORS sachets in homes of 17 (35%) health facility decedents and 14 (36%) community decedents. No respondents reported use of homemade sugar–salt solution by decedents.

Median time from illness onset to death was 20 hours (range 3 hours–7 days) for health facility decedents and 12 hours (range 2 hours–8 days) for community decedents. Twenty-two (46%) health facility decedents died on day of admission and 26 (54%) died after spending ≥1 night in the facility (Table 2). Twenty-three (59%) community decedents never sought care, 8 (21%) died en route to care, and 8 (21%) died after discharge. Of those who sought care, 29 (60%) health facility decedents and 7 (44%) community decedents waited <2 hours to visit a health facility. Family members of community decedents reported the following reasons for not seeking care: no need for care (19 [69%]), long distance to the health facility (6 [26%]), too ill to travel (4 [17%]), lack of transport (3 [13%]), unsafe to travel at night (3 [13%]), and cost of transport (1 [4%]).

Of 48 health facility decedents, 38 (79%) were treated in hospital and 10 (21%) at a health center or dispensary. Decedents received intravenous fluids (35 [73%]), ORS (27 [56%]), both (20 [42%]), or neither (3 [9%]).

Household members of 33 (69%) health facility decedents and 30 (81%) community decedents reported receiving information about cholera after the outbreak started. The most common information sources for families of health facility and community decedents, respectively, were radio (26 [79%] vs. 26 [89%]), friend (6 [18%] vs. 8 [27%]), cellular telephone text message from MSPP (4 [12%] vs. 4 [13%]), community meeting (2 [6%] vs. 2 [7%]), and CHWs (1 [3%] vs. 3 [10%]). Fewer than half of family members of health facility (23 [48%]) and community (19 [49%]) decedents believed cholera was treatable. Of these, 16 (70%) health facility decedents and 17 (90%) community decedents knew to seek care at a health facility.

Conclusions

Our findings suggest that, early in the cholera epidemic in Haiti, death occurred rapidly, and care was either inadequate or nonexistent. We found several possible explanations for this situation.

First, early in the outbreak, the population knew little about cholera. Many decedents did not know to seek care immediately. Knowledge, availability, and use of ORS were inadequate. Although many families acknowledged receiving cholera messages, their understanding was incomplete. Few reported receiving cholera messaging or ORS from CHWs. Global deficiencies in the distribution and use of ORS in recent years have impeded the ability of CHWs to initiate treatment (*7*).

Table 1. Reported cholera ORS treatment received at home for health facility versus community decedents, Haiti, October–November 2010*

ORS use	Health facility decedent, no. (%), n = 48	Community decedent, no. (%), n = 39	Total, no. (%), n = 87
ORS used at home	23 (48)	9 (23)	32 (37)
Place from which ORS was obtained			
Health center	15 (65)	5 (56)	20 (63)
Pharmacy	0	1 (11)	1 (3)
Red Cross/nongovernment organization	5 (22)	0	5 (16)
Friend	3 (13)	3 (13)	6 (19)
ORS sachets observed	16 (35)	13 (35)	29 (33)

*Health facility decedent, cholera case-patient who died in a health facility; community decedent, cholera case-patient who died at home or en route to a health facility; ORS, oral rehydration solution.

Table 2. Time and location of death from cholera for health facility versus community decedents, Haiti, October–November 2010*

Time and location	Health facility decedent, no. (%), n = 48	Community decedent, no. (%), n = 39
At home before receiving care	NA	23 (59)
En route to a health facility	NA	8 (20)
On day of admission	22 (46)	NA
After overnight admission	26 (54)	NA
At home after discharge	NA	8 (20)

*Health facility decedent, cholera case-patient who died in a health facility; community decedent, cholera case-patient who died at home or en route to a health facility; NA, not applicable.

Second, CHWs probably lacked sufficient information, experience, and resources to provide proper treatment early in the outbreak. Identification and aggressive treatment of dehydration is critical for effective cholera treatment. Deaths in health facilities in Haiti might have resulted from problems commonly observed elsewhere: overwhelming patient load, inadequate supplies, and health worker shortages (*8*).

Third, decedents' relatives identified several commonly observed barriers to care: distance to health facility, lack of transport, and unaffordable transport (*9*). Research suggests that the effect of distance and lack of transport on cholera-related death can be mitigated by local treatment with ORS by CHWs (*10*,*11*). Finally, the epidemic strain, which was particularly virulent, might have contributed to deaths (*12*).

Active case finding detected 87% of community decedents. This finding suggests that cholera-related deaths might have been underreported, particularly in more remote communities.

Our study had several limitations. First, time and logistics limited our ability to visit remote communities where more deaths might have occurred. Second, our geographically circumscribed convenience sample might not have been representative of all cholera deaths. Third, medical records at cholera treatment facilities were incomplete or absent. Finally, our data were limited to reports from decedents' families.

Findings from this assessment suggested several practical actions that could mitigate the risk for death from cholera. CHWs, particularly in remote settings, should receive training in cholera treatment and referral and adequate supplies of ORS; similar efforts for HIV and tuberculosis in Haiti have been promising (*13*,*14*). Health providers should receive sufficient cholera training and treatment supplies. Cholera education should be disseminated through multiple communication channels. Longer term efforts to increase health facility staffing and improve access to care should be prioritized.

In response to the epidemic, training and supplies have been provided to health workers in all 10 departments of Haiti. By April 2011, the cholera CFR had declined to <1% (www.mspp.gouv.ht/site/index.php?option=com_content&view=article&id=57&Itemid=1).

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Epidemic Cholera in a Crowded Urban Environment, Port-au-Prince, Haiti

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We conducted a case–control study to investigate factors associated with epidemic cholera. Water treatment and handwashing may have been protective, highlighting the need for personal hygiene for cholera prevention in contaminated urban environments. We also found a diverse diet, a possible proxy for improved nutrition, was protective against cholera.

Epidemic cholera remains a problem in poor countries that lack adequate water and sanitation infrastructure, particularly among populations in crowded, unsanitary conditions (*1*–*4*). On January 12, 2010, a magnitude 7.0 earthquake struck metropolitan Port-au-Prince, Haiti, killing >200,000 persons and destroying vital water and sanitation infrastructure (*5*). Epidemic cholera had not been reported in Haiti in the past century, but on October 21, 2010, toxigenic *Vibrio cholerae* serogroup O1, serotype Ogawa, biotype El Tor, was identified as the cause of a large outbreak of acute watery diarrhea in Artibonite Department, ≈150 km north of Port-au-Prince (*6*). By November 7, the outbreak had reached Port-au-Prince, where >1 million persons were living in internally displaced person camps or crowded slums. By December 15, ≈20,000 cases had been reported in the capital (*7*). We conducted a case–control study during December 15–19, 2010, to investigate illness transmission and guide public health actions.

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The Study

We defined a case as acute, watery diarrhea in a person ≥5 years of age admitted to the Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections (GHESKIO) cholera treatment center (CTC) in Cité de Dieu slum after November 1, 2010. Enumerators administered a standard questionnaire in Haitian Creole to CTC patients or their caregivers to gather demographic, clinical, and treatment information; food and beverage exposures in the 3 days before illness onset; and water, sanitation, and hygiene practices. Enumerators visited each case-patient's household to observe living conditions; water storage and treatment practices; and handwashing technique, which included an assessment of soap use, lathering, and drying procedure. Enumerators enrolled 2 sex-, age group– (5–15 years, 16–30 years, 31–45 years, and >45 years), and neighborhood-matched controls per case-patient by skipping the immediate neighbor and going house to house until 2 controls were identified. An identical questionnaire that included household observations was administered to controls.

We used exact conditional logistic regression to compute matched odds ratios (mORs) with 95% confidence intervals (CIs). For protective food exposures, we calculated a food diversity score for each participant based on the total number of distinct food items consumed in the 3 days before illness onset. We created a 2-level categorical diversity score variable based on the median score. The study protocol was approved by the Haitian Ministry of Public Health and Prevention and the GHESKIO institutional review board.

We enrolled 53 case-patients and 106 controls. The median ages of case-patients and controls were 29 (range

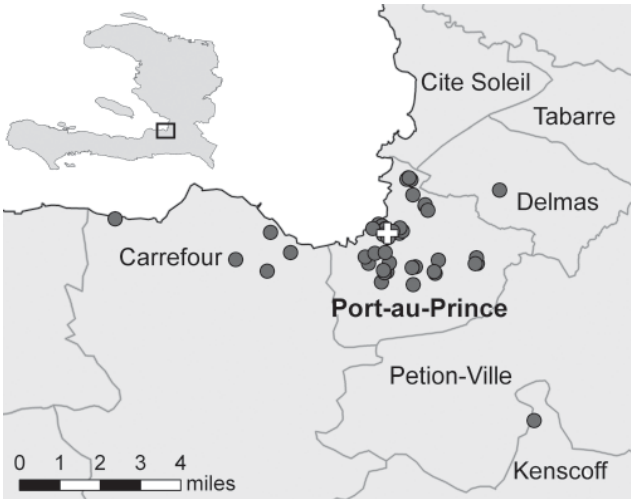

Figure. Locations of the Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections Cholera Treatment Center and case-patient households in Port-au-Prince, Haiti, 2010. Cross indicates cholera treatment center location; circles indicate households.

6–80) and 30 (range 6–85) years, respectively; 45% of case-patients and controls were female. Of participants >15 years of age, 84% self-reported as literate; 37% of case-patients and 57% of controls spoke French (mOR 0.3, 95% CI 0.1–0.8). Participant households were located in the greater Port-au-Prince area (Figure).

All case-patients reported having acute, watery diarrhea; other signs and symptoms included vomiting (81%), rice-water stool (66%), and leg cramps (64%). Reported illness onset dates ranged from December 10 through 19. Of 53 case-patients, 20 (38%) were treated with oral rehydration solution at home prior to admission, and 39 (74%) sought care at a GHESKIO CTC on the first day of symptom onset. CTC treatment included ORS (85%), intravenous fluids (55%), and antimicrobial drugs (9%).

Water sources, which included purchased bags, purchased bottled or filtered water, piped water, and water collected from a tanker, did not differ between case-patients and controls (Table 1). Bladder water (chlorinated water stored in flexible plastic tanks in internally displaced person camps) seemed protective (mOR indeterminate, 95% CI 0–0.9), although exposure frequency was limited and a point estimate could not be calculated. Controls were more likely than case-patients to have treated their drinking water by boiling or chlorinating before the outbreak began in Port-au-Prince (mOR 0.3, 95% CI 0.1–0.9) and to have used proper handwashing technique (mOR 0.2, 95% CI 0.03–0.7).

Food exposures implicated as risk factors for transmission in previous cholera outbreaks in the Americas were not associated with illness, including food or drink purchased from a street vendor, cold leftover food, cold rice, raw food, and seafood (Table 1). Of 60 food exposures included in the questionnaire for the 3 days before illness onset, 29 (48%) were protective against cholera; CIs did not overlap. The median food diversity score for case-patients and controls in the 3 days before illness was 23 (range 4–50). A higher percentage of controls (56%) than case-patients (28%) consumed more than the median number of 23 items in the 3 days before illness (mOR 0.3, 95% CI 0.1–0.6).

Table 1. Characteristics of cholera case-patients and controls, Port-au-Prince, Haiti, December 2010*

Variable	No. (%) case-patients, n = 53	No. (%) controls, n = 106	mOR (95% CI)
Socioeconomic			
Completed primary school†	5 (29)‡	13 (36)‡	0.2 (0.0–1.7)
Literate†	37 (84)‡	72 (84)‡	1.0 (0.3–3.9)
French speaking†	16 (36)‡	49 (57)‡	0.3 (0.1–0.8)
Has electricity	29 (55)	71 (67)‡	0.5 (0.2–1.2)
Owns a radio	37 (70)	77 (73)	0.8 (0.3–2.2)
Owns a television	26 (50)‡	58 (55)‡	0.8 (0.4–1.8)
Owns a car/motorcycle	3 (6)‡	12 (12)‡	0.4 (0.1–1.9)
IDP camp (self-reported)	15 (28)	24 (23)	2.1 (0.5–8.7)
IDP camp (observed)	10 (24)‡	19 (24)‡	0.7 (0.1–7.3)
Tarp roof	11 (21)	19 (18)	1.6 (0.3–8.8)
Unemployed§	5 (13)‡	14 (18)‡	0.7 (0.2–2.4)
Water sources			
Purchased bags (sachets)	9 (17)	9 (9)	2.6 (0.7–10.2)
Purchased bottles/filter	12 (23)	25 (24)	0.9 (0.4–2.3)
Piped (house, yard, public tap)	25 (47)	58 (55)	0.6 (0.2–1.6)
Tanker	7 (13)	11 (10)	1.4 (0.4–5.0)
Bladder	0	8 (8)	NA (0.0–0.9)
Water treatment and handwashing			
Boiling water or using a chlorine product ≤3 d before illness	37 (70)	86 (81)	0.5 (0.2–1.2)
Boiling water or using a chlorine product before November 1, 2010	37 (70)	90 (85)‡	0.3 (0.1–0.9)
Proper handwashing	8 (15)	31 (29)	0.2 (0.0–0.7)
Sanitation: access to toilet/latrine	45 (85)‡	97 (92)‡	0.5 (0.1–1.7)
Foods			
Food or drink from a street vendor	23 (47)‡	52 (55)‡	0.7 (0.3–1.7)
Cold leftover food	23 (44)‡	59 (56)	0.6 (0.2–1.3)
Cold rice	30 (58)‡	59 (56)‡	1.1 (0.5–2.5)
Raw food	3 (6)‡	5 (5) ‡	1.2 (0.2–6.2)
Seafood	12 (23)	37 (35)	0.5 (0.2–1.2)
Food diversity (>23 items)	15 (28)	59 (56)	0.3 (0.1–0.6)

*mOR, matched odds ratio; CI, confidence interval; IDP, internally displaced person.
†Respondents >15 y of age.
‡Denominator does not include all respondents.
§Respondents >17 y of age.

Food diversity score, proper handwashing, and treating drinking water were included in a multivariate model (Table 2). All 3 remained protective against illness, although treating drinking water did not reach statistical significance (mOR 0.4, 95% CI 0.1–1.1). All socioeconomic status variables were considered for model inclusion, but none affected the direction or effect size of predictor variables for cholera.

Conclusions

In this investigation, we identified 2 key practices that may have protected against cholera in the contaminated urban environment of Port-au-Prince: habitual water treatment and proper handwashing. These findings were biologically plausible and consistent with the findings of cholera investigations in other settings (*3*,*8*–*11*).

The protective effect of numerous food exposures was difficult to interpret, although food items such as rice, dried fish, and citrus fruit juice have been found to decrease the risk for cholera in previous outbreaks (*8*,*11*,*12*). We explored the role of food diversity through the calculated score, summarizing the relationship with illness by using crude categorization based on the median number of food items consumed, and found that food diversity was protective against illness. This finding was similar to the protective effect of diet variability observed in a case–control study of illness caused by *Escherichia coli* O157:H7 (*13*). Although differences in food diversity may serve as a proxy for socioeconomic status, other socioeconomic status variables included in multivariate models did not adjust for the protective effect of handwashing and treating water. Alternatively, food diversity differences between case-patients and controls may be a result of differential reporting by case-patients during disease incubation or may reflect the nutritional benefit of a more varied diet, which may mitigate the risk for illness. Further research into the role of diet diversity in diarrheal disease outbreaks is warranted.

This investigation revealed no risk factors for illness despite the inclusion in the questionnaire of numerous potential food and drink exposures identified in hypothesis-generating interviews, including several previously implicated in cholera outbreaks (*14*). The rapid, explosive spread of cholera across Haiti and within Port-au-Prince made it unlikely that a point source would be identified. Instead, poor sanitary infrastructure and widespread contamination created ideal conditions for propagated disease dissemination through multiple vehicles (*15*). In such circumstances, the attributable risk for individual exposures may decrease while personal protective measures, such as household water treatment and handwashing with soap, may emerge as noteworthy findings.

After the January 2010 earthquake, the population of Port-au-Prince was vulnerable to disease outbreaks because of problems with overcrowding, poverty, poor nutrition, and inadequate water and sanitation infrastructure. The cholera epidemic, which was unexpected and particularly explosive in this immunologically naive population, strained the country's capacity to respond. Personal hygiene measures taken by persons and families were crucial to protect against disease. In the long term, with cholera likely to remain a problem in Haiti, providing water and sanitation infrastructure should be a high priority for government and aid organizations.

Table 2. Practices independently associated with cholera prevention in a multivariate model case–control study, Port-au-Prince, Haiti, December 2010*

Practice	mOR (95% CI)	p value
Food diversity (>23 items)	0.3 (0.1–0.7)	<0.01
Proper handwashing	0.2 (0.03–0.90)	0.03
Boiling water or using a chlorine product	0.4 (0.1–1.1)	0.08

*mOR, matched odds ratio; CI, confidence interval.

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Toxigenic *Vibrio cholerae* O1 in Water and Seafood, Haiti

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During the 2010 cholera outbreak in Haiti, water and seafood samples were collected to detect *Vibrio cholerae*. The outbreak strain of toxigenic *V. cholerae* O1 serotype Ogawa was isolated from freshwater and seafood samples. The cholera toxin gene was detected in harbor water samples.

Epidemic cholera is caused by toxigenic strains of *Vibrio cholerae* serogroups O1 and O139, which spread most often through water contaminated with the bacterium (*1*). Cholera can also be transmitted by eating contaminated foods, including seafood (*2*). Like other *V. cholerae* strains, which are autochthonous in riverine, estuarine, and coastal ecosystems, these strains may persist in the environment (*3*). An outbreak of cholera began in Haiti's Artibonite Department in October 2010 and rapidly spread across all 10 Haitian departments and Port-au-Prince. Initial investigations indicated that drinking untreated water was the principal risk factor for infection (*4*). The ongoing risk posed to the Haitian population through contaminated water raised concern that cholera could also be introduced to other countries through transfer of *V. cholerae* by ship ballast water, contaminated seafood, or both. To better characterize the contamination of untreated surface water and seafood and to evaluate the risk for *V. cholerae* transfer from contaminated water in Haitian ports, the US Centers for Disease Control and Prevention (CDC) and the US Food and Drug Administration (FDA) collaborated with the Haitian ministries of health, agriculture, and environment to document the presence of *V. cholerae* in Haitian freshwater resources and harbors.

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The Study

In October and November 2010, water and seafood samples were collected from 2 cholera-affected communities in Haiti and tested for *V. cholerae*. Eight freshwater and 6 marine water samples were collected from 13 sites in Artibonite and Ouest Departments (Figure). Freshwater samples were collected from rivers, including the Artibonite River, and irrigation canals. Dead-end ultrafiltration, a newly developed technique that has been used to recover diverse microbes from large-volume water samples (*5*), was used to collect water samples (8–30 L each) at the freshwater sites and 3 of the marine water sites (HWS-11, -13, and -18). At the Haiti National Public Health Laboratory (LNSP), bacteria were recovered from ultrafilters by back flushing with a surfactant solution, and the solution then was added to an equal volume of 2× strength alkaline peptone water (APW). Grab samples (1 L) were collected at 3 harbor sites (HWS-15, -16, and -17) and on arrival at LNSP, they were split into two 500-mL portions for separate testing by CDC at LNSP or for shipment in chilled coolers to FDA (Dauphin Island, AL, USA). At LNSP, all water and ultrafilter back flush samples were incubated in APW at 37°C for 6 h (*6*). After APW enrichment, the culture broth was streaked onto thiosulfate citrate bile salts (TCBS) agar (Remel, Lenexa, KS, USA) and incubated overnight at 37°C. For each sample, up to 10 colonies suspected of being positive were picked from TCBS agar and grown on nonselective media for multiplex PCR testing (*7*).

Nine seafood samples were collected along the coast: 5 between Saint-Marc port and Grand Saline (1 site) and 4 from Port-au-Prince port (3 sites) (Figure). Seafood samples were obtained as convenience samples from local fishermen on the water, placed in Ziploc (SC Johnson, Racine, WI, USA) bags, and sent in chilled coolers to FDA, along with 500-mL grab samples of marine water. After enrichment, APW cultures from seafood and water grab samples were tested at FDA by using a real-time PCR specific to the cholera toxic gene (*ctxA*) of *V. cholerae* (*8*). If the APW culture was positive for the *ctxA* gene, then isolates were obtained by streaking onto TCBS agar as described for water samples.

Identification and characterization of suspected *V. cholerae* isolates were performed at CDC. Colonies or sweeps of growth typical of *V. cholerae* were selected

Figure. Locations where water and seafood samples were obtained, Haiti, October–November 2010. HWS, harbor water sample.

from TCBS plates and tested by multiplex PCR for *ctxA*, $tcpA_{\text{El Tor}}$, $tcpA_{\text{Classical}}$, *ompW*, and *toxR* genes (*9*–*11*). Colonies positive by PCR for *ctxA* or other *V. cholerae* markers were tested for agglutination in serogroup O1 antiserum and, if positive, also in Inaba and Ogawa serotype antisera. *V. cholerae* isolates were subtyped according to the PulseNet standardized pulsed-field gel electrophoresis (PFGE) protocol, using primary and secondary restriction enzymes *Sfi*I and *Not*I, respectively (*12*). The cholera toxin gene, *ctxAB*, and *tcpA* gene regions were amplified by PCR and sequenced (*13*,*14*).

V. cholerae O1, serotype Ogawa, *ctxA*-positive strains were isolated from 2 irrigation canals north of Port-au-Prince in Ouest Department (Table 1). Both of these canals were used for drinking water by the local population, and communities near the canals were heavily affected by the outbreak. *V. cholerae* O1 Ogawa *ctxA*-positive strains were isolated from 1 mixed seafood sample (sample 7, containing multiple vertebrate fish and 1 crab) and 1 bivalve sample (sample 8, containing multiple species) that were obtained from fishermen at 2 different locations in the Port-au-Prince port (Table 2). All *ctxA*-positive *V. cholerae* isolates were indistinguishable from the outbreak strain by PFGE with both enzymes (pattern combination KZGS12.0088/KZGN11.0092) (*15*). Sequence analysis for the toxigenic *V. cholerae* isolates provided additional evidence that the isolates from these samples matched the isolates from humans infected with the outbreak strain. The *tcpA* sequence of the freshwater and human isolates from Haiti matched that of CIRS 101, an altered El Tor strain from Bangladesh, and the *ctxAB* sequences matched the sequences from strains isolated in 2007 during an outbreak in Orissa, India (*15*). The *ctxAB* and *tcpA* sequences differed by 1 nt polymorphism from prototypical classical and El Tor alleles, respectively. These isolates were recovered from 30-L freshwater samples having turbidities of 11 and 16 nephelometric turbidity units, which were among those with the lowest turbidity collected during this investigation. All *V. cholerae* non-O1 *ctxA*-negative strains possessed unique PFGE patterns distinct from the outbreak pattern. In addition to samples from which toxigenic *V. cholerae* was isolated, real-time PCR testing by FDA detected the *ctxA* gene in APW culture broths for 3 seawater samples and 3 other seafood samples.

Conclusions

Isolation of the outbreak strain in seafood samples from Port-au-Prince and detection of the *ctxA* gene in APW cultures of water and seafood samples from Port-au-Prince and Saint-Marc suggest that harbor waters were contaminated with toxigenic *V. cholerae* O1. This finding underscores the need for adherence to public health recommendations disseminated during the outbreak regarding making drinking water safe and cooking seafood thoroughly to prevent infection and conducting ship ballast water exchange to limit potential transfer of the organism to other harbors. We report recovery of *V. cholerae* O1 from large-volume water samples by use of ultrafiltration.

Table 1. Results of water sampling for *Vibrio cholerae*, Haiti, October–November 2010*

Sample no.	Sample location	Sample types	Collection date	Volume sampled, L	Turbidity, NTU	APW broth real-time PCR result	Characterization of *V. cholerae* isolates
HWS-1	Liancourt River	UF	Oct 29	7.7	150	ND	No isolate obtained
HWS-2	Artibonite River	UF	Oct 29	16	250	ND	No isolate obtained
HWS-3	Obya River	UF	Oct 30	35	31	ND	Non-O1, non-O139, *ctxA* negative
HWS-5	Sipa Canal	UF	Oct 30	32	88	ND	Non-O1, non-O139, *ctxA* negative
HWS-7	Brown Root River	UF	Oct 30	21	11	ND	Non-O1, non-O139, *ctxA* negative
HWS-9	Freshwater canal (canal 2)	UF	Nov 2	30	16	ND	Isolate matched outbreak strain†
HWS-10	Freshwater canal (canal 1)	UF	Nov 2	30	11	ND	Isolate matched outbreak strain†
HWS-11	Saint Marc port marine water	UF	Nov 9	30	ND	ND	No isolate obtained
HWS-13	Saint Marc port marine water	UF	Nov 9	30	ND	ND	No isolate obtained
HWS-14	Grand Saline canal	UF	Nov 10	20	170	ND	Non-O1, non-O139, *ctxA* negative
HWS-15	Saint Marc/Grand Saline port marine water	Grab	Nov 9	1	ND	*ctxA* detected	No isolate obtained
HWS-16	Port-au-Prince port, site 1 marine water	Grab	Nov 11	1	ND	*ctxA* detected	No isolate obtained
HWS-17	Port-au-Prince port, site 2 marine water	Grab	Nov 11	1	ND	*ctxA* detected	No isolate obtained
HWS-18	Port-au-Prince port, site 3 marine water	UF	Nov 11	28	ND	ND	No isolate obtained

*NTU, nephelometric turbidity units; APW, alkaline peptone water; HWS, harbor water sample; UF, ultrafiltration; ND, no data.
†*V. cholerae* serogroup O1, serotype Ogawa, biotype El Tor, *ctxA* positive, pulsed-field gel electrophoresis–matched outbreak strain.

Although *V. cholerae* O1 was not isolated from marine water samples, real-time PCR detection of *ctxA* in these samples provided additional evidence that harbor water samples were contaminated with toxigenic *V. cholerae*. Use of this real-time PCR method has provided analytical data that reflected the presence of viable *V. cholerae* in marine water samples (*8*). Further assessment by using high-volume filtration and seafood sampling may be useful for tracking the persistence of the strain in the Haitian environment in the future.

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Table 2. Results of seafood sampling for *Vibrio cholerae*, Haiti, October–November 2010*

Sample no.	Sample location†	Seafood type	APW broth real-time PCR result	Characterization of *V. cholerae* isolates
1	Saint Marc/Grand Saline	Oysters	*ctxA* detected	No isolate obtained
2	Saint Marc/Grand Saline	Red mussels	No *ctxA* detected	No isolate obtained
3	Saint Marc/Grand Saline	Queen conch	*ctxA* detected	No isolate obtained
4	Saint Marc/Grand Saline	Conch	No *ctxA* detected	No isolate obtained
5	Saint Marc/Grand Saline	Clams	No *ctxA* detected	No isolate obtained
6	Port-au-Prince, site 1	Octopus	No *ctxA* detected	No isolate obtained
6	Port-au-Prince, site 1	Clams	*ctxA* detected	No isolate obtained
6	Port-au-Prince, site 1	Assorted gastropods	*ctxA* detected	No isolate obtained
6	Port-au-Prince, site 1	Assorted bivalves	*ctxA* detected	No isolate obtained
7	Port-au-Prince, site 2	Fish and crab combined sample	*ctxA* detected	Isolate matched outbreak strain‡
8	Port-au-Prince, site 3	Assorted bivalves	*ctxA* detected	Isolate matched outbreak strain‡
9	Port-au-Prince, site 3	Mussels	No *ctxA* detected	No isolate obtained

*ID, identification; APW, alkaline peptone water.
†Samples 1–5, sample 6, sample 7, and samples 8 and 9 were obtained at the same locations as water samples HWS-15, HWS-16, HWS-17, and HWS-18, respectively (Table 1; Figure).
‡*V. cholerae* serogroup O1, serotype Ogawa, biotype El Tor, *ctxA*-positive, pulsed-field gel electrophoresis–matched outbreak strain.

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Drug-Resistance Mechanisms in *Vibrio cholerae* O1 Outbreak Strain, Haiti, 2010

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To increase understanding of drug-resistant *Vibrio cholerae,* we studied selected molecular mechanisms of antimicrobial drug resistance in the 2010 Haiti *V. cholerae* outbreak strain. Most resistance resulted from acquired genes located on an integrating conjugative element showing high homology to an integrating conjugative element identified in a *V. cholerae* isolate from India.

Vibrio cholerae is the bacterium that causes cholera, a disease characterized by acute watery diarrhea, vomiting, muscle cramps, and severe dehydration (*1*). The bacterium has many serogroups, but only toxin-producing serogroups O1 and O139 cause epidemic cholera. The primary treatment for cholera is rehydration with oral or intravenous fluids (*2*). For severe cases, antimicrobial agents may reduce the volume and duration of diarrhea (*1*,*2*). Tetracyclines (e.g., doxycycline), fluoroquinolones (e.g., ciprofloxacin), macrolides (e.g., erythromycin), and trimethoprim/sulfamethoxazole have commonly been used to treat cholera (*2*).

Antimicrobial drug resistance can undermine the success of antimicrobial therapy. Several reports have documented tetracycline- and fluoroquinolone-resistant *V. cholerae*, and multidrug resistance is increasing (*3*). Antimicrobial drug resistance in *Vibrio* spp. can develop through mutation or through acquisition of resistance genes on mobile genetic elements, such as plasmids, transposons, integrons, and integrating conjugative elements (ICEs). ICEs integrate and replicate with the host chromosome and can excise themselves and transfer between bacteria by conjugation (*4*). ICEs commonly carry several antimicrobial drug resistance genes and play a major role in the spread of antimicrobial drug resistance in *V. cholerae* (*5*). The first *V. cholerae* ICE described was in an O139 isolate in Madras, India, in 1992 and was named SXT after the resistance phenotype it conferred (trimethoprim/sulfamethoxazole) (*6*). Many O139 and O1 isolates have since acquired SXT or a closely related ICE (*4*,*5*).

We describe antimicrobial drug resistance mechanisms in the 2010 Haiti *V. cholerae* O1 outbreak strain. Most of the resistance is caused by acquired genes located on an ICE with high similarity to an ICE identified in a *V. cholerae* O1 isolated in India.

The Study

During October 2010–January 2011, a total of 122 clinical isolates of laboratory-confirmed *V. cholerae* O1 were recovered by the National Public Health Laboratory in Haiti and submitted to the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) for characterization. Disk-diffusion antimicrobial drug susceptibility testing was performed at the National Public Health Laboratory and CDC. MICs were determined by broth microdilution at CDC by using Sensititer plates (CAMPY and CMV1AGNF; Trek Diagnostics, Cleveland, OH, USA) according to the manufacturer's instructions with the following modifications: Mueller-Hinton broth without blood was used on the CAMPY plate, and for both plates, a final inoculum concentration of 5×10^4 to 5×10^5 CFU/mL was targeted. *Escherichia coli* American Type Culture Collection (ATCC; Manassas, VA, USA) 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control testing. Where available, Clinical and Laboratory Standards Institute criteria specific for *V. cholerae* were used (*7*). For drugs lacking such criteria, manufacturers' criteria, Clinical and Laboratory Standards Institute criteria for *Enterobacteriaceae,* or consensus breakpoints used by the National Antimicrobial Resistance Monitoring System were applied (*8*,*9*). Furazolidone was tested only by disk diffusion, and azithromycin was tested only by broth microdilution.

Results for all 122 outbreak isolates were similar. They showed susceptibility to azithromycin and tetracycline, reduced susceptibility to ciprofloxacin (MIC 0.25–1.0 mg/L), and resistance to furazolidone, nalidixic acid, sulfisoxazole, streptomycin, and trimethoprim/sulfamethoxazole.

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With a common susceptibility pattern among all outbreak isolates, 1 isolate, 2010EL-1786 (deposited under ATCC BAA-2163), was chosen for molecular characterization. PCR was used to screen the isolate for the following resistance genes: *strA*, *strB*, *sul1*, *sul2*, *dfrA1*, *dfrA10*, and *dfrA12* (*10*). In addition, the *gyrA* and *parC* genes were sequenced to identify quinolone resistance–determining region mutations. PCR was performed according to standard protocols by using the HotStarTaq PCR Master Mix (QIAGEN, Valencia, CA, USA). DNA sequencing was performed by using a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

The isolate 2010EL-1786 contained *strA/B*, *sul2*, and *dfrA1*, which mediate resistance to streptomycin, sulfisoxazole/sulfamethoxazole, and trimethoprim, respectively. Nalidixic acid resistance and decreased susceptibility to ciprofloxacin were attributed to mutations in *gyrA* (Ser83Ile) and *parC* (Ser85Leu). The mechanism responsible for furazolidone resistance was not identified. Mutations in the *nfsA* and *nfsB* genes are associated with furazolidone resistance in *E. coli*, but inspection of the 2010EL-1786 sequence failed to identify these genes.

Location of the resistance genes was analyzed by whole-genome sequencing of 2010EL-1786. Single-end 454 pyrosequencing (GS FLX-Titanium; Roche Diagnostics, Indianapolis, IN, USA) reads and single-end 36-bp Illumina Solexa (GAIIe; Illumina, San Diego, CA, USA) reads were assembled de novo by using Newbler (Roche Diagnostics) and CLC Genomics Workbench (CLC bio, Cambridge, MA, USA) software. Sequence finishing was performed by using Sanger sequencing of fosmid clones (*11*).

Whole-genome sequencing identified an ICE inserted in the *prfC* gene. This ICE, designated ICE*Vch*Hai1, was 97.9 kb and contained 95 open reading frames (Figure 1). All resistance genes identified were located on ICE*Vch*Hai1. In addition, *floR*, a chloramphenicol resistance gene, was detected. The *strA*, *strB*, *sul2*, and *floR* genes were part of an ≈17-kb fragment inserted into the *rumB* gene, whereas the *dfrA1* gene was located ≈70 kb further downstream. Whole-genome sequencing also indicated a chloramphenicol acetyltransferase gene, *catB9*, that was not part of the ICE.

The genetic relatedness of ICE*Vch*Hai1 was assessed by comparison with 7 other ICE sequences (*4*). Sequence alignments were performed by using Progressive Mauve (http://asap.ahabs.wisc.edu/mauve/download.php) and visualized with PHYLIP version 3.69 (distributed by J. Felsenstein, Department of Genome Sciences, University of Washington, Seattle, WA, USA). ICE*Vch*Hai1 showed highest homology to ICE*Vch*Ind5, an ICE derived from a *V. cholerae* isolate from India (Figure 2). These ICEs differed by only 5 single-nucleotide polymorphisms.

Conclusions

In October 2010, an epidemic caused by toxigenic *V. cholerae* O1, serotype Ogawa, biotype El Tor strain,

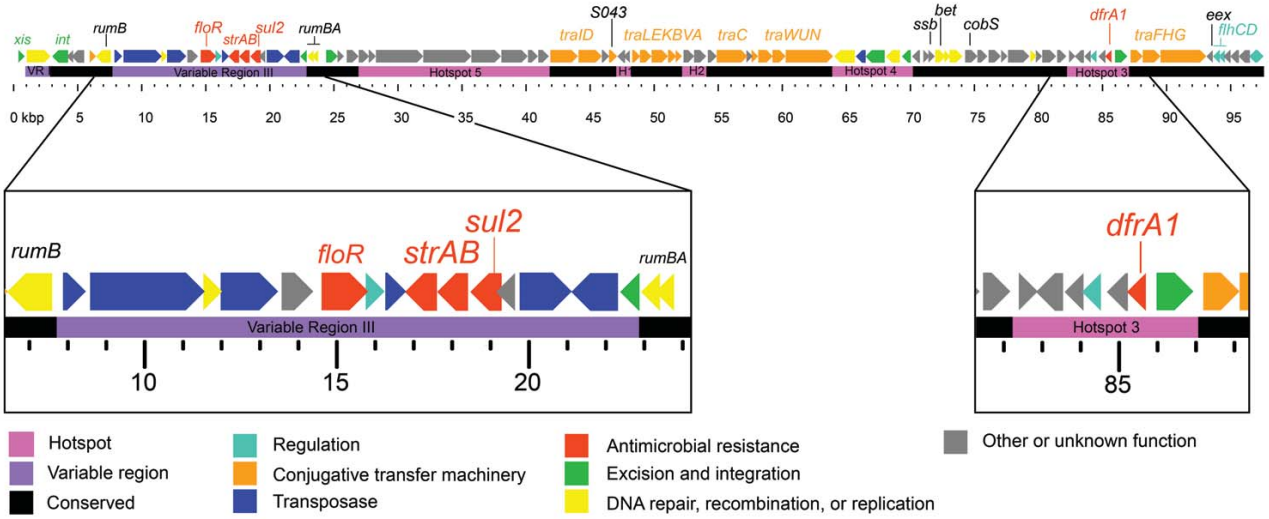

Figure 1. Genetic organization of the 2010 Haiti *Vibrio cholerae* O1 integrating conjugative element (ICE), ICE*Vch*Hai1. The ICE contained 97,915 bp and 95 open reading frames. Coding sequences were identified and manually annotated by using an in-house modified version of GenDBv2.2 (Center for Biotechnology at Bielefeld University, Bielefeld, Germany, www.cebitec.uni-bielefeld.de/groups/brf/software/gendb_info/index.html). Regions conserved among previously sequenced ICEs are indicated in black, regions of variability in purple, and previously identified hotspots of homologous recombination in pink. Conserved genes involved in conjugation are indicated in orange. Genes associated with antimicrobial drug resistance (*floR* [chloramphenicol], *strAB* [streptomycin], *sul2* [sulfamethoxazole], and *dfrA1* [trimethoprim]) are indicated in red. The complete sequence of ICE*Vch*Hai1 has been deposited into GenBank under accession no. JN648379.

was reported from Haiti. We confirmed that the outbreak strain was multidrug resistant and displayed resistance to furazolidone, nalidixic acid, sulfisoxazole, streptomycin, and trimethoprim/sulfamethoxazole and decreased susceptibility to ciprofloxacin. Genetic mechanisms responsible for resistance to 5 of these drugs were identified. Sequencing also detected *floR*, a gene commonly associated with chloramphenicol resistance in *Enterobacteriaceae* (MICs ≥32 mg/L) (*12*). However, in this study, *floR* was not associated with resistance; isolates from Haiti displayed chloramphenicol MICs of 4–16 mg/L (*7*). Clinical non-Haiti isolates lacking the *floR* gene displayed MICs ≤1 mg/L. Why the *floR* gene did not confer resistance in *V. cholerae* remains to be investigated but might be because of lower expression levels. Sequencing also showed a chloramphenicol acetyltransferase gene, *catB9*. How this gene affects chloramphenicol MICs in *V. cholerae* remains to be determined.

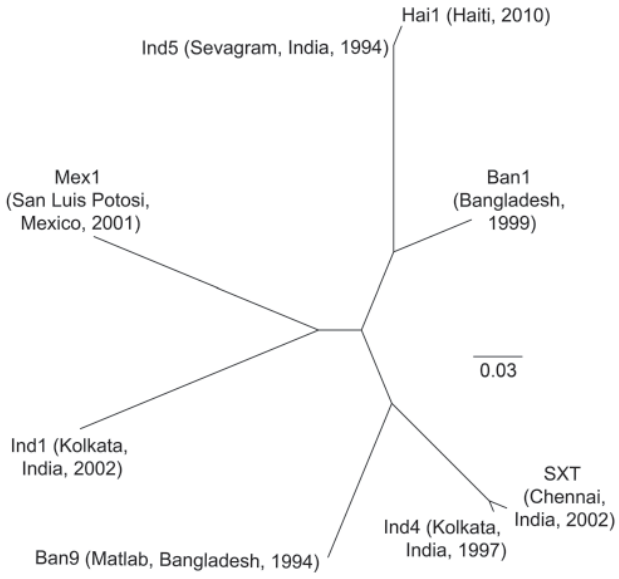

Figure 2. Phylogenetic tree illustrating the genetic relatedness between the Haiti integrating conjugative element (ICE) ICE*Vch*Hai1 and other ICEs described in *Vibrio cholerae* (ICE*Vch*Ban1, ICE*Vch*Ban9, ICE*Vch*Ind1, ICE*Vch*Ind4, ICE*Vch*Ind5, ICE*Vch*Mex1, and SXT*).* Each ICE is listed by an abbreviated name followed by geographic origin and isolation year of the isolate in this analysis. The sequence of ICE*Vch*Hai1 was aligned with the other *V. cholerae* ICE sequences by using the software Progressive Mauve (http://asap.ahabs.wisc.edu/mauve/download.php), and a neighbor-joining phylogenetic tree was constructed by using PHYLIP (PHYLIP [Phylogeny Inference Package] version 3.69; distributed by J. Felsenstein, Department of Genome Sciences, University of Washington, Seattle, WA, USA). Branch lengths indicate the genetic distance between the different ICEs. The ICE*Vch*Hai1 showed highest homology (5 single-nucleotide difference) to ICE*Vch*Ind5, an ICE first detected in an isolate of *V. cholerae* O1 in Sevagram, India, in 1994. The ICE sequences in the analysis can be accessed by using the following GenBank accession nos.: Ban1, GQ463139; Ind1, GQ463144; Ind4, GQ463141; Ind5, GQ463142; Mex1, GQ463143; Ban9, CP001485; and SXT, AY055428. The complete sequence of the ICE*Vch*Hai1 has been deposited into GenBank under accession no. JN648379. Scale bar indicates nucleotide substitutions per site.

Most of the acquired resistance genes were located on an ≈97-kbp ICE termed ICE*Vch*Hai1. The presence of an ICE in *V. cholerae* from Haiti was documented by Chin et al. in 2011 (*13*). ICE*Vch*Hai1 showed high homology to ICE*Vch*Ind5, an ICE first identified in a *V. cholerae* isolate from Sevagram, India, in 1994. Since then, ICE*Vch*Ind5 has persisted among clinical isolates in India; a recent study of O1 strains isolated in India during 1994–2005 confirmed that ICE*Vch*Ind5 was the only ICE that persisted during the study period (*14*).

Drug-resistant *V. cholerae* is a global health concern because resulting infections can be more severe and difficult to treat. Infections with drug-resistant *V. cholerae* can result in higher case-fatality rates, prolonged hospitalizations, more secondary infections, and increased health care costs. During an outbreak in Guinea-Bissau, case-fatality rates increased from 1% to 5.3% after the outbreak strain acquired multidrug resistance (*15*). To limit development and spread of antimicrobial drug resistance among *V. cholerae*, treatment with antimicrobial agents should be restricted to patients with severe dehydration or other conditions that truly warrant their use. Surveillance should continue for antimicrobial drug resistance among *V. cholerae* isolates from Haiti.

Dr Sjölund-Karlsson is a research microbiologist with the National Antimicrobial Resistance Surveillance Team at CDC. Her research interests include the characterization of antimicrobial drug–resistant bacteria, mechanisms of resistance, and studies of the biological cost of antimicrobial drug resistance.

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Cholera Management and Prevention at Hôpital Albert Schweitzer, Haiti

Silvia Ernst, Carolyn Weinrobe, Charbel Bien-Aime, and Ian Rawson

In October 2010, Hôpital Albert Schweitzer Haiti treated some of the first patients with cholera in Haiti. Over the following 10 months, a strategic plan was developed and implemented to improve the management of cases at the hospital level and to address the underlying risk factors at the community level.

Hôpital Albert Schweitzer (HAS), Haiti, built in 1956, is a 130-bed, private, nonprofit facility that serves >340,000 persons in the Artibonite region of central Haiti. The mission of HAS is to reduce illness and death by providing primary and secondary health care, building wells and latrines, and supporting community development activities.

On October 19, 2010, HAS began to receive male patients without underlying medical conditions who had acute watery diarrhea and vomiting. This clinical presentation was unusual; the rapid onset and severity of dehydration was atypical of diarrheal diseases seen at the hospital. Three days later, on October 20, 2010, an additional 24 persons arrived with the same signs, causing alarm among medical personnel, who then contacted the Ministry of Public Health and Population (MSPP) and the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA).

Concomitantly, HAS received news that the densely populated city of St. Marc, ≈20 km to the west along the Artibonite River, had a large number of persons with similar signs. Although cases of cholera had not been documented in Haiti for over a century (*1*), health care providers began to suspect cholera because of the patients' clinical signs. On October 21, 2010, the National Laboratory of Public Health officially announced a cholera outbreak in the Artibonite region. In the days after seeing the initial cases, HAS developed a strategic plan to 1) create an isolated patient area, 2) train medical personnel in cholera treatment, 3) educate the population, and 4) improve poor sanitary conditions through latrine building in the community.

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Management Strategies

A temporary isolated cholera treatment area was set up in the hospital's courtyard, and routine hospital services were relocated to prevent nosocomial spread. MSPP, with the help of international organizations, established cholera treatment centers (CTCs) at 2 nearby public hospitals, while HAS acted as a referral hospital to treat immunosuppressed patients and patients with complicated cases. However, patients with cholera continued to seek care at HAS, which required the hospital to develop a longer term plan to manage cholera patients.

The medical director implemented the World Health Organization cholera treatment protocols, which included oral rehydration solution for patients with moderate dehydration and intravenous Ringer's lactate solution and antimicrobial drugs for the patients with severe dehydration (*2*). All medical personnel received training in implementation of these protocols. Cases were defined as persons ≥5 years of age who were admitted to HAS with acute watery diarrhea, with or without vomiting.

Infection control was a top priority for HAS. Beds were removed and disinfected after each patient was discharged. With a limited number of nurses, family members were trained to be the primary caregivers for patients in the CTC; training included proper hygiene. Sanitation staff supervised handwashing stations, disinfected all buckets used for waste collection, disinfected the floors and walls, and sprayed the shoes of everyone exiting the facility with chlorinated water.

Although the initial cases were among men working in the rice fields, cholera quickly spread among persons of all age groups, both sexes, and residing in valley and mountain regions. A complicated disease course often developed in the elderly and those patients with underlying medical conditions. Older patients experienced renal failure, pulmonary edema, and heart failure; hypoglycemia developed in young children who were malnourished. Severe dehydration among pregnant women caused many to have miscarriages, a known side effect of cholera (*3*). These complications were challenging because patients could not be transferred from the CTC to the hospital for specialized care because of cholera's contagiousness. Because medical records were incomplete for almost half of the patients with cholera, an accurate overall case-fatality rate could not be calculated. Among the 2,359 patients for whom outcome data were available, the case-fatality rate at the hospital was 0.8%.

As the number of cases continued to rise in November 2010, HAS opened a permanent CTC adjacent to the hospital. By January 2011, there were only on average 6 new admissions per day to the CTC, so staffing was considerably reduced. At the same time, CTCs run by international organizations in neighboring towns were

reducing operations and shifting management responsibilities to MSPP. In late May 2011, the number of CTC admissions increased rapidly with the beginning of the rainy season; by June 29, 2011, the number of CTC admissions peaked at 132 new cases per day, a rate even greater than that during the initial outbreak (Figure 1). With no other CTCs nearby operating 24 hours a day and able to treat persons with severe dehydration, HAS served a large geographic area.

During this outbreak, patients were treated in 6 tents organized by severity of dehydration (Figure 2). As the outside temperature was >90°F, the temperature inside the tents was much higher, posing a threat to already severely dehydrated patients. Patients were closely monitored by nurses and family members to ensure adequate hydration. The CTC's waste management system was overloaded, so more latrines were built. Visiting teams of doctors and nurses supported the permanent staff in dealing with the patient surge.

Community mobilization was essential for slowing the spread of the outbreak. After initial reports that severe diarrhea was being treated with traditional remedies of guava leaves and rum and that persons were dying of "supernatural causes," HAS staff began meeting with influential community members such as traditional healers, teachers, and religious and local leaders. Community health workers, trained by HAS, educated the community on cholera signs and symptoms and disseminated prevention messages on proper hygiene while also distributing oral rehydration solution and water treatment products.

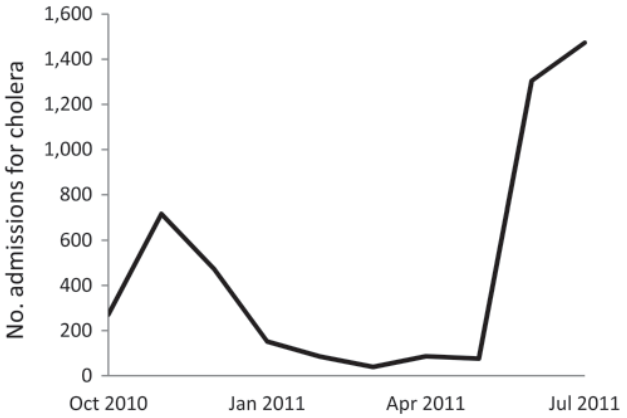

Figure 1. Cholera treatment center admissions, patients ≥5 years of age, Hôpital Albert Schweitzer, Artibonite Department, Haiti, October 17, 2010–July 31, 2011. N = 4,606. Data from cholera treatment center admissions records.

Conclusions

Poor sanitary conditions were present in the community long before the cholera outbreak (*4*). Although an informal survey of CTC patients from January 2011–May 2011 showed that most knew to drink treated water and wash their hands, only slightly more than half had access to a latrine. Soap and water-treatment products distribution addressed the acute but not chronic need for latrines. After site visits that included hygiene education and latrine assessments, HAS constructed 266 latrines. The hospital continues to work with regional partners to expand these activities.

Point-of-use water treatment has proven to be a more complicated intervention; powdered chlorine to add to water and charcoal for boiling water are expensive, and water purification tablets are donor driven and lack standardization across organizations. Different types of tablets treat different amounts of water, and low literacy rates and language comprehension make ensuring proper use of the tablets challenging.

After the surge in cholera cases in June 2011 with the start of the rainy season, it is likely that cholera has become endemic to rural Haiti. HAS is prepared to address the clinical consequences of this disease, but more needs to be done to reduce illness and death. The rise in cases signaled that the MSPP hospital infrastructure was not capable of addressing the disease independently. Without a regional plan that incorporated private and public institutions, cholera treatment was conducted primarily by HAS, a private institution. The lack of preparedness across the Artibonite region for the resurgence of cholera during the rainy season proved that a unified system must be fully equipped to manage future outbreaks. Such a system should be developed by MSPP in collaboration with private institutions and international organizations.

To reduce the effects of cholera in rural Haiti, all households should have access to latrines and clean water

Figure 2. Tents where patients with cholera were treated at Hôpital Albert Schweitzer, Artibonite Department, Haiti, October 17, 2010–July 31, 2011.

(*5*). To reduce cholera illness and death, private and public hospitals must work closely with the government and international organizations to standardize a nationwide plan of action.

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Knowledge, Attitudes, and Practices Related to Treatment and Prevention of Cholera, Haiti, 2010

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In response to the recent cholera outbreak, a public health response targeted high-risk communities, including resource-poor communities in Port-au-Prince, Haiti. A survey covering knowledge and practices indicated that hygiene messages were received and induced behavior change, specifically related to water treatment practices. Self-reported household water treatment increased from 30.3% to 73.9%.

Haiti had not experienced an outbreak of cholera for more than half a century. This changed in October 2010 when a large outbreak occurred in Artibonite Department and quickly spread to the remaining departments of Haiti, including the city of Port-au-Prince (*1*). Given the prevalence of known risk factors for explosive spread of the disease (e.g., low socioeconomic status, high population density), an emergency public health response was initiated. With crowded conditions and limited access to safe water and sanitation, persons living in the capital of Port-au-Prince were especially vulnerable to acquiring cholera (*2*–*5*). This risk was exacerbated by the January 12, 2010, earthquake, which led to >1.5 million persons seeking shelter and services at internally displaced persons settlements in and around the capital (*6*). The first cases were confirmed in Port-au-Prince on November 7, 2010. As of August 8, 2011, Port-au-Prince had reported 112,464 cholera cases and 760 deaths (*6*).

In response to the cholera outbreak, the Haiti government and partner agencies initiated emergency public health response activities aimed at treating suspected cholera cases and preventing new ones. Response activities included mass media cholera campaigns through radio and hygiene promotion activities by community health workers, distribution of water purification tablets and soap, and limited distribution of oral rehydration solution (ORS) sachets. Prevention efforts focused on internally displaced person settlements in Port-au-Prince and the poorer neighborhoods of the city where information regarding cholera knowledge, dissemination of cholera information, and distribution of treatment and prevention supplies was limited.

The Study

During December 6–7 and 14–16, 2010, we conducted a survey to assess the effectiveness of interventions implemented to prevent the spread of cholera and to improve specific response activities in these neighborhoods. Because this investigation was a public health response to an emergency, the Centers for Disease Control and Prevention determined that institutional review board review was not necessary. Informed consent was obtained from all participants.

The survey collected cross-sectional data on household demographics, communications preferences, knowledge of cholera transmission and prevention, water sources and treatment, and hygiene practices. Samples of stored water in the home were tested for chlorine residue by using the Hach Free Chlorine Test (Hach Co., Loveland, CO, USA) to provide an objective measure of water treatment. Microbiological testing for *Escherichia coli* by using IDEXX Quanti-Tray/2000 (IDEXX Laboratories, Inc., Westbrook, ME, USA) was also conducted on source water. Sampling weights according to the population size were used to improve the overall representativeness of results.

A household questionnaire (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-0818-Techapp.pdf) was pilot tested and administered to 405 households from 27 clusters from resources-limited areas of Cité Soleil, Delmas, Carrefour, and Pétion-Ville (Figure). Clusters were randomly selected by using population proportional to size sampling, with the exception of Cité Soleil, which was undersampled to provide more geographic representation in the sample. In each of the 27 selected clusters, 15 households were selected randomly along a radius from the edge to the center of the cluster.

Persons interviewed were primarily female heads of households (81%). Average household size was 5 persons, and median age of respondents was 35 years (range 17–80

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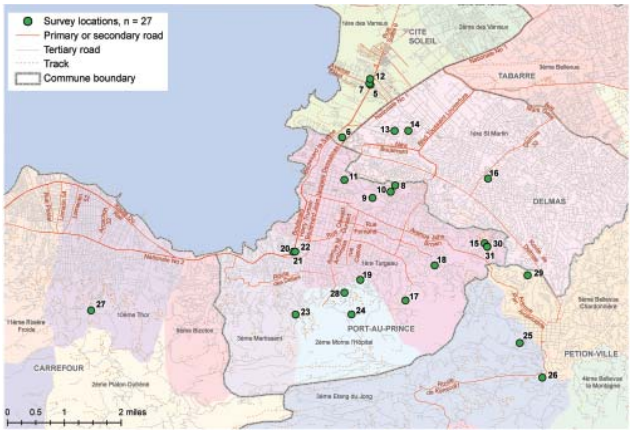

Figure. Selected clusters for the knowledge, attitudes, and practices related to treatment and prevention of cholera survey administered during December 6–7 and 14–16, 2010, Port-au-Prince, Haiti.

years). Most respondents had access to a cellular telephone (88.1%), radio (67.1%), and television (66.3%). The preferred forms of communication for receiving cholera messages were television (71.1%), radio (68.8%), and trucks with megaphones (44.0%). Knowledge of common signs of cholera was high; the 2 most common signs described were diarrhea (89.1%) and vomiting (83.4%). Respondents also showed high knowledge of transmission modes; 71.9% indicated consumption of contaminated water and 61.4% indicated consumption of contaminated food. The most common prevention method cited was handwashing (86.0%).

Before the outbreak, the most common drinking water sources were piped water to tap stands and public kiosks (Table 1). These water sources were chlorinated irregularly, and only 6.2% of respondents believed that drinking water from the piped supplies was safe. Microbiological testing of 11 unchlorinated piped water sources indicated that 7 were positive for an indicator of fecal contamination (*E. coli*). Collection of tap water decreased during the cholera outbreak, whereas collection of drinking water from private kiosks nearly doubled (47.6%). Public health messages on the health benefits of water treatment showed diffusion in these neighborhoods; water treatment practices increased from 30.3% before the cholera outbreak to 73.9% after the outbreak ($p<0.05$), and the 2 most common methods used were water purification tablets (66.6%) and bleach (57.7%) (Table 1). Water purification tablets were considered palatable by most respondents (87.7%), and 70.2% reported purchasing them in the past month (Table 2).

Among 403 (99.5%) households, ≈60% of samples from stored drinking water tested positive for residual chlorine (range 0–3.5 mg/L). Additionally, during the survey administration, nearly all (94.4%) water storage containers had a cover on them that could reduce the chance of contamination.

Hygienic practices (e.g., handwashing and latrine use) are critical for preventing the spread of diarrheal diseases (*7–10*). Active acceptance of these practices and use of soap was high among respondents. Approximately 94.1% reported washing their hands with soap; 84.1% reported having access to soap, 95.7% reported purchasing soap, and 16.5% reported receiving soap from a distribution location since the outbreak started (Table 2). Use of improved latrines was also reported by most respondents (74.0%).

ORS is a lifesaving therapy for diarrheal diseases, including cholera (*11*). Nearly 90% of respondents stated that they knew the method of ORS preparation, although only 76.0% of respondents indicated the correct volume of

Table 1. Drinking water sources and treatment before and after cholera outbreak, as reported by survey respondents, Port au Prince, Haiti, 2010*

Source or treatment	Before outbreak		After outbreak	
	No. yes/total no. respondents	Weighted % (95% CI)	No. yes/total no. respondents	Weighted % (95% CI)
Water source				
Piped public kiosk	122/396	32.5 (21.3–43.7)	84/391	21.5 (10.5–32.5)
Piped in house	101/396	26.9 (15.1–38.7)	57/391	15.1 (7.9–22.2)
Private kiosk	129/396	26.8 (18.7–34.9)	203/391	47.6 (36.2–58.9)
Tank filled by truck	11/396	4.4 (0–8.6)	12/391	5.1 (0.8–9.4)
Bladder	3/396	0.6 (0–1.2)	8/391	3.2 (0–8.0)
Other source	7/396	1.3 (0–3.3)	7/391	1.9 (0–3.9)
Treated water (any method)	130/405 (30.3)	30.3 (22.1–38.4)	307/405	73.9 (67.2–80.6)
Method of treatment†				
Water purification tablets	79.119 (66.6)	66.6 (52.8–80.4)	259/301	86.1 (80.2–92.0)
Bleach	76.132 (57.7)	57.7 (47.6–67.8)	174/347	50.1 (36.2–64.1)
Boiling	11/162 (6.8)	6.8 (2.9–10.7)	25/385	6.5 (3.4–9.6)
PuR, Gadyen Dlo, or Dlo Lavi	0	NA	1/333	0.3 (0–0.8)
Other answer	4/160 (2.5)	2.5 (0–5.5)	2/100	2.0 (1.3–3.3)

*CI, confidence interval; NA, not applicable. Sampling weights are according to population size. Before and after data were collected at 1 time point.
†Respondents selected >1 method of water purification.

Table 2. Access to soap and attitudes toward water purification tablets, Port au Prince, Haiti, December 6–7 and 14–16, 2010*

Access and attitude	No. respondents	Weighted† % (95% CI)
Soap		
Received soap	65	16.5 (3.6–29.4)
Purchased soap	381	95.7 (93.9–97.5)
Had soap at the house at time of survey	355	84.1 (81.3–86.9)
Water purification tablets		
Received in the past month	178	41.5 (29.9–53.1)
Bought in the past month, n = 403	279	70.2 (64.3–76.2)
Know how to use, n = 402	389	97.5 (96.0–99.1)
Perceptions of water purification tablets, n = 387		
Strong taste, unacceptable	25	4.7 (2.4–7.0)
Some taste, acceptable	345	87.7 (83.7–91.6)
No taste	3	0.6 (0–1.2)

*n = 404 except as indicated. CI, confidence interval.
†Sampling weights are according to population size.

water needed to prepare an ORS sachet as recommended by the World Health Organization (*12*). One fourth of respondents had ORS in their home when the survey was conducted.

This investigation had several limitations. Because of security restrictions, independent enumerators could not be used. Therefore, some of the enumerators were persons who participated in the implementation of the cholera prevention activities in these communities. Their presence might have biased certain respondent answers. Additionally, sampling was based on available population data from the 2003 census. Migration is likely to have occurred after the earthquake and might have resulted in nonproportional sampling. Finally, sanitation is a sensitive subject within Haitian culture; thus, self-reported access to latrines might be exaggerated. Despite these limitations, the survey provided valuable information reflecting the impact of the public health response to the outbreak and identified areas for improvement.

Conclusions

Overall, the knowledge of cholera symptoms, prevention, treatment, and modes of transmission indicated that public health messages had been effective. Cholera messaging was successful in promoting behavior changes to address the threat of cholera, especially in increasing acceptance of drinking chlorinated water. Recommendations include additional education campaigns to improve knowledge of correct dosing of water with water purification tablets, ORS preparation, and cholera-prevention methods. Additional follow-up is needed to ensure wide-scale availability of household water treatment products and instruction on proper dosing. Public health officials should take advantage of the substantial interest in and acceptance of chemical water treatment and develop sustainable household water treatment programs. More importantly, a concerted effort should be made to improve the safety of water sources through infrastructure upgrades and improved treatment practices. These upgrades would spur timely sanitary reform and improvements to the public health system of Haiti, which occurred over a century ago in Europe, North America, and most recently in Latin America after the introduction of cholera in the 1990s (*13*,*14*).

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Cholera Prevention Training Materials for Community Health Workers, Haiti, 2010–2011

Anu Rajasingham, Anna Bowen, Ciara O'Reilly, Kari Sholtes, Katie Schilling, Catherine Hough, Joan Brunkard, Jean Wysler Domercant, Gerald Lerebours, Jean Cadet, Robert Quick, and Bobbie Person

Stopping the spread of the cholera epidemic in Haiti required engaging community health workers (CHWs) in prevention and treatment activities. The Centers for Disease Control and Prevention collaborated with the Haitian Ministry of Public Health and Population to develop CHW educational materials, train >1,100 CHWs, and evaluate training efforts.

Because cholera can kill within hours of disease onset and access to cholera treatment centers is poor in many developing countries, community health workers (CHWs) are vital for educating community members about cholera transmission, prevention, and control and, when necessary, providing life-saving treatment. CHWs are typically laypersons selected by the community (*1*,*2*). For CHWs to effectively educate and support their communities during a cholera outbreak, they must be appropriately trained.

Cholera outbreaks typically arise in settings where water, sanitation, and hygiene infrastructures are inadequate (*3*). Even before Haiti was ravaged by an earthquake in January 2010, only 63% of the population had access to improved water sources, and only 17% had access to improved sanitation (*4*). Therefore, when the cholera outbreak began in October 2010, the Haitian Ministry of Public Health and Population (MSPP) was still actively developing and rebuilding the public health and water, sanitation, and hygiene infrastructures, and many communities were beyond the reach of these services. From the onset, MSPP initiated 3 strategies to enhance care of patients during the cholera epidemic: reinforcing existing heath care facilities with training and supplies, establishing a network of cholera treatment centers for management of severe cases, and mobilizing CHWs who could take treatment and prevention activities into the community.

The community-level strategy was particularly important in Haiti. A rapid assessment early in the outbreak indicated that among 87 cholera decendents, 39 (45%) died in the community, 60 (69%) did not suspect their illness was cholera or recognize the severity, 22 (26%) lived too far from health facilities to access care, and 30 (77%) of the 39 community decedents did not take oral rehydration solution (ORS) at home (J. Routh, pers. comm.). Populations with poor access to health care often experience higher case-fatality rates during cholera epidemics, which means that local provision of treatment and supplies through less specialized health workers such as CHWs is essential (*5*–*9*).

The Study

To assist CHWs in conducting cholera education, prevention, and treatment activities, a multidisciplinary team that included physicians, behavioral scientists, epidemiologists, engineers, and communication specialists at the Centers for Disease Control and Prevention (CDC) and MSPP developed a set of technically accurate, lower literacy, and culturally adapted training and educational materials for CHWs during the cholera outbreak in Haiti. The materials are comprehensive in scope and can be adapted for use in cholera preparedness and response activities in other countries.

Training materials were based on the World Health Organization technical guidelines for cholera; guidelines and educational materials from the International Centre for Diarrhoeal Disease Research, Bangladesh; cholera prevention messages, guidelines, and materials from CDC; online manuals and guides from various nongovernmental organizations; and information from manufacturers of point-of-use water treatment products. Consensus for all materials was reached among CDC subject matter experts and communications personal, MSPP, and CDC staff in Haiti. Amendments were made after field review and use in Haiti, and messages concerning culturally appropriate burial practices, stigma prevention, and additional water treatment options were added as the epidemic progressed and additional content needs were identified.

The materials now include comprehensive cholera prevention, treatment, and control training modules (Figure 1; Tables 1, 2); a training guide and presentation slide set for use in teaching CHWs; and community education cards and low-literacy posters for CHWs to use within the

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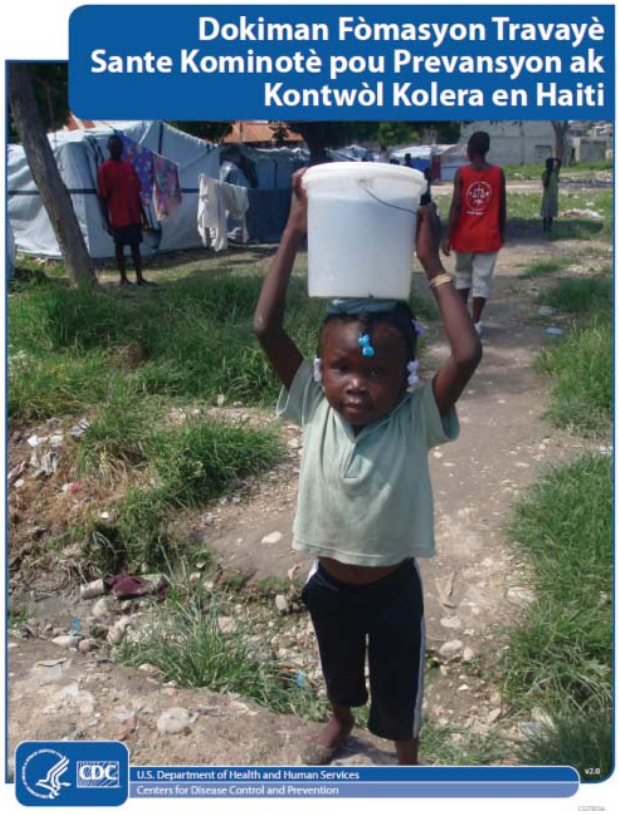

Figure 1. Cover page of community health worker cholera prevention and control training manual, Haiti, 2011.

community (Figure 2). Materials are available in Haitian Creole, French, and English.

As part of CDC's response to the cholera outbreak in Haiti, this manual was made available through both CDC's emergency website (www.cdc.gov/haiticholera) and cholera-specific website (www.cdc.gov/cholera). As of July 27, 2011, the websites had received >6,300 total views and 1,352 downloads. The training manual was also included as an appendix to CDC's national clinical cholera training course for medical staff in Haiti, which was initiated on November 15, 2010.

CDC supported MSPP in organizing a Train-the-Trainer workshop March 1–3, 2011, in Port-au-Prince, Haiti. Twenty-four master trainers, including physicians, nurses, and other health care providers, were selected from various partner organizations and MSPP regional entities with large CHW cohorts. After the master trainers completed the train-the-trainer course, they were asked to train CHWs within their own organizations in a cascading training approach. In June 2011, CDC conducted a preliminary follow-up evaluation of the train-the-trainer course among master trainers to learn how it had contributed to subsequent trainings of CHWs. Participants included representatives from 9 departments and all partner organizations who attended the March train-the-trainer workshop.

Survey questionnaires were completed by 14 of the 24 original train-the-trainer participants. Among these,

Table 1. Summary of cholera prevention and control training materials developed for community health workers, Haiti, 2011*

Training Manual
Module 1: Community Mobilization
Module 2: What You Need to Know about Cholera
Module 3: Decision Making Guide for Taking Care of People with Watery Diarrhea
Module 4: Handwashing
Module 5: Oral Rehydration Solution (ORS)
Module 6: Safe Drinking Water: Aquatabs
Module 7: Safe Drinking Water: Dlo Lavi
Module 7A: Safe Drinking Water: Gadyen Dlo
Module 8: Safe Drinking Water: PuR
Module 9: Safe Water Storage
Module 10: Safe Food Preparation
Module 11: Safe Sanitation and Cleaning
Module 12: When a Person with Cholera Dies at Home
Module 13: Preventing Cholera Stigma
Community Education Cards
Community Mobilization
What You Need to Know about Cholera
Decision Making Guide for Taking Care of People with Watery Diarrhea
Handwashing
Oral Rehydration Solution (ORS)
Safe Drinking Water: Aquatabs
Safe Drinking Water: Dlo Lavi
Safe Drinking Water: Gadyen Dlo
Safe Drinking Water: PuR
Making Drinking Water Safe With Household Bleach
Safe Water Storage
Safe Food Preparation
Safe Sanitation and Cleaning
When a Person with Cholera Dies at Home
Cleaning after Flooding
Preventing Cholera Stigma
Training Guide
Optional PowerPoint presentation to accompany materials
Low-Literacy Posters
If You or Your Family Get Sick with Cholera (2 posters: Adult and Child versions)
How to Prepare Food Safely
Wash your Hands to Stop Cholera
How to Make and Use Oral Rehydration Solution (ORS)
How to Make Water Safe Using Aquatabs (5 posters, 1 for each dosage of Aquatab)
How to Make Water Safe Using Dlo Lavi
How to Make Water Safe Using Gadyen Dlo
How to Make Water Safe Using PuR
How to Make Water Safe Using Household Bleach
How to Practice Safe Sanitation and Cleaning
How to Prevent Cholera Stigma
How to Make a Tippy Tap

*These materials are available at www.cdc.gov/cholera/materials.html.

Table 2. Key cholera questions and response messages for community health workers, Haiti, 2011

What is cholera disease?
Cholera disease causes a lot of watery diarrhea and vomiting.
Cholera diarrhea can look like cloudy rice water.
Cholera can cause death from dehydration (the loss of water and salts from the body) within hours if not treated.
How is cholera spread?
Cholera germs are found in the feces (poop) or vomit of infected people.
Cholera is spread when feces (poop) or vomit from an infected person gets into the water people drink or the food people eat.
Cholera is not likely to spread directly from one person to another.
What are the key ways to protect yourself and your family from cholera and other diarrheal diseases?
Drink and use safe water. (Safe water is water that is bottled with an unbroken seal, has been boiled, or has been treated with a chlorine product.)
Wash hands often with soap and safe water. If no soap is available, scrub hands often with ash or sand and rinse with safe water.
Use latrines or bury your feces (poop), do not defecate in any body of water.
Cook food well (especially seafood), eat it hot, keep it covered, and peel fruits and vegetables.
Clean up safely— in the kitchen and in places where your family bathes and washes clothes.
What should you do if you or your family is ill with diarrhea?
If you have oral rehydration solution (ORS), start taking it now; it can save your life.
Go immediately to the nearest health facility, cholera treatment center, or community health worker, if you can.
Continue to drink ORS at home and while you travel to get treatment.
Continue to breastfeed your baby if they have watery diarrhea, even when traveling to get treatment.

8 participants reported training a total of 1,144 CHWs before the March train-the-trainer course. Additionally, 10 participants reported training 1,170 CHWs in 9 departments of Haiti in the 3 months after the March train-the-trainer event. Among the 10 participants who trained CHWs after the train-the-trainer course, 9 reported using the CDC/MSPP CHW manual; 7 reported using the CDC/MSPP community education cards; and all demonstrated how to use soap, water treatment products, and ORS. Nine reported providing the CDC/MSPP manual to CHWs during trainings, 8 distributed water treatment products and ORS, and 7 distributed soap. All of those who trained CHWs after the March train-the-trainer session reported that by the end of the training, CHWs were able to successfully demonstrate handwashing techniques, and most (9) indicated that CHWs were able to demonstrate proper preparation of safe water and ORS. In addition, 12 of the 14 participants who completed surveys reported training other types of community workers, including midwives, professors, group leaders, nurses, brigadiers, promoters, voodoo priests, and other religious leaders.

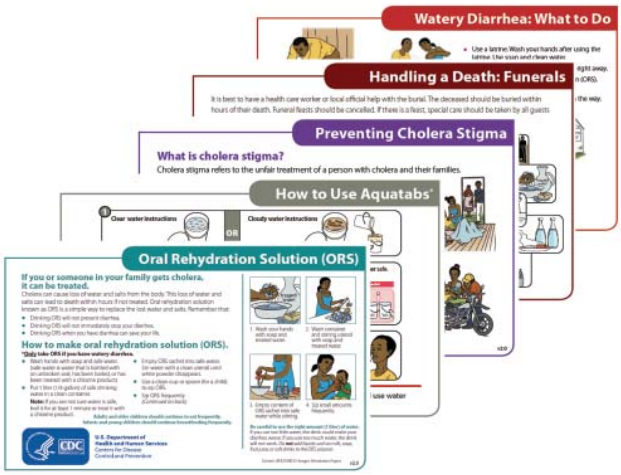

Figure 2. Series of community education cards developed for use in Haiti, 2011.

Conclusions

In an attempt to quickly and efficiently reach the most underserved areas of Haiti during a deadly cholera outbreak, we developed comprehensive training materials for CHWs and implemented training using a Train-the-Trainer model. However, this process had many challenges. The urgent need for training in Haiti required us to draft messages without the benefit of formative research or pilot testing of the materials. Translating materials into Haitian Creole, which only became an official language in Haiti in 1987 and which remains a predominantly verbal language with several dialects, was difficult and required multiple quality assurance steps. Because of resource constraints, train-the-trainer events were not initiated in Haiti until March 2011, >4 months after the epidemic began. More timely training might have helped mitigate the impact of the epidemic on remote and underserved populations. These materials can now be adapted to train CHWs in other cholera-affected countries more rapidly than we were able to do in Haiti. Additional evaluation activities are planned at the community and household levels to assess the impact of these materials on cholera knowledge, prevention activities, and treatment among CHWs and community members and to guide revisions of these materials.

In many rural areas of Haiti, CHWs are the backbone of the health care system and can play an essential role in preventing cholera illness and death among medically

underserved populations. We prepared standardized materials and training modules for CHWs that focused on prevention, treatment, and control of cholera. We conducted a train-the-trainer workshop that led to training CHWs across 9 departments of Haiti. Additional monitoring and evaluation activities are needed to assess the reach and impact of the training materials and implementation.

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Cholera in United States Associated with Epidemic in Hispaniola

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Cholera is rare in the United States (annual average 6 cases). Since epidemic cholera began in Hispaniola in 2010, a total of 23 cholera cases caused by toxigenic *Vibrio cholerae* O1 have been confirmed in the United States. Twenty-two case-patients reported travel to Hispaniola and 1 reported consumption of seafood from Haiti.

Cholera caused by toxigenic *Vibrio cholerae*, serogroup O1, serotype Ogawa, biotype El Tor, was confirmed on October 21, 2010, in Haiti and on October 31, 2010, in the Dominican Republic. These countries are on the island of Hispaniola. During October 21, 2010–April 4, 2011, >275,000 cholera cases and >4,700 deaths were reported from Hispaniola. Of these cases, 840 culture-confirmed cases and 10 deaths were reported from the Dominican Republic.

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Illness caused by toxigenic *V. cholerae* O1 has been documented in the United States since 1832. During 1965–1991, an average of 5 cases per year were reported. During the Latin American cholera epidemic that started in 1991, the number of cholera cases in the United States increased because of importation of cases related to the epidemic to an average of 53 cases per year during 1992–1994 (*1*,*2*). As the Latin American epidemic waned, during 1995–2000, the average annual case count decreased to 10 (*3*). During 2000–2010, the average number of cases was 6, and 57% of case-patients had traveled internationally (*4*). This experience raised concern that a dramatic increase in US cholera cases could result from the Hispaniola epidemic.

In the United States, cholera is confirmed by identification of toxigenic *V. cholerae* serogroup O1 or O139 or by serologic evidence of infection in a patient with diarrhea and an epidemiologic link to a culture-confirmed case. Since 2000, suspected *V. cholerae* isolates have been sent by state public health laboratories to the Centers for Disease Control and Prevention (CDC) for confirmation and characterization.

We summarize characteristics of confirmed US cases associated with the Hispaniola epidemic that were reported to the CDC Cholera and Other *Vibrio* Illness Surveillance System, a national database of all laboratory-confirmed cholera and vibriosis cases. For each case of cholera, state and local health officials submit a Cholera and Other *Vibrio* Illness Surveillance system report form that contains demographic, clinical, and epidemiologic information, including selected food and water exposures associated with cholera, travel history, and vaccination status.

The Study

The first US case associated with the epidemic in Hispaniola was laboratory confirmed on November 15, 2010, in a US resident who had traveled to Haiti and returned to Florida. The first case in a patient with history of travel to Dominican Republic was laboratory confirmed

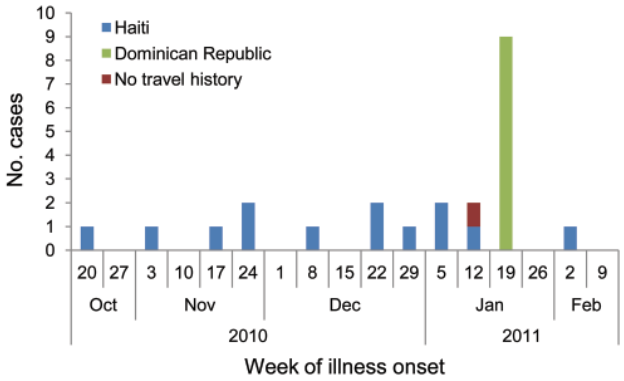

Figure 1. Confirmed cholera cases (n = 23), by onset date and travel history, United States, October 21, 2010–February 4, 2011.

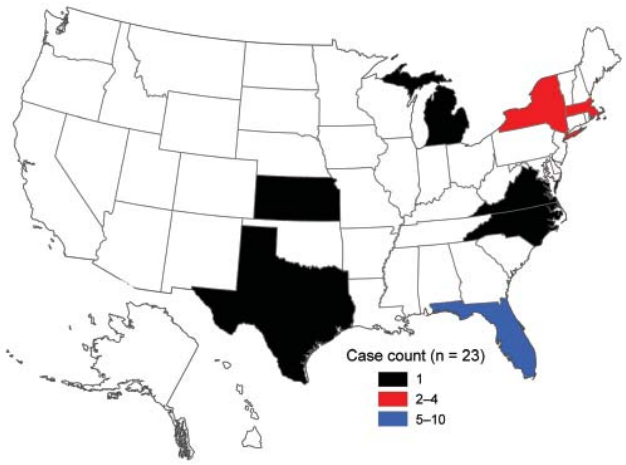

Figure 2. Geographic distribution of cholera cases in the United States associated with Hispaniola, October 21, 2010–April 4, 2011.

on January 29, 2011. As of April 4, 2011, a total of 23 cholera cases associated with the Hispaniola epidemic had been confirmed (Figure 1). Patients resided in Florida (10), Massachusetts (4), New York City (4), Kansas (1), Michigan (1), North Carolina (1), Virginia (1), and Texas (1) (Figure 2). Illness onset dates ranged from October 23, 2010, to February 2, 2011. Median age was 38 years (range 9–84 years), and 43% were female patients.

All patients were treated with antimicrobial agents, rehydration, or both; 9 (39%) were hospitalized, 6 (30%) sought care at an emergency department, and none died. Six patients had illness onset before returning to the United States, 5 had illness onset on the day of return, and 12 had illness onset 1–11 days after return (typical incubation period for cholera is 18 hours–5 days) (5).

Twenty cases were culture confirmed and 3 were confirmed by serologic testing. All 20 isolates matched the Haiti isolate outbreak pattern by pulsed-field gel electrophoresis. Susceptibility results for antimicrobial drug tested showed that all isolates were resistant to trimethoprim/sulfamethoxazole, furazolidone, nalidixic acid, sulfisoxazole, and streptomycin, and 18 isolates showed intermediate resistance to chloramphenicol, ampicillin, or amoxicillin/clavulanic acid.

Thirteen patients reported recent travel to Haiti (median length of stay 7 days, range 2–54 days) and 9 to Dominican Republic (median length of stay 4 days, range 2–9 days). One patient reported no recent travel but consumed cooked conch brought to the United States from Haiti by relatives. Travel was reported to the following departments in Haiti: Artibonite (2), Ouest (7), Centre (1), Nord (1), and Sud (1). One case-patient traveled to 2 departments, and 2 did not specify a destination. All case-patients associated with the Dominican Republic had attended a wedding in La Romana Province on January 22, 2010; an investigation conducted by the Dominican Republic Ministry of Health is ongoing. Aside from 2 case-patients who traveled to this wedding together, no other case-patients reported traveling together.

Visiting friends or relatives was the main reason for travel to Haiti (Table 1). Four patients traveled to Haiti to participate in relief activities, 2 as medical volunteers, 1 on a mission trip, and 1 to distribute canned foods. One patient immigrated to the United States from Haiti during the incubation period. A wide range of exposures was reported (Table 2); 5 patients were exposed to persons with cholera or cholera-like illness and to other risk factors for cholera acquisition. Medical volunteers participated in direct patient care. One volunteer reported no apparent lapses in safe water and food practices, although detailed information about food preparation was not available. No additional information was available for the other volunteer.

Seven of 15 patients with information available reported receiving cholera prevention information before travel. Sources included newspaper articles (4), friends (4), CDC traveler's hotline (1), and the World Health Organization website (1); 2 patients reported >1 source. None had ever received cholera vaccine. Two patients reported receiving a Travel Health Alert Notice upon arrival in the United States (M. Selent, unpub. data).

Conclusions

Six months after the Hispaniola cholera epidemic started in Haiti, 23 associated cases were recognized in the United States. All cases were associated with recent travel to Hispaniola or with consumption of seafood from Haiti. The risk for cholera transmission in the United States is low because of improved water and sanitation, and there is no evidence of secondary transmission. Florida, New York, and Massachusetts have the highest populations of persons of Haitian or Dominican ancestry (*6*). Most cases were reported from Florida, the state with the largest Haitian population. However, case-patients also resided in states with small Haitian and Dominican populations. Travel between the United States and Haiti is straightforward; 4 US airports offer daily direct flights from Florida and New York to Port-au-Prince. Many persons, including many of Haitian descent, traveled from the United States to Haiti to

Table 1. Reason for travel by destination for 22 cholera patients, United States, October 21, 2010–February 4, 2011*

Reason for travel	Haiti	Dominican Republic
To visit relatives or friends	7	9
Business	2	0
Aid travel	4	0
Immigration to United States	1	0

*Persons may have traveled for >1 reason.

Table 2. Selected exposures during 4 d (7 d for body of water exposure) before illness onset in 23 cholera patients, United States, October 21, 2010–February 4, 2011*

Exposure	No. persons exposed	No. case-patients with information available
Foodborne		
Street-vended food	1	20
Cooked seafood	9	20
Raw seafood	3	18
Waterborne		
Any body of water†	7	20
Swimming/bathing	3	6
Well water (drinking)	1	1
Other		
Seafood handled	1	7
Person(s) with cholera or cholera-like illness	5	19

*Exposures were not investigated further.
†Water exposures include local lake and stream of water running down a street in Haiti; a pool in the Dominican Republic; ocean (Dominican Republic); unspecified location in Port-au-Prince, Haiti; and a tank at a medical center in Haiti. Two case-patients did not specify body of water exposure.

help with the response to the January 2010 earthquake in Port-au-Prince.

Person-to-person transmission of cholera has only rarely been reported; cases in medical workers are almost always attributable to consumption of contaminated food or water. Person-to-person transmission is not clearly supported for either of the cases we report in medical workers, although it cannot be ruled out. Continued surveillance and detailed investigation of cases in medical workers is warranted to further define the risk, if any, of person-to-person transmission.

Echoing the Latin American cholera epidemic in the 1990s, the number of US cholera cases has increased after the cholera epidemic in Hispaniola. Travelers to cholera-affected areas should be aware of the risk and should follow prevention measures to avoid infection. In particular, travelers visiting friends or relatives may be at higher risk for travel-associated infection (*7*). Few case-patients had received cholera prevention education (educational materials available at www.cdc.gov/cholera/index.html); no cholera vaccine is licensed in the United States. Until cholera in Haiti and Dominican Republic resolves, clinicians, microbiologists, and public health workers in the United States should be prepared for more cases in travelers returning from Hispaniola.

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We thank all persons in local, state, and territorial departments of public health for their time and efforts in accomplishing laboratory-based national surveillance for cholera and epidemiologic follow-up.

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Travel Health Alert Notices and Haiti Cholera Outbreak, Florida, USA, 2011

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To enhance the timeliness of medical evaluation for cholera-like illness during the 2011 cholera outbreak in Hispaniola, printed Travel Health Alert Notices (T-HANs) were distributed to travelers from Haiti to the United States. Evaluation of the T-HANs' influence on travelers' health care–seeking behavior suggested T-HANs might positively influence health care–seeking behavior.

Travel health alert notices (T-HANs) have been used since the 1970s by the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) as a communication tool for international travelers arriving in the United States during public health emergencies. T-HANs, typically printed on yellow cards, inform travelers about possible disease exposures, advise them to seek health care if symptoms develop, and instruct them to give the T-HAN to their physicians. T-HANs also provide clinical guidance and resources. Despite their repeated use, to our knowledge the influence of T-HANs on travelers' health care–seeking behavior during an outbreak has not been evaluated.

After a large cholera outbreak was confirmed in Haiti on October 21, 2010, CDC immediately began providing health recommendations to travelers and guidance to US clinicians, primarily through the CDC website and other electronic means (*1*,*2*). By early December, 5 imported cholera cases with *Vibrio cholerae* isolates identical to the Haiti strain were confirmed in Florida; 2 case-patients had been discharged from emergency departments without cholera diagnoses, requiring subsequent reevaluation and hospitalization (*3*).

The rapidly escalating epidemic in Haiti and the historically high travel volume between Haiti and the United States during December and January prompted CDC to distribute T-HANs to travelers from Haiti to reduce the risk for delayed health care. Typically, 8–10 direct flights from Haiti arrive daily in the United States at 4 airports: Miami and Fort Lauderdale, Florida; New York (John F. Kennedy International), New York; and San Juan, Puerto Rico. Miami receives approximately half of these flights (*4*). The cholera T-HAN was written in plain language; translated to French, Haitian-Creole, and Spanish (Figure); and distributed by US Customs and Border Protection (CBP) officers at passport control booths at these 4 airports. T-HANs were not distributed to passengers on connecting flights from Haiti because these flights had fewer passengers from Haiti and flew to numerous US cities, making T-HAN distribution impractical.

The Evaluation

We evaluated the effectiveness of T-HANs through 3 methods. First, we counted the number of page views at a unique Internet address (printed only on the T-HAN and unlikely to be indexed in search engines, which redirected to CDC's Haiti Cholera Web page). Second, on January 10–11, 2011, a voluntary 5-question survey was administered to travelers from Haiti at the Miami airport. Travelers were asked whether they had received and read the T-HAN, their need for cholera health information, their likelihood of seeking health care if they had onset of diarrhea within 5 days after arrival, and whether their travel had originated in the United States. The survey was administered orally in English or Haitian-Creole by trained interviewers in the airport's Federal Inspection Station ≈10–20 minutes after T-HAN distribution. Analyses were adjusted for respondents' sex and travel origin. Third, US cholera case-patients from Haiti who traveled to the 4 airports during the T-HAN distribution period were asked by their respective health departments whether they had received a T-HAN and whether it had influenced their decision to seek health care.

From December 20, 2010, through March 31, 2011, ≈73,500 T-HANs were distributed at the 4 airports, 51,500 (70%) in Miami. Seventy-five redirects were counted at the T-HAN Web address, half within the first month. T-HAN distribution was not associated with increased calls to CDC's information hotline (printed on the T-HAN) or traffic on the CDC website.

Of 1,348 travelers from Haiti who arrived in Miami on January 10–11, 2011, a total of 882 (65%) were surveyed (Table). Receiving or reading the T-HAN was significantly

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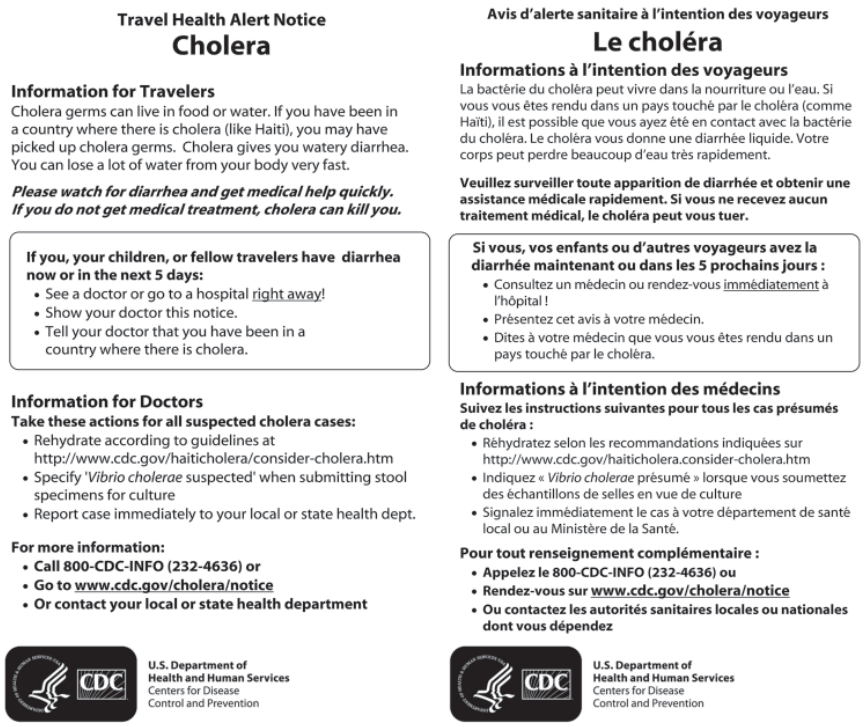

Travel Health Alert Notice

Cholera

Information for Travelers

Cholera germs can live in food or water. If you have been in a country where there is cholera (like Haiti), you may have picked up cholera germs. Cholera gives you watery diarrhea. You can lose a lot of water from your body very fast.

Please watch for diarrhea and get medical help quickly. If you do not get medical treatment, cholera can kill you.

If you, your children, or fellow travelers have diarrhea now or in the next 5 days:

- See a doctor or go to a hospital right away!
- Show your doctor this notice.
- Tell your doctor that you have been in a country where there is cholera.

Information for Doctors

Take these actions for all suspected cholera cases:

- Rehydrate according to guidelines at http://www.cdc.gov/haiticholera/consider-cholera.htm
- Specify '*Vibrio cholerae* suspected' when submitting stool specimens for culture
- Report case immediately to your local or state health dept.

For more information:

- **Call 800-CDC-INFO (232-4636) or**
- **Go to www.cdc.gov/cholera/notice**
- **Or contact your local or state health department**

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

Avis d'alerte sanitaire à l'intention des voyageurs

Le choléra

Informations à l'intention des voyageurs

La bactérie du choléra peut vivre dans la nourriture ou l'eau. Si vous vous êtes rendu dans un pays touché par le choléra (comme Haïti), il est possible que vous ayez été en contact avec la bactérie du choléra. Le choléra vous donne une diarrhée liquide. Votre corps peut perdre beaucoup d'eau très rapidement.

Veuillez surveiller toute apparition de diarrhée et obtenir une assistance médicale rapidement. Si vous ne recevez aucun traitement médical, le choléra peut vous tuer.

Si vous, vos enfants ou d'autres voyageurs avez la diarrhée maintenant ou dans les 5 prochains jours :

- Consultez un médecin ou rendez-vous immédiatement à l'hôpital !
- Présentez cet avis à votre médecin.
- Dites à votre médecin que vous vous êtes rendu dans un pays touché par le choléra.

Informations à l'intention des médecins

Suivez les instructions suivantes pour tous les cas présumés de choléra :

- Réhydratez selon les recommandations indiquées sur http://www.cdc.gov/haiticholera.consider-cholera.htm
- Indiquez « *Vibrio cholerae* présumé » lorsque vous soumettez des échantillons de selles en vue de culture
- Signalez immédiatement le cas à votre département de santé local ou au Ministère de la Santé.

Pour tout renseignement complémentaire :

- **Appelez le 800-CDC-INFO (232-4636) ou**
- **Rendez-vous sur www.cdc.gov/cholera/notice**
- **Ou contactez les autorités sanitaires locales ou nationales dont vous dépendez**

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

Figure. Travel health alert notice for 2010–2011 Haiti cholera outbreak showing English and French versions. Haitian-Creole and Spanish versions were printed on the reverse side (not shown).

associated with reported need for cholera health information (adjusted prevalence ratio 1.27 and 1.16, respectively; $p<0.05$). T-HAN readers were more likely than nonreaders to indicate that they were likely to seek health care for diarrhea (adjusted prevalence ratio 1.05; $p = 0.0127$). Of 7 confirmed cholera case-patients who met criteria for inclusion, 2 received T-HANs; both indicated that the T-HAN influenced their decision to seek health care.

Conclusions

This evaluation suggests that T-HANs had a small positive influence on travelers' health care–seeking behavior. Although more than half of survey respondents reported a need for cholera information, and receiving or reading the T-HAN was associated with this need, the low number of redirects on the T-HAN website suggests that most recipients did not use the T-HAN as a source for more information, possibly because the T-HAN information was sufficient or because they sought information elsewhere. In developing a T-HAN, complex scientific information must be reframed into simple, concise messages that grab travelers' attention. Translation for non–English-speaking travelers often is required, and imagery for lower literacy audiences might be needed. T-HANs also must raise clinicians' suspicion for uncommon communicable diseases; guide testing, treatment, and reporting in accordance with public health recommendations; and remain valid as these recommendations evolve.

Rapid T-HAN distribution to travelers during a public health emergency poses unique logistic, legal, and political challenges. CBP's assistance in distributing cholera T-HANs was invaluable; however, achieving widespread distribution was difficult. CBP officers have multiple responsibilities when reviewing travelers' documents. CBP's opinion was that T-HAN distribution was more successful during the pandemic (H1N1) 2009 outbreak than during the Haiti cholera response because T-HANs were distributed to all travelers rather than to a specific subset. With the many international travelers processed each day, an automated prompt on their computers could assist CBP officers to consistently distribute T-HANs to specific travelers.

Table. T-HAN survey of 1,348 travelers from Haiti at the Miami International Airport, Florida, USA, January 10–11, 2011*

Characteristic	No. (%)
Total travelers surveyed	882 (65)
Male travelers surveyed	504 (57)
Survey responses	
Received T-HAN	664 (75)
Read T-HAN	245 (28)
Reported need for cholera information	458 (52)
Trip originated from United States	675 (77)
Likelihood of seeking care if diarrhea developed in next 5 d	
Likely	693 (79)
Uncertain	97 (11)
Not likely	89 (10)

*T-HAN, travel health alert notice.

In-flight T-HAN distribution has been explored as an alternative to postarrival distribution. However, numerous difficulties complicate CDC's ability to secure agreements with airlines, including positioning T-HANs on aircraft with changing flight plans and airlines' concerns about negative public perceptions and possible legal and economic ramifications. Another option is predeparture distribution (e.g., in Haiti), but CDC lacks authority to require distribution of health information to US-bound travelers overseas. Public announcements on airplanes and electronic messaging or posters in airports require advance planning with airlines and airport officials and should be pursued as possible alternatives. To encourage appropriate health care visits and medical assessments, future evaluations also should assess the effectiveness of pretravel and posttravel health messages on social media sites, the CDC Travelers' Health website (www.cdc.gov/travel), and messaging aimed at clinicians.

Our results are subject to several limitations. The relatively low response rate, which reflects the operational difficulties of conducting surveys in airports, limits traveler representativeness. Interviewer or cultural bias also might have been present. Although the survey was voluntary, it was conducted in the airport Federal Inspection Station and therefore might reflect approval-seeking bias. Additionally, estimation of the T-HANs' effectiveness could have been biased because few travelers read the T-HAN, possibly because of lack of time, intent, or ability to read it (because the T-HAN was not tested with lower literacy audiences). The small number of cholera case-patients who received T-HANs might not have accurately reflected the T-HAN's effect on health care–seeking behavior, and no information was available for travelers with diarrhea who did not seek health care or in whom cholera was not diagnosed.

The Haiti cholera T-HAN response was relatively small; during a larger scale event, resource requirements for T-HAN distribution would be far greater. Given the logistical challenges of T-HAN distribution, further efforts are warranted to study the effectiveness of T-HANs and to identify alternative methods of providing health information to travelers.

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Multinational Cholera Outbreak after Wedding in the Dominican Republic

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We conducted a case–control study of a cholera outbreak after a wedding in the Dominican Republic, January 22, 2011. Ill persons were more likely to report having consumed shrimp on ice (odds ratio 8.50) and ice cubes in beverages (odds ratio 3.62). Travelers to cholera-affected areas should avoid consuming uncooked seafood and untreated water.

Over the past century, no cholera cases had been reported in the Dominican Republic. The first cholera cases were reported on November 15, 2010, associated with the epidemic in Haiti. From November 15, 2010, through January 22, 2011, a total of 1,115 suspected cases and 280 laboratory-confirmed cases were reported by the Dominican Republic Ministry of Health (*1*).

On January 25, 2011, the Dominican Republic Ministry of Health was notified that 5 Venezuelan nationals had been hospitalized with cholera-like illness. All patients had attended a large wedding reception at a luxury tourist resort in La Romana Province, Dominican Republic, on January 22. At the reception were 216 local workers and 540 guests; ≈90% were residents of Venezuela, and the others were from the Dominican Republic, Mexico, the United States, and Spain. In La Romana Province, no other cases had previously been identified, and during the week of the outbreak, all reported cases were associated with the wedding.

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The Study

We conducted a case–control study to identify foods and beverages consumed at the wedding banquet that were associated with diarrheal illness. A questionnaire developed by Dominican Republic health authorities, which addressed only food items consumed at the banquet, was sent to national health authorities in the 4 countries where guests originated. They were asked to administer it to wedding attendees and return completed questionnaires to the Dominican Republic for analysis.

Case-patients were identified from routine surveillance reports in the Dominican Republic and the United States. We also actively sought case-patients by asking the venue personnel, event organizer, and other case-patients to inform us about other persons at the banquet from the same country of origin. A patient with a suspected case was defined as a person who had watery diarrhea during January 22–28, 2011, and had consumed food and beverages at the banquet. In addition to meeting suspected-case criteria, a patient with a confirmed case also had laboratory confirmation of infection by culture for *Vibrio cholerae* O1 or agglutination for serotype Ogawa. Controls were identified by event organizers or other banquet attendees as guests or workers at the banquet who consumed food and beverages but did not have diarrhea during January 22–28, 2011. We intended to recruit at least twice as many controls as case-patients. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) regarding whether consuming particular foods and beverages was associated with being a case-patient (with suspected and confirmed cases) and controls. Food and beverage items potentially associated with illness in a univariable analysis ($p<0.10$) were included as independent dichotomous predictor variables in a multivariable logistic regression model with case status as the dependent dichotomous variable. We used StataCorp 2005 Release 9 (College Station, TX, USA) for the analysis. A Ministry of Health environmental inspector interviewed the event caterers about purchase, transport, storage, preparation, and service of food and beverages at the banquet.

We identified 42 case-patients (25 with confirmed and 17 with suspected cases) and 62 controls; all agreed to participate in the study. Questionnaires were completed between January 24 and March 3, 2011. Twenty-two (51%) case-patients and 59 (97%) controls were from the Dominican Republic (Table 1). Of case-patients, 24 (57%) were wedding guests, 16 (38%) were workers, and 2 (5%) did not attend the reception but ate leftover food from the banquet. Of 62 controls, only 6 (10%) were guests and 56 (90%) were workers (Table 1). Only data from Venezuelan case-patients who received a diagnosis in the Dominican Republic were available for analysis. We did not specify how case-patients were identified.

Table 1. Characteristics of case-patients and controls associated with a cholera outbreak after a wedding, Dominican Republic, January 2011

Characteristic	No. (%) case-patients, n = 42	No. (%) controls, n = 62	p value*
Sex			
M	33 (77)	54 (89)	0.18
F	9 (23)	8 (12)	
Age group, y			
15–24	8 (23)	9 (15)	0.07
25–34	8 (19)	20 (33)	
35–44	8 (16)	18 (28)	
45–54	6 (14)	9 (15)	
55–64	9 (21)	3 (5)	
≥65	3 (7)	3 (5)	
Role			
Guest	24 (58)	6 (8)	<0.001
Server or food handler	9 (21)	22 (36)	
Worker	9 (21)	34 (56)	
Country of origin			
Spain	1 (2)	1 (2)	<0.001
United States	10 (26)	2 (2)	
Dominican Republic	22 (51)	59 (97)	
Venezuela†	9 (21)	0	

*p value based on χ^2 test for each contingency table.
†Case-patients were identified and interviewed in the Dominican Republic.

Median age of case-patients was 42.5 years (range 16–84 years); 33 (79%) were male. All experienced watery diarrhea, accompanied by dehydration (28 patients [67%]), nausea (13 [30%]), vomiting (15 [36%]), or cramps (8 [19%]). Time to illness onset was 10 hours to 6 days (Figure). Thirty-four (81%) case-patients were given antimicrobial drugs, and 22 (52%) were hospitalized; all recovered.

Odds were higher for case-patients than controls for having consumed cooked shrimp on ice (OR 8.5, 95% CI 3.3–21.71), cooked langostinos (squat lobster) (OR 2.9, 95% CI 1.1–7.7), and beverages with ice cubes (OR 3.6, 95% CI 1.4–9.3), but odds were lower for having consumed mixed rice (OR 0.1, 95% CI 0.01–0.9) or other foods (OR 0.13, 95% CI 0.05–1.03) (online Appendix Table, wwwnc.cdc.gov/EID/article/17/11/11-1263-TA1.htm). When these variables were included in a multivariable logistic regression, cooked shrimp on ice (OR 10.8, 95% CI 3.3–35.4), ice cubes (OR 4.1, 95% CI 1.3–13.2), and mixed rice (OR 0.04, 95% CI 0.003–0.5) remained significantly associated with case status (Table 2).

Environmental inspection found that the food was prepared by a caterer outside of the resort. Shrimp and lobster came from the Dominican Republic (Higuey in La Altagracia Province), and the langostinos came from Beata Island in Perdenales Province and Anse-A-Pitre in southeastern Haiti. No samples were available for testing. Inspection of the caterer's kitchen revealed poor food-handling practices, including improper refrigeration, poor hand hygiene, and nonchlorinated water supply. On the night of the wedding reception, food was set out at 7:00 PM but not consumed until 10:00 PM. Shrimp and langostinos were served on ice or ice sculptures.

Conclusions

After a wedding banquet in La Romana Province, Dominican Republic, many persons with gastrointestinal symptoms had laboratory evidence of infection with *V. cholerae* O1 Ogawa, the cholera serotype identified in the ongoing outbreak on Hispaniola. Cases were positively associated with consumption of cooked shrimp on ice and ice cubes. However, case-patients were not more likely to have eaten shrimp in vinaigrette or shrimp kebab, which may indicate contamination during serving on ice. The

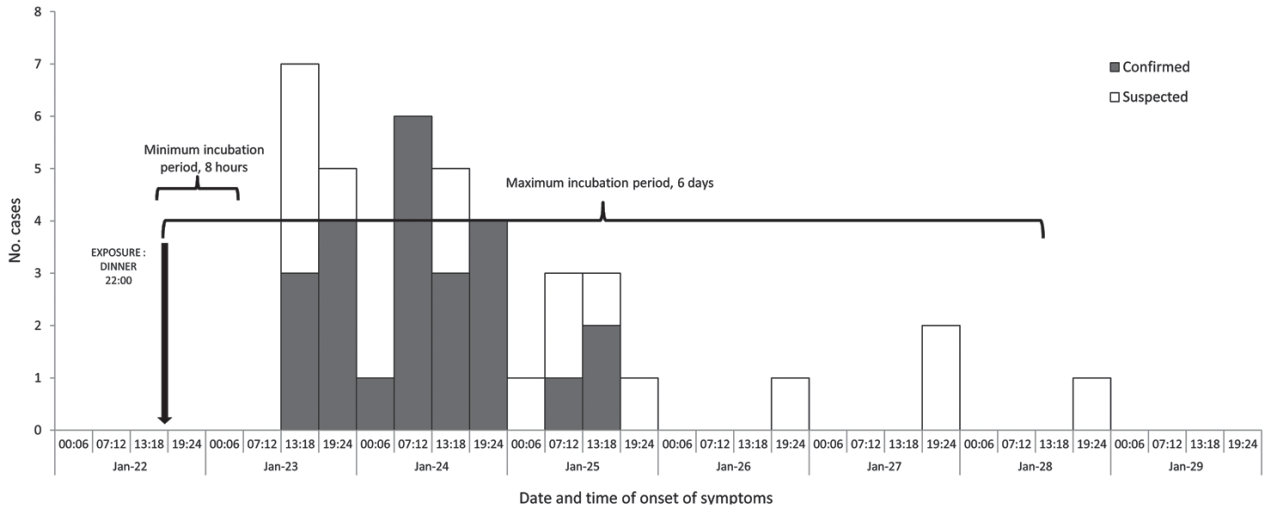

Figure. Date and time of onset of cholera cases (N = 40) associated with a wedding in La Romana, Dominican Republic, January 2011 (2 case-patients not represented here because time of symptom onset was not reported).

Table 2. Odds of consuming specific food and beverage items during a wedding, Dominican Republic, January 2011*

Food item consumed	No. (%) case-patients, n = 42	No. (%) controls, n = 62	OR (95% CI)	p value
Cooked shrimp on ice	25 (60)	9 (15)	10.82 (3.31–35.35)	<0.001
Langostinos	14 (33)	9 (15)	2.23 (0.56–8.81)	0.254
Mixed rice and vegetables	1 (2)	12 (19)	0.04 (0.003–0.45)	0.009
Other food	1 (2)	10 (16)	0.18 (0.02–1.74)	0.139
Ice cubes in beverages	16 (38)	9 (15)	4.10 (1.28–13.16)	0.018

*OR, odds ratio; CI, confidence interval. Pearson goodness-of-fit for the regression model <0.001 (10 df, χ^2 38.19). Because exponentiated coefficients from the logistic regression model are shown in the table, the regression intercept (log odds for non–case-patients) is not shown.

treatment of shrimp in vinaigrette with acid (vinegar) and shrimp kebabs with heat may have killed *V. cholerae* that was present before preparation. Seafood has long been established as a vehicle for cholera transmission (*2*–*5*).

Our investigation had several limitations. We were unable to access information from 90% of wedding guests from Venezuela, potentially introducing representation bias. We did not collect information about other meals served to guests and workers before and after the wedding banquet. We did not have itineraries of guests and could not assess whether they had traveled to other parts of Hispaniola affected by cholera before the wedding. We were unable to recruit 2 controls per case-patient, thus reducing the study's statistical power to detect any weak associations. The international nature of the investigation made it difficult to obtain information in a timely manner, potentially increasing recall bias from widespread media reports of seafood being implicated in the outbreak. Most controls were workers from the Dominican Republic, and most case-patients were guests from Venezuela, which may have led to differential food and drink preferences or differential access to food items on the menu. Moreover, nearly one fifth of case-patients were food handlers, and we cannot exclude the possibility that they cross-contaminated the food or items served. Food and drink served at the reception were unavailable for testing, and we had no information about the source of water used to make ice cubes.

Recommendations to establish a cold chain, use chlorinated water, and exclude ill food handlers were provided to the catering facility and seafood provider. After the investigation, prevention and control measures, including closer adherence to the existing prohibition of importation of high-risk food items from Haiti, were implemented in tourist hotels across the Dominican Republic.

This report highlights the need for international collaboration between public health entities during cholera epidemics. Cholera prevention materials that include information on high-risk food items, such as shellfish and ice should be provided to travelers before they visit potentially cholera-affected areas. Increased awareness and active disease surveillance can help control the spread of cholera outbreaks and prevent secondary transmission.

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Dr Jiménez is a medical epidemiologist working with the Ministry of Public Health in the Dominican Republic. She recently completed the Central America Field Epidemiology Training Program and is particularly interested in field investigations of communicable disease outbreaks.

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Haiti in the Context of the Current Global Cholera Pandemic

Edward T. Ryan

Since the early 1800s, there have been 7 cholera pandemics, and 2011 marks not only the 1-year anniversary of the reappearance of cholera in Haiti but also the 50th anniversary of the onset of the current cholera pandemic that began in Indonesia in 1961. All previous pandemics lasted 5–25 years before burning out. However, the current pandemic has shown no evidence of abating. Cholera is a disease of impoverishment, displacement, and unrest, and the 2010–2011 Haiti and global cholera milestones are integrally related. In addition to Haiti, during the past 10 years, there have been major cholera epidemics in Zimbabwe, Kenya, Nigeria, Afghanistan, Iraq, Somalia, Angola, Guinea-Bissau, Sudan, South Africa, Malawi, Liberia, and Vietnam. Cholera is endemic to >50 countries, affects 2–5 million persons each year, and kills 100,000. Most of these cases never come to public or media attention, and many of them occur in areas where cholera is deeply entrenched and often affects children. In some areas of southern Asia, most residents will have serologic evidence of infection with *Vibrio cholerae* by their teenage years.

Why has this pandemic persisted for so long? The answer is that we do not know, but several factors seem to be major contributors to its longevity. First, the organism is different from the version microbiologically associated with previous pandemics. Previous pandemics for which we have data were caused by the classical *V. cholerae* O1 biotype, but the current pandemic is caused by the El Tor biotype. *V. cholerae* persists in aquatic reservoirs, and for unclear reasons, the El Tor biotype seems to have a distinct transmission or environmental survival advantage and has replaced the *V. cholerae* classical biotype worldwide. This advantage may translate into increased likelihood that *V. cholerae* will become endemic and persist in a local environment after its introduction into new areas. The El Tor biotype is also associated with more prolonged clinical outbreaks, often featuring multiple waves, and has the ability to cause mild disease or short-term asymptomatic passage once established in a population. These features contribute to the silent introduction of cholera into new areas, as unfortunately occurred this past year in Haiti.

During the current pandemic, the El Tor biotype has continued to evolve. In the early 1990s, this biotype mutated to a new serogroup, O139, and rapidly spread to several countries in Asia, joining O1 as a cause of epidemic cholera. Previous immunity to *V. cholerae* O1 provided no protection against O139. The number of cases caused by O139 then decreased, leaving the O1 El Tor biotype as the predominant cause of cholera, perhaps again underscoring some poorly understood survival or transmission advantage of this biotype. During the past 2 decades, the organism again evolved and became hybrid, keeping its El Tor biotype characteristics but incorporating classical biotype cholera toxin, a feature that may be contributing to high case-fatality rates associated with many recent cholera outbreaks. *V. cholerae* continues to evolve, and resistance to antimicrobial drugs is complicating treatment options in areas with limited resources.

However, changes in the organism only partly explain the complexity of our current pandemic situation. Cholera is a disease of the most impoverished, but it is first and foremost a disease affected by the global economy and transportation, initially spreading from its ancestral home in southern Asia along trading and commerce routes of the nascent global economy of the early 1800s. Although cholera spreads through global interactions, it paradoxically predominantly affects those most estranged from the benefits of globalization. In the long term, economic investment and civil stability will lead to the demise of cholera, but with ≈1 billion persons currently lacking safe water, and 2.6 billion currently lacking adequate sanitation, our current war with cholera will go on for decades.

Do we just grin and bear it, or is it time to change our response strategy? No one would argue that cholera response programs need to be based on case detection,

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appropriate fluid management, and provision of safe water and improved sanitation. However, is it time to integrate new tools? Previous response plans grew from previous experience: wild fire cholera epidemics that burned out quickly, ability of rehydration strategies to decrease case-fatality rates to <1%, and a problematic parenteral cholera vaccine. However, with the propensity of the El Tor biotype to cause prolonged and recurrent outbreaks, high likelihood of becoming endemic, ability to be carried asymptomatically, association with case-fatality rates of 2%–6% among patients who receive clinical care during complex emergencies, and availabilty of improved oral cholera vaccines, is it time to rethink our plans? Should vaccines be used more broadly?

Strong evidence would support use of cholera vaccines in disease-endemic settings, and an evolving body of evidence, largely from increased interest in cholera after its appearance in Haiti, suggests that cholera vaccines might be beneficial in reactive situations, i.e., after an outbreak has started. However, such use would first require additional field and cost-effectiveness evaluations and intricate planning and commitment. Would an international stockpile of vaccine be beneficial? Who would support and manage it? What would be the triggers for its use? How would its benefit be measured? Similarly, should there be wider or more specific use of antimicrobial drugs in the initial stages of a cholera outbreak with the goal of blunting transmission? Would this buy time? Would such distribution be not only useless, but also detrimental, accelerating the development of antimicrobial drug resistance? And why is it so hard to get clean water and adequate sanitation to those who need it most? What are the obstacles? How can we improve our track record?

Quite simply, we do not yet know the answers to many of these questions, but we should not only view the cholera epidemic in Haiti as a true catastrophe, which it is of immense proportions, but we should also view it as an opportunity. Will we use the hydra-headed reappearance of cholera in Haiti as an impetus to adapt and respond, learning from our successes and failures, or will we be ill-prepared when cholera appears in the next Zimbabwe, the next Afghanistan, the next Haiti? The next Haiti will be here sooner than we think.

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Preparing Health Care Workers for a Cholera Epidemic, Dominican Republic, 2010

To the Editor: On October 21, 2010, a case of cholera was laboratory confirmed in Haiti, and within 2 months, ≈120,000 cases, 60,000 hospitalizations, and 2,500 deaths were reported (*1*). On November 16, the first laboratory-confirmed case of cholera was reported in the Dominican Republic, a country of 10 million persons sharing the island of Hispaniola with Haiti (*2*). Although the Dominican Republic has more developed sanitary infrastructure than does Haiti, spread of cholera into the country was inevitable (*3*,*4*).

Before 2010, most health care workers (HCWs) in the Dominican Republic lacked firsthand experience or training in managing cholera patients (*5*,*6*). To improve response capacity, the Ministry of Health, in collaboration with international experts, rapidly produced clinical protocols for cholera diagnosis, treatment, and infection control (*7*,*8*). Pocket guides and posters were distributed throughout each region during October and November.

During December 1–17, field teams of public health physicians visited 67 critical resource hospitals (all 11 regional referral hospitals, all 20 large provincial hospitals, and 36 additional municipal hospitals across the country). Field teams met with hospital directors and staff, conducted a survey to assess recent cholera training and knowledge based on national protocols, and offered additional training materials and resources (*7*).

At each hospital, a convenience sample of all available HCWs (e.g., physicians, nurses, cleaning staff) was assembled to discuss cholera. After giving verbal consent, participants anonymously self-administered a standardized 20-question multiple-choice survey. Immediately afterward, answer keys and explanations were distributed for participants to review and keep, along with supplemental training materials including videos, posters, and pocket guides.

Responses to questionnaires were scanned, scored, and analyzed by using Remark Office software version 8.0 (OMR Data Center, Gravic Inc., Malvern, PA, USA). The Centers for Disease Control and Prevention determined that this evaluation was not research because it was conducted as a public health response to an emergency.

Of 233 respondents who completed surveys, 125 (54%) were physicians, 33 (14%) licensed nurses, 57 (24%) auxiliary nurses, and 18 (8%) another type of hospital staff. At least 58 (25%) believed that at least 1 of their 100 most recent patients had illness that met the definition for a suspected case of cholera.

Eighty (34%) respondents answered ≥80% of questions correctly; 97 (42%) answered 60%–79% of questions correctly, and 56 (24%) answered <60% of questions correctly. Nationally, the average overall test score was 71%.

Most (174 [75%]) respondents reported having received cholera protocol training after the start of the outbreak in Haiti, and 146 (63%) already carried their personal copy of the national pocket guide to cholera diagnosis and treatment. Respondents who had received cholera training answered a mean of 76% of questions correctly, whereas respondents who had not received cholera training answered a mean of 62% of questions correctly. Of respondents who reported receiving training, the largest group was physicians (111 [64%]). Most (>90%) participants correctly answered questions about case definitions for suspected cholera and the need for immediate rehydration for all cholera patients (Table).

Most HCWs had received cholera protocol training through public health efforts, which improved knowledge in key topic areas. Improving health care response capacity is an important way to reduce cholera case-fatality rates (*9*). The World Health Organization recommends that intervention strategies ideally should aim to reduce case-fatality rates to <1% by ensuring access to treatment and controlling the spread of disease (*10*). As of December 18, 2010, cholera had been confirmed in 59 persons in the Dominican Republic; 46 of these persons were hospitalized, and none died (*1*). Efforts to prepare HCWs likely contributed to the initially low case-fatality rate by improving clinical case management and response capacity. These successful collaborative efforts to train HCWs strengthened relationships and communication within local, national, and international public health networks.

This analysis has at least 4 limitations. First, although all major hospitals in the country were visited and all geographic regions are represented, hospital staff were not randomly selected; thus, results are not generalizable to hospital staff throughout the country. Second, denominator information for types of HCWs was not available. Third, biases are possible in self-reported survey results. Finally, HCWs at primary care centers, which may be commonly visited by persons seeking acute medical care especially in rural areas, were not systematically assessed.

To strengthen the capacity of HCWs to respond to threats such as epidemic cholera, our approach included site visits, dissemination of national clinical guidelines, and rapid assessment of knowledge deficiencies that could be addressed by providing immediate training and educational materials. Training should involve not solely physicians but also hospital

Table. Percentage of 233 hospital staff correctly answering cholera knowledge assessment questions near the start of a cholera epidemic in the Dominican Republic, 2010

Topic	No. (%)
Importance of rehydration	207 (89)
Suspected cholera case definitions	206 (88)
Nutrition for cholera patients	198 (85)
Ideal type of intravenous fluid	189 (81)
Infection control measures	175 (75)
Environmental cleaning	168 (72)
Risk factors for disease	157 (67)
Handling cadavers	146 (63)
Quantity and timing of intravenous fluid	128 (55)
Uses of bleach solution	128 (55)
Treatment of mild dehydration	125 (54)
Identification of severe dehydration	122 (52)
Treatment of severe dehydration	108 (46)
Disinfection methods	100 (43)

staff who may provide direct care to patients, such as nurses, and other personnel who may share infection control responsibilities. Hospital staff should maintain ongoing communication with public health leadership.

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Understanding the Cholera Epidemic, Haiti

To the Editor: After the devastating outbreak of cholera in Haiti in mid-October 2010, several hypotheses have emerged regarding the origin of the outbreak. Some articles and media reports pointed to the United Nations peacekeepers from Nepal as the source. Piarroux et al. drew a similar conclusion from their epidemiologic study (*1*). Nepal did experience an outbreak of cholera during August–October 2010, in which 72 cases of infection with *Vibrio cholerae* O1, serotype Ogawa, were confirmed, mostly among young adult males. The cases peaked from mid-September to early October (Figure; online Appendix Figure, wwwnc.cdc.gov/EID/article/17/11/11-0981-FA1.htm), and no deaths occurred. Despite this similarity in timing, I believe several points need to be considered before a firm conclusion is reached.

Cholera strains isolated in Haiti were genetically most similar to strains detected in Bangladesh in 2002 and 2008; thus, cholera was most likely introduced into Haiti from southern

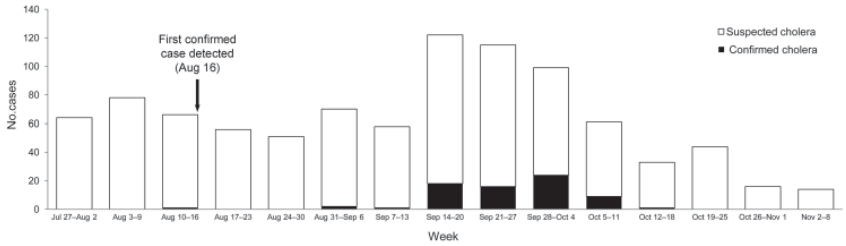

Figure. Patients with confirmed and suspected cases of cholera admitted to Sukraraj Tropical and Infectious Disease Hospital, by week, Katmandu, Nepal, July–November 2010. Case definitions: suspected cholera, acute watery diarrhea, with or without vomiting, in a child >5 years of age; confirmed cholera, isolation of *Vibrio cholerae* O1 or O139 from feces of any patient with diarrhea.

Asia (*2*). Despite the genetic similarity in the strains, no attempt was made by the researchers to ascertain and rule out the source of the outbreak in Bangladeshi policemen stationed at Mirebalais between September and October 2010. Another, although less likely, source for the introduction of cholera into Haiti could have been travelers or relief workers who may have recently been to southern Asia. Most relief workers probably come from countries without endemic cholera, but they cannot definitely be ruled out as a source of cholera in Haiti. For example, in industrialized countries, cholera has been detected among travelers, albeit in smaller numbers, returning home from cholera-endemic areas (*3*,*4*). However, Piarroux et al. offered no information about travelers or relief workers or whether they had been screened for *V. cholerae* infection before coming to Haiti (*1*). Of note, the United Nations reported that none of the Nepalese peacekeepers was found to be positive for the strain in Haiti (*5*); hence, other possible explanations for the origin of the outbreak simply cannot be overlooked.

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In Response: We read with great interest the letter by Pun, which suggests that Bangladeshi policemen in Mirebalais could have introduced cholera into Haiti (*1*). However, we want to emphasize that the first Haitian cholera case occurred in Meille, just next to the Nepalese military camp—not in Mirebalais or Hinche, where Bangladeshi policemen served. The location of the first case was stated in our article (*2*) and confirmed by the United Nations (UN) panel of experts on the cholera outbreak in Haiti (*3*). The UN panel also reported that major sanitation deficiencies likely resulted in contamination of a stream flowing within a few meters of the Nepalese camp. No other humanitarian forces were working in the small hamlet of Meille.

As acknowledged by Pun, Nepalese soldiers left for Haiti just when a cholera epidemic was raging in their country. According to the UN panel report, "a careful analysis of the MLVA [multilocus variable-number tandem-repeat analysis] results and the *ctxB* gene indicated that the strains isolated in Haiti and Nepal during 2009 were a perfect match." Nepalese strains had been made available to the UN Panel from the International Vaccine Institute in Seoul, South Korea (*3*).

Referring to UN press conferences, Pun stated that "none of the Nepalese peacekeepers was found to be positive for the [*V. cholerae*] strain in Haiti." However, it should be remembered that no testing of the soldiers was performed. Although the UN panel reported that "no cases of severe diarrhea and dehydration occurred among MINUSTAH [United Nations Stabilization Mission in Haiti] personnel during this period," the panel provided no information concerning mild or moderate diarrhea.

Overall, evidence overwhelmingly supports the conclusion that the UN military camp in Meille was the source of the Haitian cholera

epidemic. The person who brought cholera into Haiti could not be identified because of the lack of an early, independent investigation in the camp.

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Correction

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In the article Reassortment of Ancient Neuraminidase and Recent Hemagglutinin in Pandemic (H1N1) 2009 Virus (P. Bhoumik, A.L. Hughes), errors were made in selection of the hemagglutinin (HA) and neuraminidase (NA) sequences for the initial and subsequent data sets. As a result, the authors incorrectly concluded that the NA gene of the pandemic (H1N1) 2009 virus is of a more ancient lineage than the HA. Other researchers (and the authors) have not been able to reproduce the findings when using HA and NA matched pairs from viruses chosen on the basis of geography and time and correctly have pointed out errors in the data set that make the original conclusions invalid.

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Georges Desarmes (b. 1950) *The Bathers* (2006) (detail) Acrylic on canvas (61 cm × 50.8 cm). Courtesy of Patrick Lammie

Persistence of Memory and the Comma Bacillus

Polyxeni Potter

"That anyone should condescend to die of cholera at the bidding of so insignificant a creature as the comma bacillus," wrote Marcel Proust, should not be astonishing to those in the know. Plagued by illness from childhood, the author was very much in tune with medicine, which he pondered often in Remembrance of Things Past (1913), his monumental novel on the nature of memory. He also knew about cholera. His father, eminent physician and public health pioneer Achille-Andrien Proust, dedicated much of his life to promoting *cordon sanitaire* for the control of the disease, convinced that "questions of international hygiene reach beyond the borders established by politics." The elder Proust's idea, ahead of his time, challenged free trade so it was not until the Bombay cholera epidemic of 1877 that the concept of quarantine prevailed over commercialism and self-interest.

Marcel Proust was proud of his father. If visitors to the house were ever unwell, he was wont to ask them, "Would you like Papa to come to see you?" Once, when he made the offer to Anatole France, the man of letters replied, "My dear young friend, I should never dare to consult your father; I'm not important enough for him. The only patients he takes on nowadays are river basins!" Indeed, Dr. Proust had turned to public health. At great personal risk, he had cared for many a cholera patient during the 1866 epidemic in France and came to understand that individual treatment could not defeat this disease but prevention might control it. An early authority on epidemiology and a tireless advocate of an international sanitation system against disease spread, he is known in the history of medicine for his single-minded devotion to achieving the exclusion of cholera from the borders of Europe. This, some 20 years before Robert Koch identified the causative agent of the disease.

A severe infection spread by water contaminated with human waste, cholera was likely known in antiquity. Hippocrates and Galen described a compatible set of symptoms, and many sources point to similar illness, always present and frequently epidemic, in the plains of the Ganges River. In his landmark paper identifying the cause of the disease, Koch pinpointed the Ganges Delta as the *Heimat* (homeland) of cholera.

Many studied the scourge. In 1854, Filippo Pacini in Florence described vibrios in the intestinal contents of cholera victims and was amazed at their large numbers in

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the mucus and desquamated epithelial cells. He described the culprit as "an organic living substance of parasitic nature which can reproduce itself and thereby produce a specific disease." In London, John Snow demonstrated the role of water as carrier of the disease, prompting local authorities to, reluctantly, remove the handle of the notorious Broad Street pump. But it was not until 1884, during the outbreak in Egypt, when Koch noted that the disease was a specific gastrointestinal infection caused by a comma-shaped bacillus, which he isolated in the laboratory and named *Vibrio cholerae*. And it was not until 1965 that the Judicial Committee on Bacteriological Nomenclature ruled that the organism should be known as *Vibrio cholerae* Pacini 1854.

Cholera can strike anywhere when sanitary conditions are compromised and the causative agent is present. After a short incubation period of 2 or 3 days, the patient becomes ill with serious diarrhea and nausea followed, in severe cases, by extreme dehydration and death. Early European observers were struck by the patients' mummified appearance due to the draining of fluid from soft tissues.

Modern knowledge about cholera dates from the beginning of the 19th century, with seven major pandemics from 1800 to 1995, and has seen great progress in treatment if not prevention. With oral rehydration therapy, few patients should die if clean water is available. But floods and other natural disasters, along with social and economic ills favoring unsanitary conditions, compromise clean water supplies. Increased travel, population movements, and global conflict facilitate microbial traffic. Far from disappearing, cholera shows its ugly head as soon as the opportunity arises.

In October 2010, an outbreak of cholera was confirmed in Haiti. The two required conditions for emergence were present: *V. cholerae* introduced into the population and breaches in the water, sanitation, and hygiene infrastructure permitting exposure to contaminated water.

The Bathers, on this issue of Emerging Infectious Diseases, painted by Georges Desarmes, provides a glimpse of life on the Artibonite River and the bordering communities before the outbreak. Desarmes, born Yves Michaud in Port-au-Prince, began his artistic career working with Nehemy Jean, a well-traveled Haitian artist with diverse training in the United States and elsewhere. In the mid-1970s, Michaud met and went to work with Carlo Jean-Jacques, a Haitian impressionist; and in 2000, he started to paint in an entirely new style and assumed the name Georges Desarmes. Since then he has painted impressions of Haitian life.

In *The Bathers*, the artist captures the ease and communal charm of living along the river bank lined with tiny homes and populated with locals relating to each other on a personal level as they cool off. The scene is lyrical, spare, sunny, pre-cholera. On this day, this is the center of the universe, and this is the life. The artist captures the gist of it in a way only an impressionist could. As Proust would put it, "it's a country to be happy in." Yet this scene has since moved to another sphere, much like Proust's impressionist moments in time.

Refusing to recognize false boundaries, cholera encompasses the frailties of political conflict and the aftermath of mass travel and increased human contact. Since no outbreaks were seen in the Caribbean since the mid-19th century, it was said that Haiti had no memory of or experience in handling cholera. But Proust would disagree. The memory was there: Ships pull in harbors with unknown pestilent cargo. Sanitary conditions are not optimal. Contraband microbes hop off and settle in new areas among populations with no immunity or infrastructure to prevent rapid spread of disease.

Desarmes' lyrical impression of the waterfront meets Remembrance of Things Past. Proust struggled with the concept of involuntary memory, in which everyday cues evoke recollection of things past. But human history benefits more from voluntary memory, a deliberate effort to recall the past. Unless that happens, no one should be surprised if an inconsequential microbe causing a preventable and treatable disease continues to kill so many people.

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EMERGING INFECTIOUS DISEASES

Upcoming Issue

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November 16–19, 2011
7th World Congress of the World Society for Pediatric Infectious Diseases (WSPID 2011)
Melbourne, Victoria, Australia
http://www.kenes.com/wspid2011/mailshot/ms3.htm

March 5–8, 2012
19th Conference on Retroviruses and Opportunistic Infections (CROI 2012)
Washington State Convention Center
Seattle, WA, USA
http://www.retroconference.org

March 11–14, 2012
ICEID 2012
Atlanta, GA, USA

June 13–16, 2012
15th International Congress on Infectious Diseases (ICID)
Bangkok, Thailand
http://www.isid.org/15th_icid

Announcements

To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

Announcements may be posted on the journal Web page only, depending on the event date.

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Earning CME Credit

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 70% passing score) and earn continuing medical education (CME) credit, please go to **www.medscape.org/journal/eid**. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.org. If you are not registered on Medscape.org, please click on the New Users: Free Registration link on the left hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/category/2922.html. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for *AMA PRA Category 1 Credits*™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the certificate and present it to your national medical association for review.

Article Title

Deaths Associated with Pandemic (H1N1) 2009 among Children, Japan, 2009–2010

CME Questions

1. You are seeing a 4-year-old girl with a 2-day history of fever, malaise, and cough. Two other children in her day care were diagnosed with pandemic (H1N1) 2009 last week. Her parents are concerned with the possibility of serious outcomes, including death, associated with a potential pandemic (H1N1) 2009 infection.

What was the most common symptom among children in the current case series of fatal pandemic (H1N1) 2009 cases?

A. Cough
B. Rhinorrhea
C. Altered mental status
D. Fever

2. What were the 2 most common causes of death in the current case series of children with pandemic (H1N1) 2009 infection?

A. Respiratory failure and viral sepsis
B. Encephalopathy and viral sepsis
C. Myocarditis and respiratory failure
D. Encephalopathy and cardiopulmonary arrest

3. What other characteristics of the specific causes of death associated with pandemic (H1N1) 2009 infection should you consider in the evaluation and treatment of this patient?

A. 90% of children who died of cardiopulmonary arrest had important preexisting medical conditions
B. Chest radiographs demonstrated bilateral infiltrates among children who died of cardiopulmonary arrest
C. All children with encephalopathy had evidence of brain edema on neuroimaging studies
D. Children who died of respiratory failure were generally free of preexisting medical conditions

4. Which of the following variables was most significantly related to the cause of death of children in the current study?

A. Older age was associated with a higher risk of cardiopulmonary arrest
B. Cardiopulmonary arrest occurred close to the onset of illness compared with other causes of death
C. Treatment with neuraminidase inhibitors reduced the overall risk of mortality
D. Respiratory or neurologic disorders were more frequent among children with death due to respiratory failure

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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Article Title

Global Distribution and Epidemiologic Associations of *Escherichia coli* Clonal Group A, 1998–2007

CME Questions

1. Which of the following statements regarding clonal group A (CGA) and resistance to trimethoprim/sulfamethoxazole (TMP/SMZ) in the current study is most accurate?

A. Most *Escherichia coli* isolates resistant to TMP/SMZ were from CGA worldwide
B. Most *E. coli* isolates resistant to TMP/SMZ were from CGA only among children
C. The prevalence of CGA was higher among *E. coli* isolates resistant to TMP/SMZ
D. The overall prevalence of CGA was approximately 25% among all *E. coli* isolates

2. Which of the following countries had the highest prevalence of CGA in the current study?

A. United States
B. Taiwan
C. Nigeria
D. Zambia

3. Which of the following variables had the most significant impact on the prevalence of CGA in the current study?

A. Geographic location
B. Age (adult vs. child)
C. Inpatient vs. outpatient status
D. Isolates from urine vs. nonurine specimens

4. Which of the following statements regarding the prevalence of CGA between 1998 and 2007 in the current study is most accurate?

A. The overall prevalence remained roughly similar between the periods from 1998 to 2002 and 2003 to 2007
B. The overall prevalence doubled between the periods from 1998 to 2002 and 2003 to 2007
C. The overall prevalence increased fivefold between the periods from 1998 to 2002 and 2003 to 2007
D. The overall prevalence declined by more than half between the periods from 1998 to 2002 and 2003 to 2007

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
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Article Title

Close Similarity between Sequences of Hepatitis E Virus Recovered from Humans and Swine, France, 2008–2009

CME Questions

1. Based on the French study by Dr. Bouquet and colleagues, which of the following statements about epidemiologic features of autochthonous hepatitis E virus (HEV) infections is most likely correct?

A. Most cases are in women
B. Most cases are in adolescents and young adults
C. Genotypes 1 and 2 are the cause of sporadic cases in industrialized countries
D. Waterborne outbreaks of HEV in developing countries may differ epidemiologically and genetically from cases reported in industrialized countries

2. Based on the study by Dr. Bouquet and colleagues, which of the following statements about the genetic identity of HEV strains found in humans and swine during an 18-month period in France is most likely correct?

A. About half of sequences belonged to genotype 3
B. Similarity of about 75% was found between HEV sequences of human and swine origins
C. Subtype 3c was the largest cluster
D. Both human and swine populations had the same proportions of subtypes 3f, 3c, and 3e

3. You are asked to consult with a public health department in southern France regarding a recent increase in autochthonous HEV cases. Based on the study by Dr. Bouquet and colleagues, which of the following statements is most likely to appear in your report?

A. Spread of swine HEV infection within a herd occurs primarily by respiratory droplet transmission
B. The highest risk to humans eating pork products is associated with eating pork chops
C. Environmental factors are a more likely source of exposure than ingestion of pork products
D. A surveillance and control plan, either at the level of pig production or at the level of food processing, is recommended

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Use terms as listed in the National Library of Medicine Medical Subject Headings index (www.ncbi.nlm.nih.gov/mesh).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Submit figures as separate files, in the native format when possible (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .jpg or .tif files. Other file formats may be acceptable; contact fue7@cdc.gov for guidance. Figures should not be embedded in the manuscript file. Use color only as needed. Use Arial font for figure lettering. Figures, symbols, lettering, and numbering should be clear and large enough to remain legible when reduced to print size. Large figures may be made available online only. Place figure keys within the figure; figure legends should be provided at the end of the manuscript file.

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Types of Articles

Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome.

Research. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no abstract, figures, or tables. Include biographical sketch.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research, are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. No biographical sketch is needed.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only and should contain 500–1,000 words. They should focus on content rather than process and may provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Announcements. We welcome brief announcements of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.